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ORIGINAL PAPER

JMS Journal of Medical Science

Effect of glibenclamide, catechin and ethanolic neem leaf extract on pacreatic beta cell regeneration in alloxan-induced diabetic rat

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ABSTRACT

Introduction. Type 1 diabetes mellitus is characterized by the destruction of pancreatic β-cells, leading to insulin deficiency and persistent hyperglycemia. This study investigates the regenerative potential of glibenclamide, catechin, and ethanolic neem leaf extract on β-cell function and architecture in alloxan-induced diabetic rats.

Material and methods. Thirty-five male Sprague-Dawley rats were divided into five groups: normal control (Group A), diabetic control (Group B), glibenclamide-treated (Group C), catechin-treated (Group D), and ethanolic neem leaf extract -treated (Group E). Diabetes was induced using alloxan monohydrate (150 mg/kg, i.p.), and treatments were administered orally for 14 days.

Results and conclusions. Biochemical analysis revealed marked hyperglycemia and hypoinsulinemia in diabetic controls, alongside elevated oxidative stress (↑MDA, ↓GSH, SOD, CAT, TAC, TP) and inflammatory markers (NF-κB, IL-6). Treatment with glibenclamide, catechin, and ethanolic neem leaf extract significantly ameliorated these disturbances, with neem producing the most notable improvements. Ethanolic neem leaf extract -treated rats showed near-normal insulin levels, enhanced antioxidant status, and suppressed inflammatory responses. Furthermore, key regenerative markers (IGF-1, GLP-1, EGF, HGF, and betatrophin) were favorably modulated, particularly in the neem group, indicating stimulation of β-cell neogenesis and survival pathways. Histological examination supported the biochemical findings: ethanolic neem leaf extract-treated pancreases exhibited well-preserved islets and restored tissue architecture, contrasting with the degenerative features seen in diabetic controls. These findings suggest that ethanolic neem leaf extract, beyond its hypoglycemic and antioxidant effects, promotes β-cell regeneration through anti-inflammatory and growth factor-mediated mechanisms. This positions neem as a promising phytotherapeutic agent for diabetes management and β-cell restoration.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Among its major forms, type 1 diabetes is marked by autoimmune-mediated destruction of pancreatic β-cells, while type 2 involves progressive β-cell dysfunction alongside insulin resistance [1]. A central pathological hallmark of both forms is the impairment or loss of pancreatic β-cell mass, which compromises insulin production and glucose homeostasis [2].

In type 1 diabetes, chronic oxidative stress and inflammation contribute significantly to pancreatic β-cell destruction, impairing their survival and regenerative capacity. Markers such as Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT), Total Antioxidant Capacity (TAC), and Total Protein (TP) reflect the oxidative balance, with elevated MDA and reduced antioxidant enzymes indicating oxidative damage [3,4]. Inflammatory cytokines like Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Interleukin-6 (IL-6) further exacerbate β-cell apoptosis and insulin dysfunction [5,6]. Additionally, regenerative and hormonal factors—Insulin-like Growth Factor 1 (IGF-1), Glucagon-like Peptide 1 (GLP-1), Epidermal Growth Factor (EGF), Hepatocyte Growth Factor (HGF), and betatrophin—are vital in promoting β-cell proliferation, differentiation, and function [7–9]. Evaluating these markers is crucial, as they directly influence β-cell loss and recovery.

Alloxan, a urea derivative, is widely employed in experimental models to induce type 1-like diabetes by selectively destroying insulin-producing β-cells via reactive oxygen species (ROS) generation and oxidative stress [10]. This oxidative mechanism mirrors key aspects of human diabetic pathology, making it suitable for evaluating β-cell regeneration and protective interventions.

Current antidiabetic drugs, such as glibenclamide—a sulfonylurea—stimulate insulin secretion by enhancing residual β-cell activity [11]. Phytochemicals with antioxidant, anti-inflammatory, and cytoprotective properties have attracted attention for their potential in β-cell preservation and regeneration. Catechin, a flavonoid found in green tea and cocoa, has demonstrated β-cell protective effects by mitigating oxidative damage and enhancing insulin secretion [12]. Similarly, ethanolic neem (*Azadirachta indica*) leaf extract, rich in bioactive compounds like nimbolide and nimbin—both classified as limonoids—exhibits antidiabetic, antioxidant, and pancreatic regenerative properties [13,14].

This study investigates and compares the effects of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic β-cell regeneration in alloxan-induced diabetic rats. By integrating biochemical, histological, and functional assessments, this research aims to elucidate the regenerative potential of these agents, offering insight into novel therapeutic avenues for restoring pancreatic function in diabetes.

Material and methods

Thirty-five mature male Sprague-Dawley rats, seven weeks old were utilized for this study. The rats were given unrestricted access to food and water and were housed in plastic cages under typical laboratory conditions with a 12-hour day/12-hour night cycle. Ethical approval for the use of animal for research was obtained from Olabisi Onabanjo University Teaching Hospital Human Research Ethics Committee (OOUTH-HREC) with the number OOUTH/HREC/010//026/E120/2024AP. Strict adherence was made to all criteria regarding the use and care of laboratory animals. After the induction of diabetes, the animals were weighted and randomly assigned to groups followed by treatment for a period of 14 days as showed in the **Table 1**.

Plant material collection, preparation and extraction

Neem leaves (*Azadirachta indica*) were collected from Sagamu. The leaves were identified at the Department of Pharmacognosy, Olabisi Onabanjo University. The leaves were air dried in the Physiology laboratory for two weeks and were crushed using mechanical grinder.

The extraction was carried out according to the method of Nazir et al. [19] using 100 g of the leave powder dissolved in 500 ml of 99% ethanol for 3 days kept in the refrigerator with periodic shaking. The resulting mixture was then filtered using muslin cloth followed by whatman filter

Table 1. Animal grouping and experimental design.

	Group	No of rats	Treatment	References
A	Normal control	7	Distilled water only	Yakubu et al., [15]
B	Diabetes control	7	Alloxan (150 mg/kg bw) i.p	Yakubu et al., [15]
C	Glibenclamide treatment group	7	Alloxan (150 mg/kg bw) i.p + glibenclamide (5 mg/kg bw) p.o	Shan & Khan, [16]
D	Catechin treatment group	7	Alloxan (150 mg/kg bw) i.p + CTN (40 mg/kg bw) p.o	Nazir et al., [17]
E	Ethanolic neem leaf extract treatment group	7	Alloxan (150 mg/kg bw) i.p + neem leave extract (250 mg/kg bw) p.o	Dholi et al., [18]

p.o – orally; i.p – intraperitoneal; bw – body weight

paper. The filtrate was concentrated into a semi-solid mass at 40 °C under reduced pressure in the rotary evaporator. The concentrate was reconstituted in 70%ethanolto prepare the dose to be used for the study.

Induction of diabetes

The induction of diabetes was done according to the method described by Yakubu et al., [15]. Stock solution of alloxan monohydrate was prepare by dissolving alloxan monohydrate (0.9 g) in 3 ml of distilled water to give a stock concentration of approximately 150 mg/0.5 ml. Diabetes was induced by single i.p administration of alloxan monohydrate (150 mg/kg b.w.). The rats with blood glucose level greater than 200 mg/dl, 72 hours post-induction, was considered diabetic and used for this research work.

Preparation and administration of Glibenclamide

10 mg glibenclamide tablet was crushed and dissolved in 5 ml of distilled water to give a concentration of 2 mg/ml. 5 mg/kg bw dose of glibenclamide was administered from the stocked solution according to Shan & Khan [16].

Preparation and administration of Catechin

160 mg catechin (Central Drug House Ltd. India) was dissolved in 10 ml of distilled water to give a concentration of 8 mg/ 0.5 ml. 40 mg/kg bw dose of catechin was administered from the stocked solution according to Nazir et al. [17].

Preparation and administration of ethanolic neem leaf extract

4.9 g of ethanolic neem leaf extract was dissolved in 49 ml of 70% ethanol to give a concentration of 50 mg/ 0.5 ml. 250 mg/kg bw dose of ethanolic neem leaf extract was administered from the stocked solution according to Dholi et al. [18].

Measurement of fasting blood glucose

Fasting blood glucose was determined with a drop of blood from the rat tail using a glucometer (Accu-Check, Roche, Germany), after an overnight fast of 14 hours every 48 hours.

Procedure for blood collection

Blood was collected into sample bottle from the retro-orbital plexus using heparinized capillary tube according to the method of Diehl et al. [20].

Animal sacrifice and determination of organ weight

The animals were sacrificed by cervical dislocation after the expiration of research. The organs of study were harvested following midline abdominal incision, the organs weight was determined using a weighing scale.

Determination pancreatic tissue Antioxidant Enzymes Activity

Approximately 0.1 g of pancreatic tissue was excised and immediately washed with ice-cold saline to remove blood residues. The tissues were then homogenized in 4 mL of phosphate buffer solution (PBS; 0.1 M, pH 7.2). The homogenates were centrifuged at 3,000 rpm for 10 minutes at 4 °C using a refrigerated centrifuge (Thermo Scientific™ Heraeus™ Megafuge™ 16R, USA). The resulting supernatants were aliquoted and stored at -80 °C until further analysis.

GSH levels were determined using the Cayman Chemical Glutathione Assay Kit (Cat. No. 703002, USA), based on the reaction of GSH with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) forming a yellow-colored product measurable at 405

nm. Absorbance was read using a microplate reader (BioTek Epoch™, USA).

SOD activity was assayed using the Cayman Chemical Superoxide Dismutase Assay Kit (Cat. No. 706002). This method employs xanthine oxidase to generate superoxide radicals, which then react with a tetrazolium salt. SOD inhibits this reaction, and activity is inversely proportional to absorbance at 450 nm.

Catalase activity was measured with the Cayman Chemical Catalase Assay Kit (Cat. No. 707002). This kit uses the peroxidatic function of catalase to react with methanol in the presence of H_2O_2, forming formaldehyde, which is colorimetrically detected at 540 nm.

TAC was determined using the Abcam Total Antioxidant Capacity Assay Kit (AB65329, UK). This kit measures antioxidant capacity via the reduction of Cu^{2+} to Cu^{+} by antioxidants present in the sample, with absorbance measured at 570 nm.

Total protein concentration was quantified using the Bradford Protein Assay Kit (Bio-Rad, Cat. No. 5000006). The assay is based on the binding of Coomassie Brilliant Blue G-250 to proteins, forming a complex detectable at 595 nm.

MDA levels, an index of lipid peroxidation, were measured using the TBARS Assay Kit (Cayman Chemical, Cat. No. 10009055). This assay detects the MDA-TBA adduct, with absorbance read at 532 nm.

Quantification of pancreatic tissue Inflammatory Markers [Nuclear Factor kappa B (NF-κB), Interleukin-6 (IL-6)]

Levels of NF-κB in pancreatic tissue were quantified using a Rat NF-κB p65 ELISA Kit (Elabscience®, Cat. No. E-EL-R0676, USA). The assay is based on the sandwich ELISA principle using pre-coated 96-well plates with an NF-κB-specific capture antibody. After adding standards and samples, a biotinylated detection antibody was applied, followed by HRP-conjugated streptavidin and TMB substrate. Absorbance was measured at 450 nm using a microplate spectrophotometer (BioTek Epoch™, Agilent Technologies, USA). Concentrations were calculated against a standard curve.

Pancreatic IL-6 concentrations were determined using a Rat IL-6 ELISA Kit (Elabscience®, Cat. No. E-EL-R0015, USA). Following the manufacturer's protocol, standards and samples were incubated in wells pre-coated with a rat IL-6 monoclonal antibody. Detection was performed using biotin-conjugated antibodies and HRP-streptavidin, followed by TMB substrate development. The reaction was terminated with stop solution, and absorbance was recorded at 450 nm. Values were derived from a standard curve.

Determination of Betatrophin, IGF-1, GLP-1, EGF, and HGF in Pancreatic Tissue Homogenates Using ELISA

The quantification of pancreatic regeneration-associated proteins, including Betatrophin, Insulin-like Growth Factor-1 (IGF-1), Glucagon-like Peptide-1 (GLP-1), Epidermal Growth Factor (EGF), and Hepatocyte Growth Factor (HGF), was performed using enzyme-linked immunosorbent assay (ELISA) on pancreatic tissue homogenates.

Pancreatic tissues were excised promptly post-sacrifice, rinsed in ice-cold physiological saline to remove excess blood, and blotted gently on sterile filter paper. Approximately 0.1 mg of each tissue sample was homogenized in 4 mL of cold phosphate buffer solution (PBS, 0.1 M, pH 7.2) under ice-cold conditions to preserve protein integrity. The homogenates were then centrifuged at 3,000 rpm for 10 minutes at 4 °C using a refrigerated centrifuge (Thermo Scientific™ Heraeus™ Megafuge™ 16R). The resulting supernatants were carefully collected and stored at -80 °C until further analysis.

ELISA assays were carried out using rat-specific commercial kits according to the manufacturer's instructions. The following ELISA kits were used: Rat Betatrophin ELISA Kit (Elabscience®, Cat. No. E-EL-R2543), Rat IGF-1 ELISA Kit (Elabscience®, Cat. No. E-EL-R0022), Rat GLP-1 ELISA Kit (Cloud-Clone Corp., Cat. No. CEB887Ra), Rat EGF ELISA Kit (R&D Systems, Cat. No. RGE00), and Rat HGF ELISA Kit (Elabscience®, Cat. No. E-EL-R0713). These kits utilize the sandwich ELISA technique, wherein samples were loaded into 96-well microplates pre-coated with specific monoclonal antibodies against the target analytes.

After incubation with biotinylated detection antibodies and horseradish peroxidase (HRP)-conjugated streptavidin, tetramethylbenzidine

(TMB) substrate was added to induce a colorimetric reaction. The reaction was stopped with an acidic stop solution, and absorbance was read at 450 nm using a microplate reader (BioTek Epoch™, Agilent Technologies, USA). Concentrations of each analyte were calculated from standard curves generated using the supplied standards in each kit. All measurements were conducted in duplicates to ensure reproducibility and accuracy.

Histological Procedure for Pancreatic Tissue

For histological analysis, tissue samples were fixed in 10% neutral buffered formalin (NBF) for 24–48 hours, followed by dehydration in a series of ethanol solutions (70%, 80%, 90%, and 100%) and clearing in xylene. The tissues were then embedded in paraffin wax, sectioned into 5-µm thick slices using a microtome, and deparaffinized in xylene. The sections were rehydrated in a series of ethanol solutions, stained with Harris' hematoxylin solution for 5–10 minutes, and then stained with eosin Y solution for 1–2 minutes. After dehydration and clearing, the sections were mounted on glass slides using a mounting medium (DPX) and examined under a light microscope to observe tissue morphology and architecture.

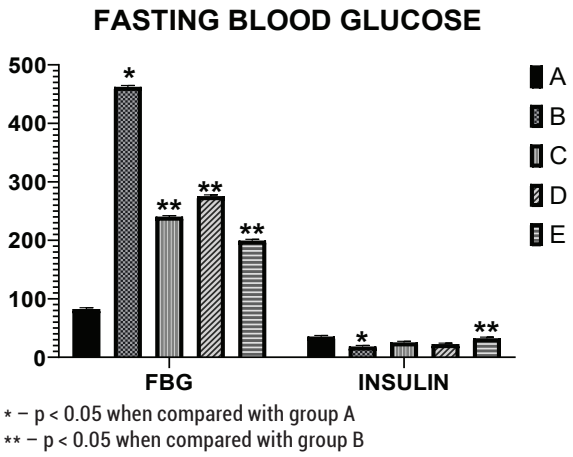
Statistical analysis

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using graph pad prism version 8 for windows. Differences between groups were analyzed by one-way anova followed by Bonferronipost-hoc test. Differences were considered significant when $p < 0.05$.

Results

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on glucose homeostasis in Alloxan-induced diabetic rat

Figure 1 presents the effects of glibenclamide, catechin, and ethanolic neem leaf extract on glucose homeostasis in alloxan-induced diabetic rats. The results revealed a significant increase in blood glucose levels (462 ± 2.64) and a significant decrease in insulin level (18 ± 2.3) in the diabetic group B compared to the control group A (82 ± 2.70; 35 ± 2.2). Conversely, treatment with glibenclamide (group C), catechin (group D), and ethanolic neem leaf extract (group E) resulted in a significant decrease in blood glucose levels (240 ± 2.12, 275 ± 1.73, and 199 ± 2.71, respectively) compared to group B (462 ± 2.64) And a significant increase in insulin level in group E (32 ± 2.4) when compared with group B. All the values are expressed as mean ± standard error of mean (SEM).



* – $p < 0.05$ when compared with group A
** – $p < 0.05$ when compared with group B

Figure 1. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on glucose homeostasis in Alloxan-induced diabetic rat.

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Oxidative Stress Markers in Alloxan-induced diabetic rat

The results presented in **Table 2** demonstrate the effect of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic tissue oxidative stress markers in alloxan-induced diabetic rat. A significant decrease in GSH, SOD, and CAT activities, as well as TAC and TP levels, was observed in the diabetic group (Group B) compared to the control group (Group A). Conversely, treatment with glibenclamide, catechin, and ethanolic neem leaf extract (Groups C, D, and E, respectively) resulted in a significant increase in antioxidant enzyme activities (GSH, SOD, and CAT), TAC, and TP levels. Additionally, a significant decrease in MDA levels was noted in the treatment groups. All the values are expressed as mean ± standard error of mean (SEM).

Table 2. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Oxidative Stress Markers in Alloxan-induced diabetic rat.

Groups	GSH	SOD	CAT	TAC	MDA	TP
A	0.94 ± 0.11	0.86 ± 0.14	7.42 ± 0.88	8.89 ± 0.85	1.11 ± 0.57	36.14 ± 0.11
B	0.46 ± 0.71*	0.51 ± 0.79*	6.01 ± 0.91*	6.99 ± 0.65*	1.95 ± 0.82*	32.38 ± 0.58*
C	0.12 ± 0.78**	0.11 ± 0.13**	7.38 ± 0.88**	10.21 ± 0.96**	1.14 ± 0.64**	36.52 ± 0.37**
D	0.15 ± 0.71**	0.16 ± 0.14**	7.47 ± 0.87**	9.05 ± 0.78**	1.01 ± 0.63**	36.94 ± 0.51**
E	0.12 ± 0.60**	0.14 ± 0.94**	7.41 ± 0.91**	10.92 ± 0.93**	0.99 ± 0.58**	37.94 ± 0.51**

* – $p < 0.05$ when compared with group A; ** – $p < 0.05$ when compared with group B
All the values are expressed as mean ± standard error of mean (SEM).

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Inflammatory Markers in Alloxan-induced diabetic rat

Table 3 presents the effects of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic tissue inflammatory markers, specifically NF-κB and IL-6 in Alloxan-induced diabetic rat. The results indicate a significant increase in NF-κB and IL-6 levels in the diabetic group (Group B) compared to the control group (Group A). In contrast, treatment with glibenclamide, catechin, and ethanolic neem leaf extract (Groups C, D, and E, respectively) resulted in a significant decrease in NF-κB and IL-6 levels compared to Group B.

Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Beta Cell Regeneration Factors in Alloxan-induced diabetic rat

Table 4 presents the effects of glibenclamide, catechin, and ethanolic neem leaf extract on pancreatic beta cell regeneration factors in Alloxan-induced diabetic rat. The results indicate a significant increase in betatrophin and HGF levels, alongside a significant decrease in IGF-1, GLP-1, and EGF levels, in the diabetic group (Group B) compared to the control group (Group A). Conversely, treatment with glibenclamide, catechin, and ethanolic neem leaf extract (Groups C, D, and E, respectively) resulted in a significant decrease

Table 3. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Tissue Inflammatory Markers in Alloxan-induced diabetic rat.

Groups	NF-kB	IL-6
A	1.56 ± 0.71	144.9 ± 0.51
B	3.52 ± 0.37*	524.7 ± 0.75*
C	1.98 ± 0.93**	330.3 ± 0.51**
D	0.77 ± 0.51**	284.4 ± 0.20**
E	1.58 ± 0.51**	226.5 ± 0.32**

* – $p < 0.05$ when compared with group A; ** – $p < 0.05$ when compared with group B
All the values are expressed as mean ± standard error of mean (SEM)

Table 4. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic Beta Cell Regeneration Factors in Alloxan-induced diabetic rat.

Groups	Betatrophin	IGF-1	GLP-1	EGF	HGF
A	6.7 ± 0.3	12.4 ± 0.24	14.9 ± 0.46	0.92 ± 0.08	1.2 ± 0.26
B	12.2 ± 0.37*	5.2 ± 0.20*	7.11 ± 0.24*	0.22 ± 0.13*	2.8 ± 0.26*
C	10.7 ± 0.49	7.9 ± 0.46**	11.2 ± 0.37**	0.53 ± 0.24**	2.1 ± 0.24**
D	11.52 ± 0.33	6.42 ± 0.26	8.3 ± 0.30	0.81 ± 0.20**	2.2 ± 0.37**
E	8.5 ± 0.31**	8.21 ± 0.20**	10.2 ± 0.37**	0.72 ± 0.27**	1.7 ± 0.30

* – $p < 0.05$ when compared with group A; ** – $p < 0.05$ when compared with group B
All the values are expressed as mean ± standard error of mean (SEM).

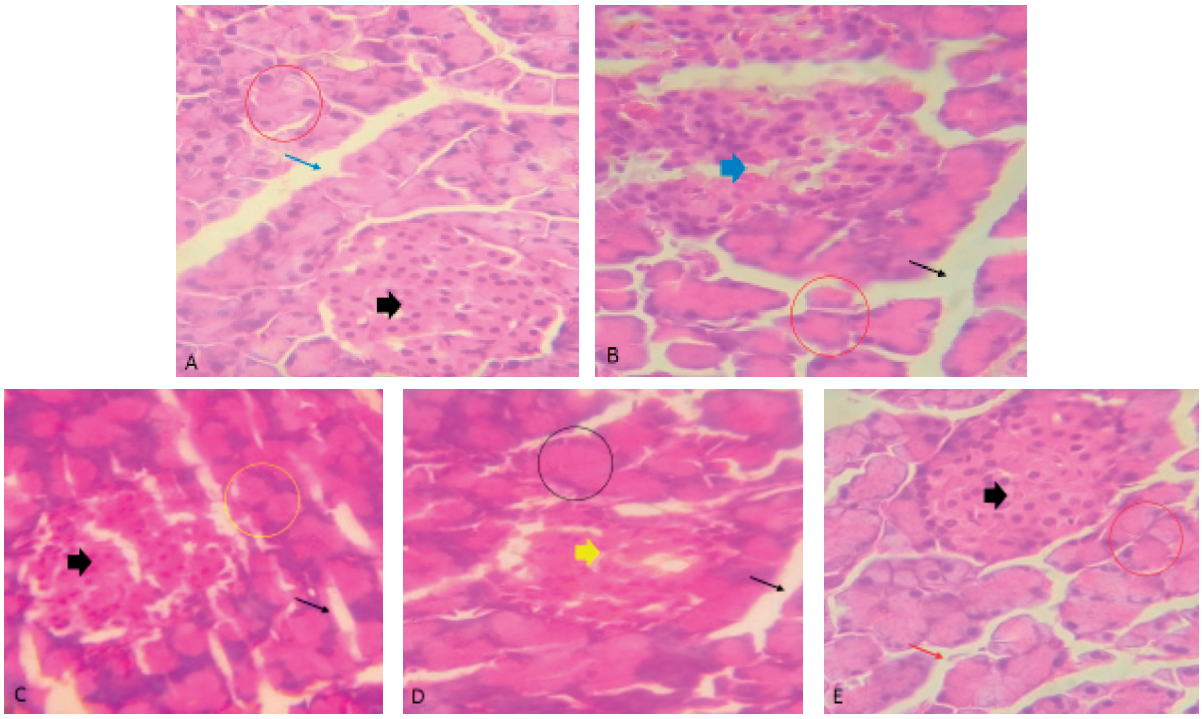


Figure 2. Effect of Glibenclamide, Catechin, and Ethanolic neem leaf extract on Pancreatic tissue histology Alloxan-induced diabetic rat. Photomicrograph of pancreatic histology showing: A – Control group showing a well differentiated and organized acinar cells (red circle), interlobular ducts (blue thin arrow) and islets of langerhans (black thick arrow); B – Diabetes shows degenerated islets of Langerhans (blues thick arrow), dilated interlobular ducts and irregular distributions of the acinar cells (red circle); C – Glibenclamide treated group shows congested interlobular ducts (black thin arrow), degenerated islets of Langerhans (black thick arrow) and acinar cells (yellow circle); D – Catechintreaed g roup shows congested and degenerated islets of Langerhans (yellow thick arrow), interlobular ducts (black thin arrow) and acinar cells (black circle); E – Ethanolic neem leaf extracttreated shows well differentiated islets of Langerhans (black thick arrow), acinar cells (red circle) and interlobular ducts(red thin arrow). H/E X 400.

in betatrophin and HGF levels, as well as a significant increase in IGF-1, GLP-1, and EGF levels, compared to Group B.

Discussion

Diabetes mellitus, particularly Type 1 diabetes, is marked by the selective destruction of pancreatic β-cells, resulting in insulin deficiency and persistent hyperglycemia. Alloxan, a toxic glucose analogue, is frequently used to induce experimental diabetes in animals due to its specific cytotoxicity to β-cells through oxidative stress mechanisms. This study examines the potential regenerative effects of glibenclamide, catechin, andethanolic neem (*Azadirachta indica*) leaf extract on β-cell function and integrity in alloxan-induced diabetic rats. Through a multifaceted physiological assessment—encompassing glucose-insulin homeostasis, oxidative stress, inflammatory markers, β-cell regenerative factors, and histological observations—the study offers deep insights into therapeutic prospects for restoring pancreatic function in diabetes.

Hyperglycemia and hypoinsulinemia are cardinal features of diabetes. In this study, Group B (diabetic rats) exhibited a significant elevation in blood glucose (462 ± 2.64 mg/dL) and a corresponding decrease in insulin levels (18 ± 2.3 µIU/mL), reflecting β-cell destruction and impaired insulin secretion. Alloxan'sdiabetogenicity stems from its selective accumulation in pancreatic β-cells via GLUT2 transporters, where it generates reactive oxygen species (ROS), leading to DNA fragmentation and apoptosis [10].

Treatment with glibenclamide, catechin, and ethanolic neem leaf extract significantly lowered blood glucose and increased insulin levels, with neem (group E) showing insulin levels nearing the control (32 ± 2.4 µIU/mL). Glibenclamide, a sulfonylurea, enhances insulin release by inhibiting ATP-sensitive K^+ channels on β-cells [21] while catechin—a flavonoid—exerts antioxidant effects and

improves insulin sensitivity [22]. Neem's superior efficacy likely arises from its multifaceted action, including β-cell protection, enhancement of insulin secretion, and peripheral insulin sensitivity [23].

Oxidative stress plays a critical role in β-cell dysfunction due to their inherently low levels of antioxidant enzymes. In diabetic rats, significant reductions in GSH (glutathione), SOD (superoxide dismutase), CAT (catalase), total antioxidant capacity (TAC), and total protein (TP) were observed, alongside increased MDA (malondialdehyde), a marker of lipid peroxidation. These findings reflect profound oxidative damage in pancreatic tissues.

All three treatments reversed oxidative damage by elevating endogenous antioxidant enzyme activities and reducing MDA levels, indicating restored redox homeostasis. Catechin and ethanolic neem leaf extract are particularly potent in activating the Nrf2 pathway, a master regulator of antioxidant gene expression [24]. Neem's efficacy may also be attributed to its polyphenolic compounds, such as nimbolide and quercetin, which directly scavenge ROS and inhibit oxidative stress-induced β-cell apoptosis [25].

Chronic inflammation exacerbates β-cell damage in diabetes. The diabetic group displayed significant elevations in NF-κB (nuclear factor kappa B) and IL-6 (interleukin-6), two key inflammatory mediators involved in β-cell dysfunction and apoptosis. NF-κB, when activated by oxidative stress, promotes transcription of pro-inflammatory cytokines like IL-6, thereby perpetuating cellular injury.

Treatment with glibenclamide, catechin, and ethanolic neem leaf extract significantly downregulated NF-κB and IL-6 levels. While glibenclamide shows modest anti-inflammatory action, catechin and neem offer more robust effects through direct inhibition of inflammatory signaling pathways. Neem's anti-inflammatory potency has been linked to inhibition of NF-κB activation, thereby reducing downstream cytokine production and preserving islet architecture [13,23].

A unique aspect of this study is its focus on β-cell regenerative markers: betatrophin, hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), glucagon-like peptide-1 (GLP-1), and epidermal growth factor (EGF). The diabetic group showed an increase in betatrophin and HGF, but a decrease in IGF-1, GLP-1, and EGF. Betatrophin and HGF upregulation may represent a compensatory but ineffective attempt at β-cell regeneration in response to injury [26].

Treatment groups showed normalized or reduced betatrophin and HGF levels, alongside increases in IGF-1, GLP-1, and EGF—markers associated with effective β-cell neogenesis, survival, and proliferation. IGF-1 promotes β-cell mass expansion through PI3K/Akt signaling, while GLP-1 enhances β-cell proliferation and reduces apoptosis [27]. EGF stimulates islet cell regeneration and has been shown to restore β-cell architecture in injured pancreases. Neem's ability to significantly boost these regenerative markers indicates its potential in restoring endocrine pancreatic function beyond mere symptom control.

Histopathological assessment of pancreatic tissue corroborated biochemical findings. Diabetic rats displayed disrupted islets of Langerhans, dilated ducts, and distorted acinar cells—consistent with alloxan-induced cellular degeneration. The glibenclamide and catechin groups showed partial restoration but retained signs of congestion and islet degeneration.

Strikingly, neem-treated rats exhibited near-normal pancreatic histology, with well-defined islets of Langerhans, intact acinar architecture, and preserved ductal structures. This morphological recovery aligns with the observed upregulation of regenerative and anti-inflammatory factors, indicating that neem not only protects but also actively restores β-cell architecture and function.

The physiological relevance of these findings lies in their demonstration that β-cell regeneration is a viable therapeutic target in diabetes management. The reversal of hyperglycemia, restoration of insulin secretion, normalization of redox and inflammatory states, and upregulation of pro-regenerative factors collectively suggest that ethanolic neem leaf extract offers a promising, multi-targeted approach to diabetes therapy. Unlike glibenclamide, which primarily enhances existing β-cell function, and catechin, which offers antioxidant support, neem exhibits both protective and regenerative capacities.

Such comprehensive β-cell restoration could translate into prolonged remission of diabetes symptoms, reduced dependence on exogenous insulin, and prevention of long-term complica-

tions. These findings underscore the potential of phytotherapeutics like neem as adjuncts or alternatives to conventional anti-diabetic drugs, especially in resource-limited settings.

Conclusion

This physiological study reveals the potential of glibenclamide, catechin, and ethanolic neem leaf extract in reversing pancreatic damage in alloxan-induced diabetic rats. While all treatments offer benefits, ethanolic neem leaf extract stands out for its ability to restore glucose-insulin balance, enhance antioxidant and anti-inflammatory responses, and stimulate β-cell regeneration both biochemically and histologically. These findings support neem's traditional use in diabetes and warrant further preclinical and clinical studies to explore its integration into mainstream diabetes management.

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Conflict of interest statement
The authors declare no conflict of interest.

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ORIGINAL PAPER

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Differentiation of benign and malignant breast lesions using shear wave elastography; estimation of the most accurate parameters

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ABSTRACT

Aim. Breast elastography is a sonographic imaging technique, used additionally in diagnosis of breast lesions. The place of shear-wave elastography (SWE) in breast imaging is still unclear, the literature is limited and the interpretation of SWE results is undefined. The aim of our study was to evaluate the diagnostic accuracy of SWE in relation to histopathology and to estimate the probable cut-off value of SWE parameters, which would indicate malignancy.

Material and methods. The study included 53 consecutive patients with suspicious breast lesions. Each patient underwent the SWE of the breasts, and every visualized lesion was biopsied.

Results. 56 lesions were found; 24 of them were classified as malignant and then confirmed as cancer. Malignant tumours presented with significantly higher SWE parameters, except elasticity value of fat tissue surrounding the lesion (Efat), as compared to benign lesions. The optimal cut-off point was determined using the Youden Index. The receiver-operating characteristic curve (ROC) curve analysis established cut-off val-

ues of: Emax 63.4 kPa (p < 0,000001, AUC 0.94), Emean of 40.8 kPa (p = 0.000003, AUC 0.87), Emax/Efat ratio of 5.64 kPa (p < 0.000001, AUC 0.92) and Emean/Efat ratio of 4.31 kPa, (p = 0.000006, AUC 0.86), which indicate malignancy. The sensitivity and specificity were 100% and 87.5% respectively for Emax and 100% and 75% for Emean. There were no differences in SWE parameters between cancer subtypes.
Conclusions. In our study SWE indicated correctly all malignant lesions. Moreover, we established cut-off values of SWE parameters that may be useful in differentiating malignant and benign breast lesions.

Introduction

Breast elastography is a technique that has been recently used more frequently as an additional tool in the diagnosis of breast lesions. Elastography allows for the measurement of the lesion's elasticity and comparison of its stiffness to the surrounding tissue. Malignant lesions are usually stiffer than normal breast tissue, therefore elastography may potentially increase the specificity of B-mode ultrasonography (US) [1]. There are two main methods used to measure tissue stiffness: strain elastography (SE) and shear-wave elastography (SWE). SE is based on manual compression and provides a qualitative tissue elasticity assessment via color display analysis (for example, using the Tsukuba Score) [2]. SWE, on the other hand, uses acoustic waves emitted from the transducer and propagated throughout the tissue. SWE provides not only a qualitative analysis by means of color display but also a quantitative measurement of tissue elasticity in kilopascals [kPa] or wave propagation speed in meters per second [m/s] [2].

B-mode US, while highly sensitive with a sensitivity rate of up to 95%, suffers from a low specificity range of 13–81%. This high sensitivity indicates that B-mode US is effective in detecting abnormalities, but its low specificity results in a high rate of false positives, leading to unnecessary biopsies and anxiety for patients. A meta-analysis conducted by Park et al. revealed that incorporating SWE into the diagnostic process could enhance US specificity by 28% while causing only a minor decrease in sensitivity (between 1 to 5%) [3]. Other studies have also highlighted the ability of SWE to improve the specificity and overall diagnostic accuracy of US [4–6].

As a supplementary tool to B-mode US, elastography can assist in determining which lesions warrant a biopsy. Given that SWE is a more objective and reproducible method compared to SE, it holds promise for reducing the number of unnecessary biopsies [3,7,8]. This reduction in redundant procedures is beneficial not only for patient comfort and mental well-being but also for the efficiency and cost-effectiveness of healthcare systems.

Despite its potential, the role of SWE in breast imaging remains unclear, and the current literature is limited. No standardized cut-off values for kPa have been established for different breast lesions, leading to ambiguity in the interpretation of SWE results. This lack of clarity underscores the need for further research to refine the diagnostic parameters and establish robust guidelines for SWE application in breast imaging.

Aim

The aim of our study was to evaluate the diagnostic accuracy of SWE in breast lesions in relation to histopathology and to estimate the probable cut-off value of SWE parameters indicating malignancy. By doing so, we hope to contribute to the growing body of evidence supporting the clinical utility of SWE and to provide insights that could lead to more precise and reliable diagnostic protocols.

Materials and methods

Patients

This prospective study was approved by the local Bioethical Committee, and the requirement for informed written consent was necessary. Patients involved in the study were invited to participate after being recalled from the breast cancer screening programme. As recommended by the guidelines, patients with BIRADS (Breast Imaging Reporting and Data System) score 0 or ≥ 4 were recalled from screening [9, 10]. Addi-

tionally, patients referred to our institution due to palpable breast tumours were included as well.

Specific exclusion criteria were applied to ensure the accuracy and relevance of findings. Patients with a history of breast cancer or prior breast surgery were excluded from the study, as these factors could potentially affect the elasticity measurements of breast tissue. Additionally, the study did not include patients who did not provide consent.

Upon agreeing to participate, each patient underwent a comprehensive evaluation that included both ultrasonography and shear-wave elastography.

Methods

All the examinations were performed between April 2021 and May 2022 by an experienced radiologist using a Canon Aplio i600 ultrasound system. The imaging protocol included capturing multiple measurements of each lesion to ensure accuracy and reliability. All lesions identified during the imaging sessions were histopathologically verified either by a US-guided core-needle biopsy or a US-guided vacuum-assisted biopsy. The choice between core-needle and vacuum-assisted biopsy was based on the lesion's characteristics, size, and location. Core-needle biopsies involve the use of a hollow needle to extract tissue samples from the lesion, while vacuum-assisted biopsies employ a vacuum-powered instrument to collect larger tissue samples, which can be particularly useful for small or complex lesions.

The histopathological results of the biopsies were classified according to the B code classification system, as established by the UK National Coordinating Committee for Breast Screening Pathology (NCCBSP). This classification system categorizes biopsy results into several categories (B1 to B5), ranging from normal tissue (B1) to malignant lesions (B5). This standardized approach ensured a consistent and accurate interpretation of the biopsy results across all cases [11].

Following the biopsy, the cancer lesions were further analyzed based on their immunohistochemistry results. This analysis included the assessment of estrogen receptor (ER) and progesterone receptor (PR) expression levels, HER2 status, and Ki67 proliferation index. ER and PR expression levels were categorized as increased if more than 1% of the tumor cells were positive and decreased if 1% or fewer cells were positive. HER2 status was determined using immunohistochemical staining and/or fluorescence in situ hybridization (FISH), with results classified as positive or negative based on established guidelines. The Ki67 index, which indicates the proportion of tumor cells undergoing mitosis, was also recorded as part of the tumor characterization process.

US and SWE examinations

Examinations were performed using a Canon Aplio i600 device (Canon Medical Systems Europe B.V., The Netherlands) with a PLT-1005-BT linear probe (frequency range of 5–14 MHz). All lesions were examined using B-mode US, followed by SWE.

The SWE parameters obtained were maximum elasticity value (Emax), mean elasticity value (Emean), elasticity value of fat tissue surrounding the lesion (Efat), the Emax/Efat and Emean/Efat ratios. Emax value was obtained by placing a circular, 2–3 mm wide region of interest (ROI) on the stiffest area of the lesion. Emean value was obtained by drawing a free-hand ROI following the margins of the entire lesion. Efat value was obtained by placing a circular ROI on the fat lobule near the lesion. The device's software calculated the ratios.

A radiologist with 15 years of experience in breast imaging, including an 8-month training period in SWE examinations prior to the study, performed all examinations.

By employing a rigorous methodology and thorough analysis, our study aimed to provide a comprehensive evaluation of SWE's diagnostic accuracy in breast lesion assessment and contribute to the development of standardized guidelines for its use in clinical settings.

Statistical analysis

The calculations were made using Statistica 13 by TIBCO and PQStat by PQStat Software. The level of significance was $\alpha = 0.05$. The result was considered statistically significant when $p<\alpha$. The normality of the distribution of variables was tested with the Shapiro-Wilk test. Quantitative variables were compared in two groups using the Student's t-test (for a normal distribution of a variable in the groups under analysis) or the

Mann-Whitney's test (for a variable with non-normal distribution). Associations between continuous variables were evaluated using Spearman's rank correlation coefficient (R). Receiver-operating characteristic curve (ROC) analysis was performed to determine the optimal cut-off point. The optimal cut-off point was determined using the Youden Index. Sensitivity and specificity were determined for such a point. The determined areas under the curve were compared with each other using the Z statistics.

The area under the curves (AUC) with 95% confidence intervals was determined. The non-parametric DeLong method was used for this.

Results

53 consecutive patients with suspicious breast lesions were included in the study. Each patient underwent the SWE of the breasts, and every visualized lesion was biopsied.

56 lesions were found, 32 of them were classified as B2 (benign) and 24 as B4 (suspicion of malignancy). Finally, all 24 suspicious tumors were histopathologically confirmed as cancer. The average age of the patients was 60.2 years, and the average lesion size was 11.4 mm.

Benign vs malignant comparison

The comparison of SWE results of benign and cancer lesions is presented in **Table 1**. Malignant tumors presented with significantly higher Emax, Emean, Emax/Efat ratio and Emean/Efat ratio than benign lesions. There were no statistical differences in lesion size and Efat between groups.

Cut-off value

Based on the ROC curve analysis, cut-off values of different SWE parameters for differential

Table 1. Comparison of SWE parameters between benign and malignant lesions. Values are presented as median (standard deviation).

	Benign	Malignant	P value
Number Of Lesions	32	24	>0.05
Average Lesion Size [Mm]	10.19 (4.19)	12.96 (8.74)	>0.05
Emax [Kpa]	39.94 (27.65)	101.49 (25.17)	0.00001
Efat [Kpa]	10.18 (6.26)	10.40 (4.30)	>0.05
Emax/Efat Ratio [Kpa]	4.45 (2.44)	11.08 (4.86)	0.00001
Emean [Kpa]	36.2 (26.25)	71.64 (20.70)	0.00001
Emean/Efat Ratio [Kpa]	4.00 (2.39)	7.63 (2.91)	0.00001

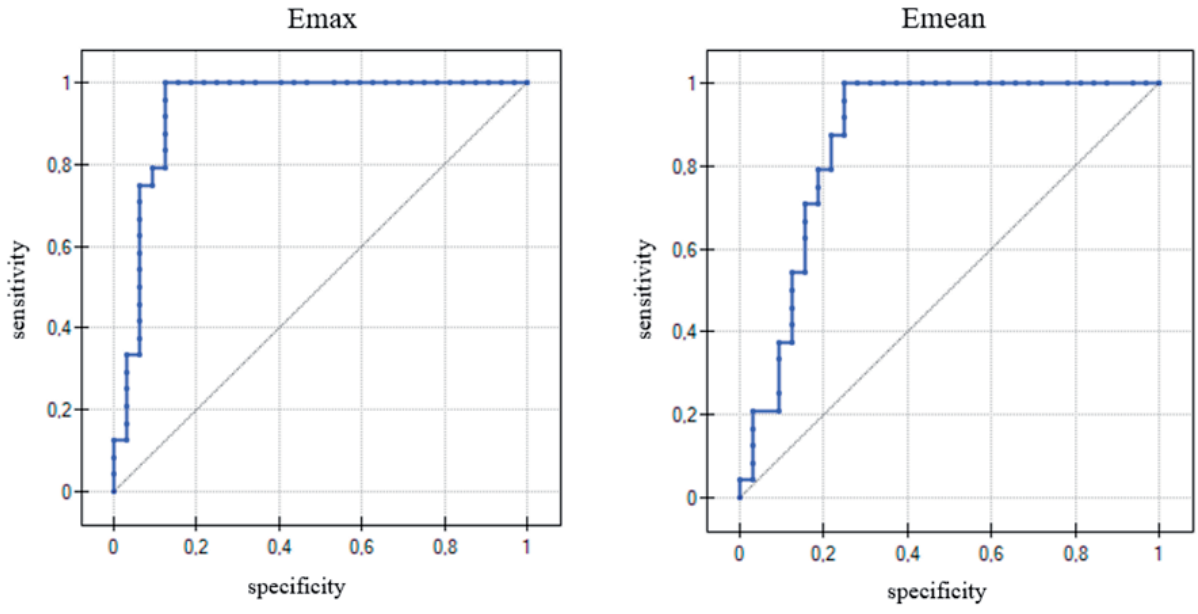

Figure 1. The ROC curve analysis estimated cut-off values of Emax and Emean for differential diagnosis of benign and malignant lesions.

diagnosis of benign and malignant lesions were established. The Emax cut-off value was 63.4 kPa (p < 0,000001), with AUC 0.94; the sensitivity and specificity were 100% and 87.5% respectively. The Emean cut-off value level was 40.8 kPa (p = 0.000003), with an AUC of 0.87, a sensitivity of 100% and a specificity of 75% (see **Figure 1**). The Emax/Efat ratio level was 5.64 kPa (p < 0.000001), with an AUC of 0.92, a sensitivity of 95.8% and a specificity of 65.6%. The Emean/Efat ratio level was 4.31 kPa, (p = 0.000006), with AUC 0.86, a sensitivity of 100% and a specificity of 68.8%.

Malignant lesions analysis

Malignant lesions were divided and evaluated depending on ER and PR expression, as well as HER2 status. There were 19 lesions with increased ER and 5 with decreased ER expression. The statistical analysis indicated no significant differences between groups in the Emax, Emean, Emax/Efat ratio and Emean/Efat ratio. Likewise, in the context of PR expression (17 lesions with increased and 7 with decreased PR expression) there were no significant differences in Emax, Emean, Emax/Efat ratio and Emean/Efat ratio. There were 19 HER2-positive cancer lesions and 5 HER2-negative lesions, and the statistical analysis indicated no significant differences in Emax, Emean, Emax/Efat ratio and Emean/Efat ratio between groups. What is more, the Ki67 level did not affect SWE results either (there was no significant correlation between Ki67 level and either Emax, Emean, Emax/Efat ratio and Emean/Efat ratio level (p > 0.05)). Adequate data are presented in **Table 2**.

Discussion

In our study, the SWE parameters directly related to the lesion's stiffness (Emax, Emean, Emax/Efat, and Emean/Efat ratios) had significantly higher mean values in malignant lesions compared to benign ones. Conversely, there was no significant difference in Efat mean values for both benign and malignant findings (see **Table 1**). This indicates that while lesion stiffness is a key differentiator between benign and malignant lesions, the surrounding fat tissue stiffness (Efat) does not significantly vary between these categories.

As a result of the ROC curve analysis, we established cut-off values for two main SWE parameters: Emax at 63.4 kPa and Emean at 40.8 kPa (see **Figure 1**). These results differ from those stated in the literature, likely due to differences in the devices used. For example, Song et al. reported Emax and Emean cut-off values of 145.7 kPa and 89.1 kPa, respectively, using the Aixplorer (Hologic) system [12]. Similarly, Au et al., using the same system, estimated cut-off values of 46.7 kPa for Emax and 42.5 kPa for Emean [13]. Kim et al., working with a Toshiba device, established values of 50.85 kPa and 42.08 kPa for Emax and Emean, respectively [14].

In Aixplorer systems, the SWE parameters such as maximum elasticity, mean elasticity, and elasticity ratio are automatically calculated by the device's software after measuring the stiffest part of a lesion [13]. However, in Canon and Toshiba systems, the measurement process differs. We obtained Emax by placing a circular ROI on the stiffest part of a lesion and Emean by

Table 2. Comparison of SWE parameters between different malignant lesions subtypes. Values are presented as median (standard deviation). No significant differences were found (p > 0.05).

	ER+	ER-	PR+	PR-	HER2+	HER2-
Emax [Kpa]	98.28 (24.37)	116.63 (30.36)	97.35 (24.80)	113.15 (26.89)	114.20 (33.04)	98.79 (24.15)
Emean [Kpa]	70.48 (21.60)	82.98 (11.22)	69.65 (22.51)	81.15 (10.94)	76.86 (12.32)	71.76 (22.05)
Ratio Emax/Efat [Kpa]	10.88 (4.32)	12.24 (8.16)	10.85 (4.57)	11.88 (6.38)	13.47 (7.45)	10.62 (4.40)
Ratio Emean/Efat [Kpa]	7.68 (3.02)	8.06 (2.69)	7.61 (3.18)	8.13 (2.21)	8.62 (2.32)	7.56 (3.04)

drawing a free-hand ROI along the lesion margins. Efat was measured by placing a circular ROI on a fat lobule surrounding the examined lesion (see **Figure 2**). The standard deviations of each parameter as well as the Emax/Efat and Emean/Efat ratios were automatically calculated by our device's software. These technical differences in measurement processes between US systems could account for the different results observed.

In our clinical experience, Emax proved to be the most accurate parameter for differentiating benign and malignant lesions. The nature of obtaining Emean measurements, which requires a free-hand drawn ROI, makes it a problematic parameter for routine use. Malignant lesions often have irregular margins, complicating the drawing of the Emean ROI and making the process time-consuming. Emax, in contrast, is relatively easier and faster to obtain while maintaining good predictive value for distinguishing between malignant and benign lesions [12, 14].

We also analyzed cancer lesions and evaluated the correlation between SWE parameters and immunohistochemistry characteristics. No correlation was found between ER and PR expression and SWE variables. Furthermore, HER2 status and Ki67 levels had no significant effect on any of the examined SWE measurements (see **Table 2**). In contrast to our findings, Chang et al. reported that more aggressive types of breast cancer (e.g., tri-ple-negative) and tumours with higher histological grades exhibited higher mean stiffness [15]. Kim et al. found that PR-negative tumours had higher Emax values and that the Emax/Efat ratio was higher in tumours with Ki-67 ≥ 14% [14]. However, some researchers found no significant correlation between SWE parameters and immunohistochemistry characteristics in breast cancer tumours, as well as in breast cancer axillary metastases [16, 17, 18]. The conflicting results from different studies highlight the need for more research on the correlation between SWE parameters and breast cancer immunohistochemistry characteristics.

As previously mentioned, obtaining SWE measurements varies between different US systems.

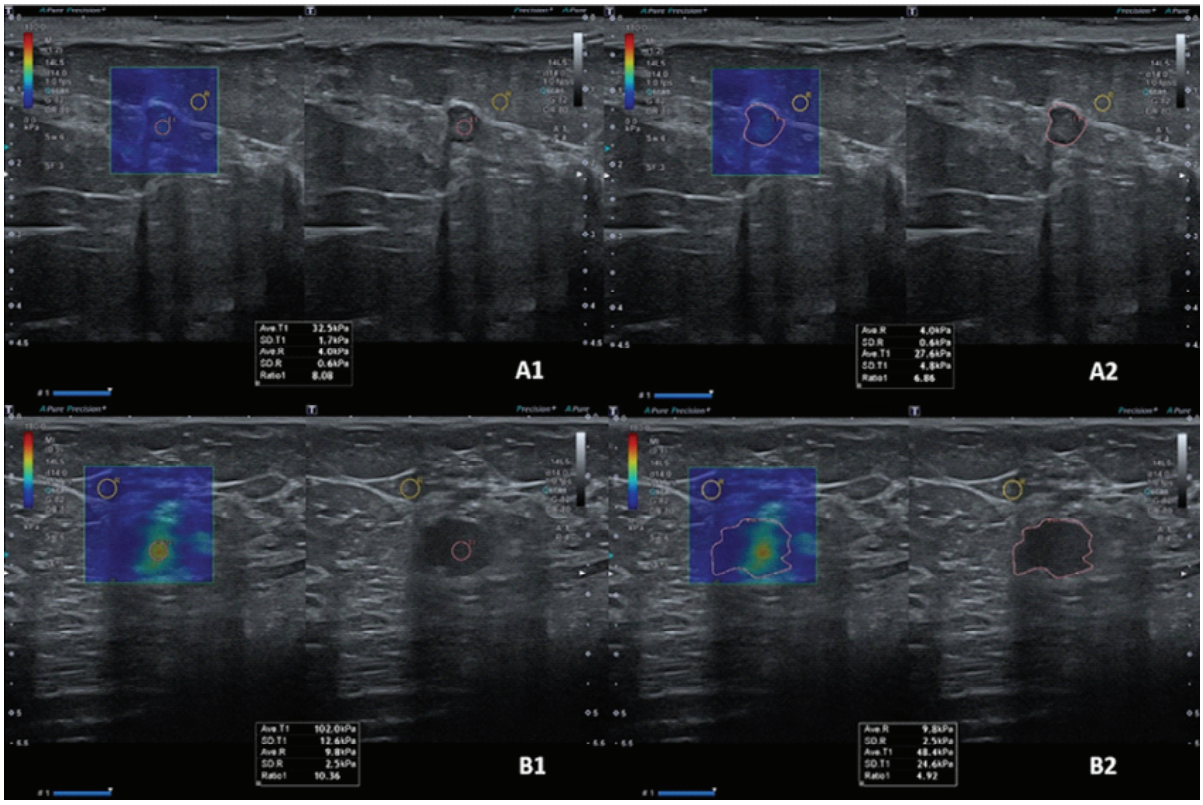

Figure 2. Comparison of benign and malignant lesions in SWE. Lesion A presented with Emax of 32.5 kPa (A1), Emean of 27.6 (A2) and Efat of 4 kPa. It was later biopsied and histopathologically verified as breast papilloma. Lesion B presented with Emax of 102 kPa (B1), Emean of 48.4 kPa (B2) and Efat of 9.8 kPa. The lesion was biopsied and histopathologically verified as intermediate-grade ductal carcinoma in situ.

To our best knowledge, there are significantly fewer studies using Can-on/Toshiba devices compared to Aixplorer systems. As the use of SWE increases, there is a growing need for universal guidelines for SWE interpretation, regardless of the US system manufacturer. If establishing strict cut-off values proves infeasible, proposing universal interpretation principles, such as the proportion of lesion's Emax to Efat, could be beneficial.

It is well known that SWE presents certain limitations. Lesions located too close to the skin or chest wall often display incorrect wave propagation, resulting in false-positive SWE results [19]. Such outcomes could be due to precompression occurring during SWE examinations of lesions in these areas [20, 21]. The distribution of lesion depths using descriptors proposed by Stavros is presented in Figure 3 [22]. The influence of location on SWE results can be seen in **Figure 3**, comparing wave propagation in two papillomas located at different depths of breast tissue. This aspect limits the possible clinical application of SWE based on lesion location. What is more, some malignant lesions exhibited stiffness exceeding the maximum kPa values of our US system. Expanding the maximum range of kPa in US systems could help overcome this limitation. We also found that in malignant lesions surrounded by significant edema, wave propagation was incorrect.

Feldman et al. demonstrated on Aixplorer system that malignant lesions were more heterogeneous on SWE stiffness maps, exhibiting higher stiffness and ratio values compared to benign lesions [23]. However existing literature indicates that invasive ductal or lobular carcinomas, as well as mucinous or intraductal carcinomas, can sometimes present as false-negative cases in SWE imaging [24]. However, numerous studies have highlighted that both qualitative and quantitative parameters obtained using SWE show significant differences between benign and malignant breast lesions [24–28]. Jiang et al. proved that the benign lesion group had significantly lower SWE parameters, and the diagnostic value

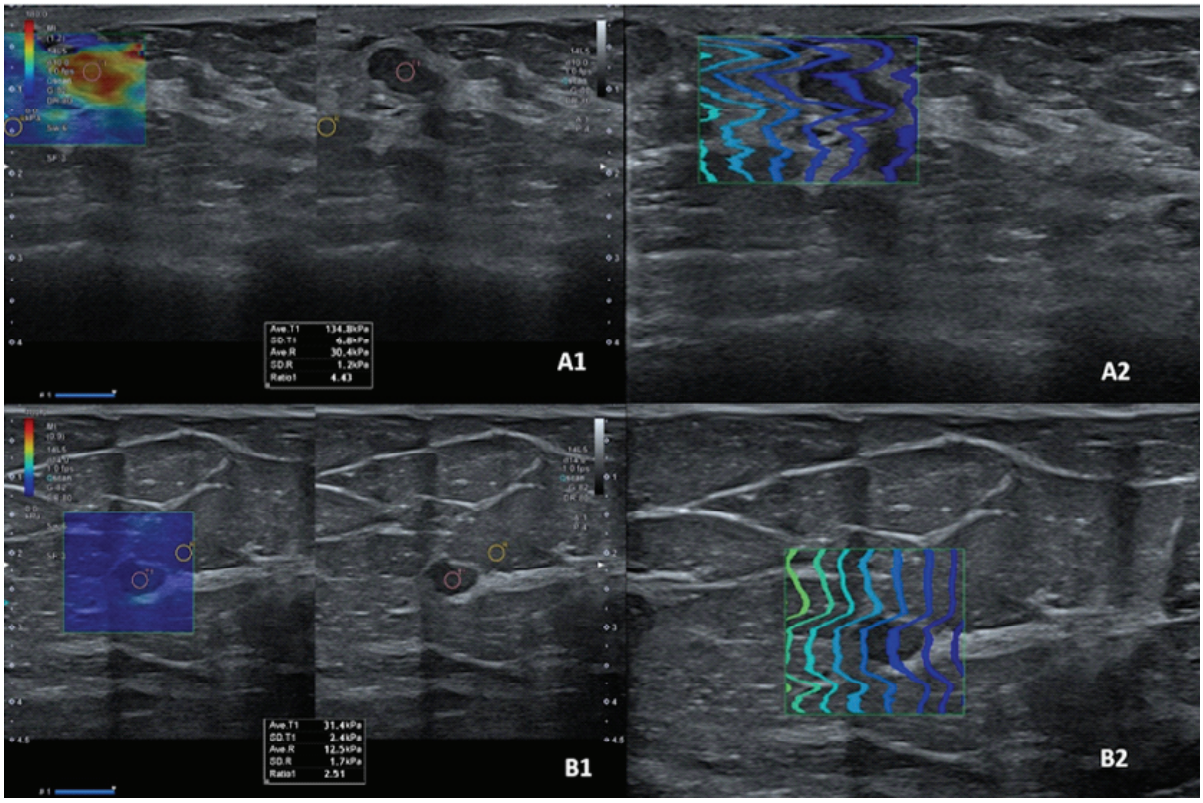

Figure 3. Comparison of wave propagation in papillomas located on different depths. Lesion A was located near the skin, which resulted in falsely increased SWE parameters (Emax of 134.8 kPa; A1), due to incorrect wave propagation (A2). Lesion B was located in the middle depth of the breast, which resulted in accurate SWE parameters (Emax of 31.4 kPa; B1) and correct wave propagation (B2).

of SWE in combination with string elastography exceeded SWE alone [29]. When SWE is incorporated into the BI-RADS 4a and 4b lesions assessment, it significantly increases the ultrasound's specificity without a corresponding sensitivity loss [30]. This enhancement in diagnostic accuracy suggests that SWE could play a crucial role in patient management by reducing the number of unnecessary biopsies, thereby sparing patients from invasive procedures and reducing healthcare costs. Such advancements underscore the potential of SWE to refine breast cancer screening and diagnosis, ultimately improving patient outcomes.

Screening is widely regarded as one of the most successful approaches to reducing breast cancer mortality in average-risk women and is recommended by the World Health Organization (WHO) [31]. However, breast density presents a significant challenge in breast cancer screening. Breast density is an independent risk factor for the development of breast cancer and also decreases the sensitivity of mammography, leading to potential underdiagnosis. This issue is particularly pronounced in women with extremely dense breast tissue, where the dense tissue can mask tumours, making it harder to detect cancer early [32]. As a result, there is an urgent need to establish more specific and sensitive imaging modalities that can improve the early detection of breast cancer in these patients, thereby decreasing the number of late-diagnosed cases.

By incorporating SWE into the screening process, particularly for women with dense breast tissue, clinicians can potentially improve the specificity and sensitivity of breast cancer screening. This could lead to earlier detection and treatment of breast cancer, reducing the mortality rate associated with the disease. Furthermore, the enhanced diagnostic accuracy provided by SWE can help reduce the number of unnecessary biopsies, which are often performed due to the lower specificity of traditional imaging techniques in dense breasts.

Our study has several limitations. It is a single-center study with a relatively small number of patients. All the examinations were performed by one radiologist, even if we take into account that SWE is considered to be highly reproducible. A multi-center study with a larger sample size is warranted to confirm our findings. As stated above, SWE measurements vary between different US systems. Therefore, a study comparing different methods of obtaining SWE measurements could be beneficial in establishing universal cut-off values. Additionally, including a more diverse patient population could help generalise the findings to a broader clinical context. These future studies could provide more definitive evidence and potentially lead to standardized SWE guidelines for breast lesion assessment.

Perspectives

Our study demonstrated the excellent performance of shear-wave elastography (SWE) in correctly characterizing breast lesions as benign or malignant. Through meticulous analysis, we established cut-off values for maximum elasticity (Emax) and mean elasticity (Emean) that effectively facilitate the differentiation of benign from malignant breast lesions.

Moreover, our data suggest that Emax is superior to Emean in everyday clinical practice. The superior performance of Emax not only simplifies the diagnostic workflow but also enhances the accuracy of SWE, thereby potentially reducing the number of unnecessary biopsies. SWE can improve patient comfort and reduce healthcare costs by providing a more precise and less invasive diagnostic tool.

In summary, our findings highlight the significant role of SWE in breast cancer diagnosis. The cut-off values established in our study provide a practical and reliable tool for clinicians, supporting the integration of SWE into routine clinical practice. Further multi-centre studies with larger sample sizes are recommended to validate our results and refine the diagnostic criteria for SWE. As SWE technology evolves, its application could lead to more accurate, non-invasive breast cancer diagnostics, ultimately improving patient outcomes and streamlining clinical workflows.

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Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Antonina Godlewska, Natalia Andryszak and Anna Pasiuk-Czpeczynska. The first draft of the manuscript was written by Antonina

Godlewska and Natalia Andryszak, all authors commented on previous versions of the manuscript. The final draft of the manuscript, the supervision, review and editing: Dariusz Godlewski, Marek Ruchała, Rafał Czepczyński. All authors read and approved the final manuscript.

Conflict of interest statement
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REVIEW PAPER

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Indications and timing for genetic testing in ovarian cancer

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ABSTRACT

Modern management of ovarian cancer (OC) relies on molecular diagnostics, with genetic testing playing a central role in therapeutic decisions. High-grade serous ovarian cancer (HGSOC) is frequently associated with mutations in the BRCA1 and BRCA2 genes, as well as other alterations within the homologous recombination repair (HRR) pathway. The identification of pathogenic variants is critical for selecting patients eligible for treatment with poly (ADP-ribose) polymerase inhibitors (PARPi), which significantly improve progression-free survival, especially in individuals with BRCA mutations and homologous recombination deficiency (HRD).

Current guidelines recommend BRCA testing at diagnosis for all patients with HGSOC, followed by HRD testing. Various techniques are used to assess genetic alterations and HRD status. Commercial tests assess mutations in genes in HRR pathways, genomic instability, or HRR functional status to quantify HRD.

Despite the availability of these assays, challenges remain regarding test standardisation, predictive accuracy, and cost-effectiveness. Moreover, emerging research highlights the potential for artificial intelligence (AI) to enhance molecular profiling, utilising whole-slide imaging (WSI) and deep learning to predict homologous recombination deficiency (HRD) and other tumour characteristics.

The integration of molecular subtypes, as defined by The Cancer Genome Atlas (TCGA), into routine clinical practice holds promise for tailoring therapy beyond BRCA or homologous recombination deficiency (HRD) status. As the field advances, comprehensive genetic testing combined with AI-driven analytics may become the cornerstone of precision oncology in ovarian cancer.

Introduction

The aetiology of ovarian cancer (OC) involves a combination of genetic, reproductive, hormonal, and environmental factors. Genetic predispositions, particularly mutations in the *BRCA1* and *BRCA2* genes, play a significant role in its development [1]. High-grade serous epithelial ovarian

cancer (HGSOC) is the most common and aggressive subtype. Characteristic molecular abnormalities in HGSOC include germline and somatic mutations in the *BRCA1* or *BRCA2* genes, *BRCA1* promoter methylation, and alterations in other genes involved in DNA repair through homologous recombination (HR) [2,3]. *TP53* gene mutations are found in up to 96% of HGSOC cases [4]. Among many identified genes whose alterations are related to OC pathogenesis are *NF1*, *CDK12*, *RB1*, *CHEK2*, *RAD51*, *BRIP1*, *PALB2*, and *CCNE1* [5–11]. Alterations in *BRCA* and other genes associated with homologous recombination play a crucial role in determining appropriate adjuvant therapy [12] and genetic counselling for affected individuals' families [13].

Modern OC treatment is not possible without genetic diagnostics. Recent targeted therapies, such as PARP inhibitors (PARPi), exploit genetic disorders associated with *BRCA* mutations and other genes involved in DNA repair through homologous recombination [14,15]. This underscores the importance of research on molecular disorders in OC and the ongoing efforts to integrate these findings into clinical practice. This study aims to summarise the genetic diagnostics used in managing OC.

Relevance of molecular testing in treatment planning

The management of OC depends on the stage of the disease. Primary debulking surgery is performed for operable tumours, followed by adjuvant chemotherapy, potentially combined with an antiangiogenic agent – bevacizumab. If complete cytoreduction is not possible, treatment begins with neoadjuvant chemotherapy, followed by interval debulking surgery [16,17]. Patients with

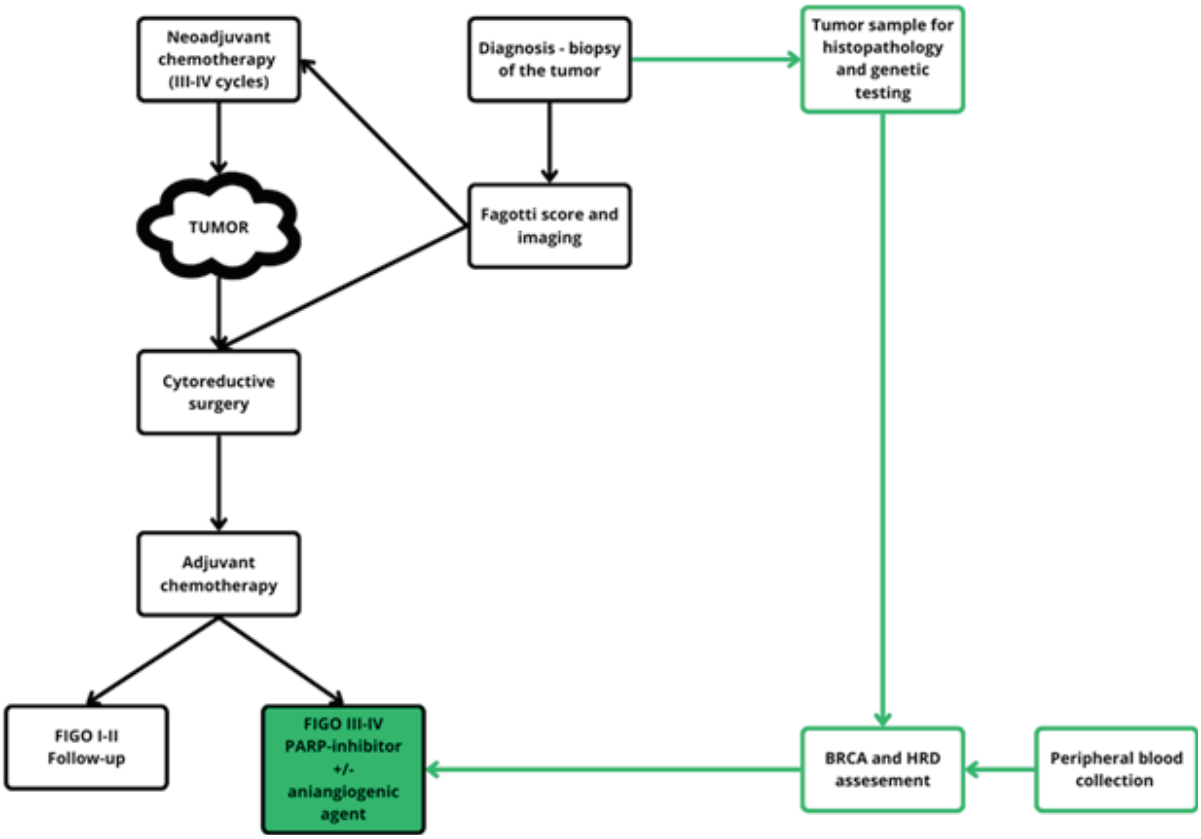

Figure 1. Ovarian cancer treatment algorithm including molecular diagnostics. Treatment of ovarian cancer must be preceded by histopathological confirmation. Molecular diagnostics should be performed on the initial biopsy, which should contain a sufficient amount of tumour tissue—at least 30% tumour cells—to ensure material for analysis. This enables the assessment of eligibility for PARP inhibitor therapy. The result should ideally be available by the third cycle of chemotherapy, as this is when the decision is made regarding the use of the PAOLA-1 treatment regimen. Peripheral blood analysis is used to determine whether the detected mutations are of somatic or germline origin.

advanced disease (FIGO III, IV) who have responded to platinum-based chemotherapy are eligible for maintenance therapy with poly (ADP-ribose) polymerase (PARP) inhibitors [14,15]. Patients with BRCA1/2 mutation or another HR deficiency benefit more from PARPi than from maintenance therapy with bevacizumab. Therefore, genetic testing results are necessary for the treatment decision process. For this reason, every patient diagnosed with OC should be tested for *BRCA1/2* gene variants. The assessment should determine whether the abnormality is somatic or germline in origin. In the case of a typical BRCA1/2 sequence, a homologous recombination deficit (HRD) status evaluation is required [18].

BRCA1 and *BRCA2* genes

The *BRCA* genes belong to the class of tumour suppressor genes. Germline mutations in these genes significantly increase the familial risk of developing breast and OC, known as hereditary breast and ovarian cancer syndrome (HBOC) [19]. A mutation in the *BRCA1* gene increases the lifetime risk of developing OC to 39–58%, while a *BRCA2* mutation raises this risk to 13–29% [20]. *BRCA1/2* gene mutations are present in approximately one-quarter of patients with OC [21]. Approximately three-quarters of these mutations are germline, while the remaining one-quarter are somatic [22].

In clinical practice, detecting a pathogenic *BRCA* gene variant enables the implementation of PARP inhibitor therapy [4,14]. Based on the results of the SOLO-1 trial, olaparib is indicated as a first-line maintenance treatment in women with somatic or germline *BRCA*-mutated advanced OC after first-line platinum-based chemotherapy [23]. The SOLO-2 trial demonstrated the benefits of olaparib for second-line maintenance treatment in patients with germline *BRCA* mutations who had responded to platinum-based chemotherapy [24]. The PRIMA trial showed the benefit of niraparib across all patient populations, including those with HR proficiency, though the effect was moderate in this group [25].

It is worth noting that mutations in other genes that interact with the *BRCA* genes may also be associated with an increased risk of ovarian cancer. *BRIP1*, also referred to as *BACH1* (*BRCA1*-Associated C-Terminal Helicase), was identified during investigations of *BRCA1* gene functions. The BRCT domain of *BRIP1* is essential for its interaction with *BRCA1*, forming a protein complex that facilitates the repair of double-stranded DNA breaks through, among others, HR pathways. Mutations affecting the BRCT domains disrupt this interaction, thereby impairing DNA repair processes [26,27]. *BRIP1* pathogenic variants have been related to hereditary breast and ovarian cancers that are independent of *BRCA1/2* mutations. Individuals carrying heterozygous deleterious variants in *BRIP1* have an elevated risk of developing ovarian cancer [26]. The carriers have an estimated 5–15% lifetime risk, significantly higher than the approximate 2% risk observed in the general population [28]. The PALB2 protein (Partner And Localizer of *BRCA2*) plays a crucial role in HR. Its primary function is to act as a molecular bridge linking the *BRCA* complex, comprising *BRCA1*, *PALB2*, *BRCA2*, and *RAD51*, and to support the activity of RAD51, a key protein involved in strand invasion during HR [29]. Women harbouring *PALB2* mutations face a lifetime ovarian cancer risk of approximately 5% [30]. Given shared mechanisms, carriers of *BRIP1* and *PALB2* pathogenic variants should be included in *BRCA1/2*-based therapies and trials as they can potentially benefit from them [31,32].

BRCA variants testing

The diagnosis of pathogenic variants in the *BRCA* genes can be performed using various techniques. Classical methods, such as Sanger sequencing or quantitative polymerase chain reaction (qPCR), are used as a first step in population-based screening or for confirming variants identified through next-generation sequencing (NGS) [33–36]. A key limitation of these techniques is their ability to detect only selected pathogenic variants, typically those most common in a given population, including so-called founder mutations [36]. Hence, some less common but pathogenic variants remain undetected. A negative result from those methods should prompt further diagnostic evaluation using NGS. This approach allows the comprehensive analysis of the entire coding sequence of the *BRCA* genes. This is particularly important given their

large size. Moreover, clinically significant variants can be distributed throughout the whole coding region [36–38]. Another advantage of NGS is the possibility of analysing other genes associated with OC pathogenesis in panel sequencing that, in addition to *BRCA1/2*, may include *RAD51C/D*, *BRIP*, and *PALB2* [39,40]. Multiplex ligation-dependent probe amplification (MLPA) is typically employed to detect large chromosomal rearrangements in *BRCA* genes [41].

The clinical relevance of specific *BRCA* gene variants is classified. These include pathogenic or likely pathogenic variants (*BRCAmut*) and the absence of such variants, referred to as wild type (*BRCAwt*) [26]. Further diagnostic steps determine whether the mutation is somatic (*sBRCAmut*) or germline (*gBRCAmut*) [27]. Tumour-only testing (*tBRCAmut*) [28] cannot determine the somatic or germline nature of the mutation. Molecular testing of the host genome, typically from peripheral blood, is required to identify the germline nature of the variant. According to The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines, variants are classified using a five-tier scale as benign (B, class 1), likely benign (LB, class 2), variant of unknown significance (VUS, class 3), likely pathogenic (LP, class 4), and pathogenic (P, class 5) [29]. Pathogenic variants (PVs) in the *BRCA1* and *BRCA2* genes are detected in 10–15% of unselected epithelial OC cases [42]. Patients with likely pathogenic and pathogenic variants are eligible for treatment with PARP inhibitors [30]. It should be noted, however, that specific variants of unknown significance may be considered pathogenic in the future as the number of patients with such a variant grows. Therefore, those patients and their families may require increased monitoring, especially if other cases appear to suggest a hereditary syndrome.

Homologous recombination deficit

From a practical perspective, detecting HRD allows qualifying patients for PARPi therapy [14,15]. Based on the results of the PRIMA trial, niraparib can be used as maintenance therapy for all patients, including those with HRD and HRP tumours [25]. The PAOLA-1 trial demonstrated the efficacy of olaparib in combination with bevacizumab compared to bevacizumab monotherapy. Based on the results of this study, the drug combination has been approved for HRD-positive patients who respond to first-line platinum-based chemotherapy [43].

The detection of recombination defects remains a significant challenge. Identification of patients, beyond carriers of *BRCA1/2* gene mutations, who may benefit from maintenance therapy remains ineffective [44]. HRD test results are often inconclusive due to differences between available tests and the lack of standardised criteria for defining HRD [18]. Studies involving niraparib, olaparib, and veliparib utilised the *myChoice* test developed by Myriad Genetic Laboratories [45–47]. This test detects mutations in the BRCA1/2 genes. It determines the Genomic Instability Score (GIS), which is based on the extent of loss of heterozygosity, the number of subchromosomal regions with allele imbalance extending to the telomere, and the number of large-scale genomic rearrangements. [44]. Different clinical trials have applied varying threshold values for the GIS determined by the *myChoice* test to define the presence of homologous recombination deficiency (42 in the PAOLA-1 trial and 33 in the VELIA trial), highlighting the lack of clarity in patient stratification based on this metric [45,47]. Another test, *FoundationFocus CDxBRCA* by Foundation Medicine, is based on the assessment of subchromosomal loss of heterozygosity and the detection of *BRCA1/2* mutations in tumour tissue [44] and was utilised in the clinical trial evaluating the efficacy of rucaparib [48]. In contrast, the PRIME clinical trial of niraparib employed the *BGI Genomics* test [49]. There are substantial differences among these assays, and their negative predictive value remains low [44]. Consequently, accurately identifying patients who will not respond to treatment remains challenging.

The currently used methods rely on so-called genomic "scars" (indicators of genomic instability), which are static and may not accurately reflect the current status of DNA repair in the tumour. These genetic features may change throughout the disease and in response to applied treatments [50]. Tests may yield false-positive or false-negative results (estimated in 10–15% of cases). Moreover, the heterogeneity within tumour cells

can lead to different classifications of the same tumour depending on the biopsy site [51].

HRD testing should be performed as early as possible following the diagnosis of OC, ideally at the time of primary diagnosis. A stepwise diagnostic approach is also acceptable if molecular testing of the *BRCA* genes is conducted first. If the tumour is *BRCA* wild type (*BRCAwt*), HRD testing is subsequently performed. Economic considerations primarily justify this approach. However, current reports on cost-effectiveness are inconsistent [52–54].

HRD testing methods

The currently used tests for HRD status assessment can be categorised into three main groups – assessing mutations in genes in HRR pathways, genomic instability, or HRR functional status by nuclear RAD51 tests [18,55–57].

The first group is based on the detection of typical causes of HRD. They assess the loss of function of germline and somatic mutations in the HRR pathway genes, including *BRCA1/2* [18] and *BRCA1* promoter methylation [57]. However, it is worth noting that the lack of mutations in those genes should not be considered equivalent to HRP status.

The second group determines the HRD by calculating the genomic instability (GI) score [58]. It is calculated as the sum of events collectively referred to as "genomic scars". These are loss of heterozygosity (LOH), large-scale state transition (LST), and telomere allele imbalance (TAI) [18,55,58]. These disorders reflect the abnormalities occurring in HRD cells.

LOH is a frequent genetic condition in cancer cells [59]. It occurs when a heterozygous genetic locus loses one of its parental alleles, resulting in homozygosity. The remaining allele's dysfunction can lead to a neoplastic transformation. LOH can be categorised into two primary types: LOH with copy number loss (CNL-LOH) and copy number neutral LOH (CNN-LOH). During cancer progression, tumour cells may experience the loss of an allele due to partial chromosomal deletion, which characterises CNL-LOH. Subsequently, CNL-LOH may undergo recombination, utilising the homologous chromosome as a template for repair, leading to copy number neutral LOH (CNN-LOH) [18,59,60].

Large-scale transitions (LST) refer to significant chromosomal modifications, including translocations, inversions, and deletions resulting from chromosomal breakage events. These alterations involve chromosomal gains or losses of at least 10 megabases (Mb) in size [61,62].

In cells with proficient DNA repair mechanisms, double-strand breaks are accurately repaired through homologous recombination, using the identical sister chromatid as a template, thereby preventing telomeric allelic imbalance (TAI). However, error-prone pathways are utilised when DNA repair is impaired in HRD, resulting in chromosomal rearrangements and abnormal radial chromosome formations. After mitotic division, this defective repair leads to TAI, characterised by an unequal contribution of parental telomeric chromosome segments in the daughter cells [63].

The third group of tests assesses the HRR status by nuclear RAD51 functional tests [55,18]. The RAD51 family comprises five paralogous proteins (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3) that mediate DNA damage signalling to facilitate break repair. RAD51 is the key protein in homologous recombination, playing a crucial role in cellular damage sensing and checkpoint signalling pathways [64,65]. Consequently, cell phenotypes resembling those of *BRCA*-mutated cells can also result from other, less common alterations, such as mutations in *PALB2*, *RAD51C*, and *RAD51D* or the epigenetic silencing of HR genes [66]. When homologous recombination repair functions correctly, RAD51 assembles into nuclear foci, indicating HRR proficiency (HRP). Conversely, the absence of nuclear foci signifies HRR deficiency [64].

Numerous commercial tests are available to assess HRD in OC patients. *MyChoice® CDx Plus* and *FoundationOne® CDx* were the first tests approved by the FDA for this purpose. *MyChoice® CDx Plus* is based on sequencing 15 HRR genes and a genome-wide single-nucleotide polymorphism-based assay (GW-SNP). HRD is determined based on a *BRCAmut* result or a genome instability score (GIS) ≥ 42. GIS is evaluated as a combined score of LOH, TAI, and LST [67]. *FoundationOne® CDx* analyses 324 genes using next-generation sequencing (NGS) and a genome-wide single-nucleotide polymorphism-based assay (GW-SNP). HRD is defined by the presence of a *BRCAmut*

variant or a genome-wide loss of heterozygosity (gLOH) score of ≥ 16% [67,68].

Several other commercially available tests are designed to assess homologous recombination deficiency (HRD). *OncoDEEP®* utilises next-generation sequencing (NGS) to assess 638 genes and an RNA-based 20-gene panel for detecting gene fusions and splicing events. The test evaluates *BRCA* status and determines the genome instability score (GIS) based on a developed algorithm [67,69]. *SeqONE HRD* utilises NGS and shallow whole genome sequencing (sWGS) to assess *BRCA* and HRD status, which is based on a composite score of LOH and LGA (large genomic alterations) and genes *CCNE1* and *RAD51B* amplification at two specific locations [67,70]. *SOPHiA DDM™ Dx HRD CE-IVD* performs next-generation sequencing (NGS) analysis of 324 selected genes and shallow whole-genome sequencing (sWGS). HRD assessment is conducted based on a proprietary algorithm [67,71]. *HRD Focus* utilises next-generation sequencing (NGS) to detect *BRCA* gene mutations and assess genomic instability using a genome-wide single-nucleotide polymorphism-based assay (GW-SNP). HRD is defined by the presence of *BRCAmut* or a genome scar score (GSS) ≥ 50 [67,72]. *Caris HRD Status* determines the presence of *BRCA* mutations and assesses a GSS based on gLOH and LST [67]. *The AmoyDx® HRD Complete Panel* detects genetic alterations in 20 HRR genes and determines overall HRD status. Its proprietary GIS algorithm, based on machine learning, evaluates genomic instability by analysing multiple types of copy number variations across the genome [73]. In addition to the tests described above, several established assays are currently used for academic purposes. These include the *Geneva HRD Test*, *NOGGO GIS Assay*, *GIScar*, *Leuven HRD test*, *Shallow HRDv2*, and *BRCA-Like Classifier* [67].

Molecular subtypes of ovarian cancer according to TCGA analysis – potential expansion of genetic diagnostics

The TCGA (The Cancer Genome Atlas) database and its analysis have significantly benefited gynecologic oncology by identifying molecular subtypes of endometrial cancer, which now directly influence clinical decision-making [74,75]. Given these advancements, it is no surprise that gynecologic oncologists are increasingly interested in further utilising the resources of this database. Based on the analysis of data from TCGA, four molecular subtypes of OC have been identified: mesenchymal, proliferative, immunoreactive, and differentiated [4]. The mesenchymal subtype is characterised by high expression of *HOX* genes (a group of genes responsible for the morphological development of specific body parts during early embryonic stages) and markers suggesting increased stromal components (such as *FAP*, *ANGPTL2*, and *ANGPTL1* genes). The proliferative

Table 1 – Summary of commercial and academic tests for assessing HRD based on "Homologous recombination deficiency in ovarian cancer: Global expert consensus on testing and a comparison of companion diagnostics" [67].

Tests for assessing HRD			
Approved Commercial Tests	**HRD definition**	**Academic Tests**	**HRD definition**
MyChoice® CDx Plus	BRCAm and/or GIS ≥42	Geneva HRD Test	GIS ≥15
OncoDEEP®	GIS >39	NOGGO GIS Assay	NOGGO GIS ≥83
SeqONE HRD	BRCAm and/or HRD status (probability ≥50%; based on composite score and gene amplification at two locations)	GIScar	GIScar score ≥0.48
SOPHiA DDM™ Dx HRD CE-IVD	GII >0	Leuven HRD test	BRCAm and/or GIS ≥56
FoundationOne® CDx	BRCAm and/or gLOH score ≥16%	Shallow HRDv2	>20 LGAs
HRD Focus	BRCAm and/or a GSS ≥50	BRCA-Like Classifier	Posterior probability >0.5
Caris HRD Status	BRCAm or high GSS		

BRCAm, BRCA mutation; CDx, companion diagnostic; GI, genome instability; GII, genome instability index; GIS, genome instability score; gLOH, genomic loss of heterozygosity; GSS, genome scar score; HRD, homologous recombination deficiency; HRR, homologous recombination repair; indel, insertion or deletion; LGA, large genomic alterations

subtype exhibits high expression of transcription factors such as HMGA2 and SOX11 and proliferation markers like MCM2 and PCNA. However, it shows low expression of OC markers, including MUC1 and MUC16.

Additionally, this subtype is associated with a reduced frequency of *MYC* amplification and *RB1* deletion. The presence of T-cell ligands CXCL11 and CXCL10, along with their receptor CXCR3, defines the immunoreactive subtype. Moreover, 3q26.2 amplification (MECOM) occurs more frequently in this subtype. The differentiated subtype is characterised by higher differentiation features, including increased expression of MUC1, MUC16, and the secretory fallopian tube marker SLPI [4].

The current standard of care does not use information on molecular subtypes of OC defined in the TCGA project. It has been demonstrated that patients with mesenchymal and proliferative subtypes derive more significant benefits from bevacizumab treatment [76–78]. Furthermore, the mesenchymal subtype is more responsive to dose-dense taxane chemotherapy, which suggests that the preferred treatment regimen should be dose-dense paclitaxel with carboplatin (ddTC) [79,80]. The possibility of routine molecular subtype profiling could be helpful in clinical decision-making. The main obstacles include costs and technical challenges, particularly those related to standardising the methodology [79,81]. Conducting specialised tests, such as using microarrays solely for this purpose, may be challenging [82]. Attempts have been made to histopathologically profile ovarian tumours based on their molecular subtypes, which could facilitate access to knowledge about specific tumour biology [80].

Artificial intelligence in molecular profiling of ovarian cancer

Artificial intelligence (AI), particularly machine learning, is increasingly utilised in medicine to support diagnostics and treatment planning. Learning algorithms can identify patterns that may be imperceptible to human experts. These techniques are applied in areas such as radiological image analysis, disease progression prediction, and personalised therapy [83,84]. Several studies have integrated genomics, epigenomics, transcriptomics, and clinical or pathological data to enhance the diagnosis, prognosis, and prediction of treatment response in OC. Approaches using machine learning and deep learning demonstrated that multiomics models outperform single-omics models in tasks such as survival prediction, subtype classification, and response to therapy [85,86]. AI techniques have the potential to effectively surpass classical methods of identifying patients with HRD.

An example of this is DeepHRD, a platform trained to predict HRD from hematoxylin and eosin (H&E)–stained histopathological slides. Compared to four standard molecular tests, this model identified more tumours exhibiting HRD-related features [87]. AI utilises the analysis of histopathological images obtained through Whole Slide Imaging (WSI), which involves scanning and digitising entire histology slides [88–90]. Algorithms identify morphological patterns associated with HRD, such as hemorrhagic necrosis at tumour margins, lymphocytic infiltration, fibrosis, and high tumour cell density [89]. There are also ongoing efforts to apply machine learning and neural networks for classifying ovarian cancers into distinct subgroups and for analysing data derived from single-cell image analysis [91–93]. It is essential to emphasise the critical role of building large-scale databases that include macroscopic and microscopic images and omics data, such as TCGA.

Conclusions

Molecular diagnostics are now essential for planning the treatment of patients diagnosed with OC. Molecular profiling is crucial for implementing maintenance therapy as part of first-line treatment. *BRCA* and HRD testing are fundamental in guiding treatment decisions, particularly in selecting patients for PARP inhibitor therapy. *BRCA1* and *BRCA2* mutations and homologous recombination deficiency (HRD) are key predictive biomarkers that determine responsiveness to targeted therapies.

Focusing on alternative molecular pathways is equally essential as targeting *BRCA* mutations and HRD-related alterations in OC. This is particularly relevant for patients who are resistant to PARP inhibitors or do not meet the criteria for this treatment. Additionally, research into

OC subtypes other than HGSOC is crucial, as BRCA mutations and HRD are less prevalent in these tumours. Targeted therapies tailored to the unique molecular characteristics of non-HGSOC remain underdeveloped, highlighting the need for further studies. AI models play an increasingly important role in the diagnosis, prognosis, and personalisation of OC treatment, particularly by integrating omics, imaging, and clinical data.

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REVIEW PAPER

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Innovative approaches in axial spondyloarthritis: exploring emerging trends in treatment and management

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ABSTRACT

Background. Axial Spondyloarthritis (axSpA) is a chronic inflammatory disease affecting the axial skeleton, resulting in chronic pain, reduced mobility, and potential spinal fusion. Traditional therapies emphasise symptomatic control via nonsteroidal anti-inflammatory drugs (NSAIDs) and physical therapy. Recent advances, however, including biologic agents and JAK inhibitors, have opened new therapeutic possibilities.
Material and methods. A comprehensive literature review was conducted using databases such as PubMed and Scopus. The literature search included English-language publications between January 2018 and February 2024. We included clinical trials, meta-analyses, systematic reviews, cohort studies, and significant basic science studies. Publications outside these criteria or those in other languages were excluded. Methodological rigour and limitations of included studies were critically discussed.
Results. Key recent advances include biologic therapies targeting IL-17 and IL-23 cytokine pathways, demonstrating significant efficacy in controlling inflammation and improving function. Additionally, precision medicine, microbiome-based interventions, and advanced imaging techniques enhance personalised treatment strategies.
Conclusions. Integrating novel pharmacological approaches with lifestyle modifications presents a promising strategy for optimising axSpA management. Long-term studies are required to assess the full impact of these innovations on disease progression.

Introduction

Axial Spondyloarthritis is a chronic, progressive inflammatory disorder primarily affecting the axial skeleton, including the spine and sacroiliac joints. This condition often leads to significant pain, stiffness, and reduced mobility, with severe cases resulting in spinal fusion, known as "bamboo spine" [1]. Typically presenting in young adulthood and demonstrating male predominance, however,

recent studies indicate that 9.9% of axSpA patients present over the age of 45, suggesting a broader age distribution than previously assumed [2]. While the exact aetiology of axSpA remains unclear, it is strongly associated with the HLA-B27 gene, suggesting a genetic predisposition [3].

Traditionally, the management of axSpA has focused on controlling symptoms and maintaining functional ability through NSAIDs and physical therapy. Contrary to prior assumptions, NSAIDs may also have a modest disease-modifying potential, delaying radiographic progression in some patients [4,5]. The introduction of biologic therapies, specifically tumour necrosis factor (TNF)-alpha inhibitors, significantly improved the advancement in axSpA treatment, offering better control over inflammation and slowing disease progression. However, TNF-alpha inhibitors are not considered traditional treatments but rather biological therapies.

Recent developments introduce novel biologic therapies targeting interleukin pathways (IL-17 and IL-23) and Janus kinase (JAK) inhibitors, providing new mechanisms to manage axSpA [6]. These treatments target different inflammatory pathways, giving additional options for patients who do not respond to traditional therapies. Despite pharmacological advances, there is growing recognition of the importance of comprehensive management strategies that include lifestyle modifications, such as exercise, diet, and mental health support, to improve overall patient outcomes [7].

Aim

The purpose of this review is to explore these emerging trends in axSpA treatment, examining the efficacy and potential of new therapeutic approaches, discussing clinical efficacy, potential limitations, and areas necessitating further investigation. The article also highlights the importance of integrating pharmacological and non-pharmacological strategies in the management of this complex disease.

Etiopathogenesis

Axial spondyloarthritis is a chronic autoimmune disorder, where the exact etiopathogenesis remains elusive. Genetic factors play a significant role, with the HLA-B27 gene being strongly linked to axSpA susceptibility [8]. However, the presence of this gene alone does not always lead to the development of the disease, indicating the involvement of other genetic and environmental factors. Recent genome-wide association studies (GWAS) have identified other significant genetic loci, such as ERAP1 (endoplasmic reticulum aminopeptidase 1) and genes within the IL-23/IL-17 signalling axis, which are crucial for the differentiation and function of Th17 cells—immune cells implicated in promoting inflammation in axSpA [8–10].

Additionally, studies indicate that Familial Mediterranean Fever and axSpA may share overlapping etiopathogenic mechanisms, suggesting potential inflammatory pathways common to both diseases [11].

The immune system's dysregulation, particularly involving Th17 cells and cytokines like IL-17 and IL-23, contributes significantly to the inflammatory process. These cells drive the chronic inflammation characteristic of axSpA, which leads to the progressive fusion of the spine. Additionally, environmental triggers such as gut microbiota, particularly *Klebsiella pneumoniae,* may exacerbate the autoimmune response by interacting with HLA-B27, supporting the hypothesis of a gut-joint axis in axSpA pathogenesis [8,9].

Moreover, non-genetic factors like vitamin D deficiency and disturbances in the hypothalamic--pituitary-adrenal axis are also thought to influence disease progression, highlighting the complexity of axSpA etiopathogenesis [12].

Characteristic symptoms and diagnosis

AxSpA presents a variety of characteristic symptoms, primarily involving pain and stiffness in the lower back and hips, particularly in the morning or after periods of inactivity. Other hallmark symptoms include progressive spinal stiffness, loss of spinal flexibility, and in severe cases, fusion of vertebrae (ankylosis), leading to reduced mobility and postural deformities [8,9]. Peripheral joints and entheses (the areas where tendons and ligaments attach to bones) may also be affected, causing pain and inflammation. Additionally, patients

may experience fatigue, weight loss, and, less commonly, extra-articular manifestations such as anterior uveitis, inflammatory bowel disease, and psoriasis [10].

Diagnosing axSpA can be challenging due to its slow progression and overlap with other forms of inflammatory arthritis, which requires a diagnostic combination of clinical evaluation, laboratory tests, and imaging. Magnetic resonance imaging (MRI) is a key tool for early detection, as it can reveal inflammation in the sacroiliac joints before significant radiographic changes are visible. Radiographs can show structural changes in later stages, including syndesmophytes and bamboo spine [8]. Blood tests may reveal elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), which are markers of inflammation, although these are not specific to axSpA. The presence of the HLA--B27 antigen can support the diagnosis, though not all patients with axSpA test positive for this gene [9].

Advances in biological therapies in axial spondyloarthritis

The treatment possibilities for axSpA have evolved with the advent of biologic therapies, offering novel options for patients who do not respond adequately to first-line treatments such as NSAIDs [14]. Among the most significant advances are therapies targeting the IL-17 and IL-23 pathways, which play crucial roles in the inflammatory processes driving axSpA [15]

The IL-17/IL-23 pathway is essential in the pathogenesis of axSpA, with IL-17A and IL-17F playing significant roles in inflammation [14,18]. Both cytokines share a receptor complex and can be targeted by the monoclonal antibody bimekizumab, which effectively neutralises their activity. IL-23 is essential for the development and maintenance of Th17 cells, which are prominent in axSpA inflammation. Research has shown a correlation between axSpA risk and polymorphisms in the IL-23 receptor, indicating its involvement in disease mechanisms [14,16].

Table 1. Diagnostic aspects of axial spondyloarthritis [8,9,13].

Aspect of diagnosis	Details
Diagnosis challenges	Early symptoms of axSpA, such as back pain and stiffness, can overlap with other conditions, making early diagnosis difficult.
Supporting diagnostic evidence	Radiographic evidence, especially **sacroiliitis**, is key for confirming the diagnosis.
Primary diagnostic criteria	Diagnosis is based on **clinical presentation**, such as chronic back pain and stiffness, typically in young adults.
Hallmark radiographic feature	**Sacroiliitis** visible on X-rays, with inflammation in the sacroiliac joints being a defining feature of axSpA.
Genetic marker	The **HLA-B27** gene is present in **80–95%** of individuals with axSpA, though it is also found in a significant portion of the general population without the disease. HLA-B27 is not exclusive to axSpA but is a strong genetic predisposition factor
Laboratory tests	A thorough **clinical examination** and detailed medical history, combined with the exclusion of other conditions, are essential for diagnosing axSpA.
MRI findings	**MRI** is valuable in detecting **non-radiographic axial spondyloarthritis**, revealing early inflammatory changes not visible on conventional radiographs. Specific **lesions**, such as erosions and sclerosis, further increase the positive predictive value
MRI findings and lesions of nr-axSpA	– Bone marrow edema in two quadrants in a single section or two consecutive sections in a single quadrant. – Erosion in two quadrants in a single section or two consecutive sections in a single quadrant. – Bone marrow edema and erosion together in any quadrant in a single section.
Degrees of sacroiliitis	– **Mild sacroiliitis**: Subtle imaging changes and minimal inflammation. – **Moderate sacroiliitis**: Pronounced inflammation and pain. – **Severe sacroiliitis**: Intense pain, stiffness, and joint changes. – **Chronic sacroiliitis**: Persistent inflammation and recurring episodes

axSpA – axial spondyloarthritis; nr-axSpA – non-radiographic axial spondyloarthritis; MRI – magnetic resonance imaging

IL-17 inhibitors, such as secukinumab and ixekizumab, have shown remarkable efficacy in controlling inflammation in axSpA. These drugs specifically inhibit IL-17A, a cytokine critical to the immune response in axSpA, reducing disease activity, pain, and improving patients' mobility. JAK inhibitors, such as tofacitinib, upadacitinib, and filgotinib, are also emerging as promising treatment options, especially for patients who do not respond to TNF-alpha or IL-17 inhibitors.

Meta-analyses have demonstrated significant improvements in the proportion of patients with at least 20% improvement in Assessment of Spondyloarthritis International Society (ASAS20) response criteria and ASAS40 (secondary response), with some patients achieving sustained remission even after 16 weeks of treatment with IL-17 inhibitors [16,17].

Meanwhile, therapies targeting the JAK-STAT pathways are emerging as promising options, especially for patients who are intractable to both TNF and IL-17 inhibitors. JAK inhibitors work by blocking Janus kinases, which are critical enzymes that enable the signalling of various cytokines involved in inflammatory processes. By inhibiting these enzymes, JAK inhibitors prevent the activation and proliferation of immune cells, reducing the production of pro-inflammatory cytokines like IL-6, IL-17, and IL-23 [18]. As mentioned before, the IL-23/IL-17 axis is critical in the pathogenesis of axSpA, with cytokines like IL-23 maintaining Th17 cells that produce pro-inflammatory IL-17A and IL-17F; therefore, the mechanism of JAK inhibitors helps alleviate inflammation and tissue damage [18–20].

As mentioned above, several JAK inhibitors, including tofacitinib, upadacitinib, and filgotinib, have shown efficacy in treating axSpA. These inhibitors work by blocking intracellular signalling pathways, allowing for the simultaneous inhibition of multiple cytokines involved in inflammation [21]. For instance, tofacitinib demonstrates preferential inhibition of JAK1 and JAK3, effectively targeting various pro-inflammatory cytokines, while upadacitinib shows strong selectivity for JAK1 [19,22]. Recent studies, including the SELECT-AXIS 2 trial, have supported the efficacy of upadacitinib in treating both radiographic and non-radiographic axial spondyloarthritis, highlighting the expanding role of JAK inhibitors in the management of axSpA [23,24].

The SELECT-AXIS 2 trial assessed the efficacy and safety of upadacitinib in patients with non-radiographic axial spondyloarthritis. The study was conducted on 314 patients who had active disease and inadequate responses to NSAIDs. Results showed a significant improvement in the Assessment of Spondyloarthritis International Society (ASAS) response with upadacitinib compared to placebo (45% vs. 23%; $p < 0.0001$), indicating its effectiveness in managing symptoms of non-radiographic axial spondyloarthritis [24].

Although much progress has been made in the last decade on JAKs and their inhibition, there are still many unanswered questions in this rapidly advancing field. The importance of selectivity for an effective treatment response and reduction of adverse events remains unclear. Moreover, safety concerns regarding JAK inhibitors must be carefully evaluated, as their use has been associated with increased risks of infections, cardiovascular events, and malignancies.

Precision medicine and genetic insights

In the current approach to managing axSpA, personalised medicine is playing an increasingly important role. Advances in genetic research, such as involving the HLA-B27 antigen, are helping clinicians tailor treatments to individual patient profiles. HLA-B27, which has been consistently linked as a key risk factor for axSpA, now provides valuable insights into disease progression and likely treatment response, supporting more targeted and effective therapeutic strategies.

Axial spondyloarthritis has seen significant advancements in understanding genetic susceptibility, particularly through GWAS, which are instrumental in identifying genetic markers associated with treatment efficacy, enabling personalised therapy approaches that consider individual genetic backgrounds [25]. These large-scale studies examine up to a million genetic variants, focusing mainly on common single-nucleotide polymorphisms that appear in over 1% of the population. A groundbreaking study conducted in 2013 by the International Genetics of Ankylosing Spondylitis [26] consortium identified 25 loci related to axSpA, while subsequent

investigations have revealed a total of 115 distinct SNPs (Single Nucleotide Polymorphism) across more than 90 genomic regions [27,28]. These findings highlight the complex genetic background of axSpA and reinforce the potential of genomics in guiding future diagnostic and therapeutic approaches.

A significant number of these genetic variants are located near genes involved in immune function, especially ERAP1 and ERAP2. These genes play a key role in processing peptide antigens for presentation by MHC class I molecules, such as HLA-B27 [28,29]. Interestingly, specific ERAP1 variants that reduce the enzyme's function have been associated with a lower risk of developing ankylosing spondylitis. This observation supports the arthritogenic peptide hypothesis, which proposes that faulty processing of self-peptides may trigger abnormal T-cell responses, contributing to the development of autoimmunity [28].

Research also indicates that HLA-B27 might interact with killer immunoglobulin-like receptors, potentially influencing the risk of axSpA [28,30,31]. Overall, current genetic findings strongly emphasise the role of dysregulated immune signalling – particularly involving cytokines and antigen presentation – in the development of axSpA. However, the underlying mechanisms remain only partially understood, and further studies are needed to fully clarify how these genetic factors contribute to disease onset and progression.

Role of gut microbiota in axSpA

Recent studies have highlighted a strong link between gut microbiome dysbiosis and the development of axSpA. Studies have demonstrated that patients with axSpA often show a significantly different composition of gut bacteria compared to healthy individuals [32]. In particular, there is a noticeable reduction in beneficial species such as *Bacteroides* and an increase in pro-inflammatory bacteria, including various strains of *Prevotella*. Among them, *Prevotella copri* has explicitly been associated with disease activity and immune dysregulation in axSpA [32]. Notably, the presence of specific bacteria like *Prevotella copri* is thought to contribute to immune responses that exacerbate joint inflammation, suggesting a mechanistic role for gut microbiota in axSpA pathology.

The HLA-B27 gene plays a crucial role in the pathogenesis of axSpA and is closely associated with gut dysbiosis [33]. In HLA-B27 transgenic rat models, research has shown that this gene significantly alters the gut microbiome – increasing the presence of *Bacteroides vulgatus* and *Paraprevotella*, while reducing levels of *Rikenellaceae* bacteria [33]. These microbial alterations are linked to immune dysregulation, including elevated Th17 cell populations, which contribute to both intestinal inflammation and joint disease [34,35]. Additionally, transgenic rats showed increased colonisation of *Akkermansia muciniphila* and IgA coating of gut bacteria, further implicating the gut microbiota in axSpA progression [33–35].

Innovative therapeutic strategies targeting the gut microbiota, such as probiotics and faecal microbiota transplantation (FMT), are currently under investigation in the context of axSpA [34]. Probiotic strains, especially those from the *Bifidobacterium* genus, have shown potential in modulating immune responses and helping to restore a healthier microbial balance [36]. Early data from FMT studies also suggest promising results. For instance, increased levels of *Parasutterella* and reduced *Escherichia–Shigella* and *Intestinibacter* align with microbiota changes seen in axSpA patients compared to healthy controls [37].

One fascinating observation is the rise in *Faecalibacterium* levels following FMT, which has been linked to decreased disease activity. This likely stems from its anti-inflammatory properties, including maintaining the Th17/Treg balance and increasing IL-10 production [38,39]. Although previous studies reported higher *Faecalibacterium* levels in axSpA patients, these paradoxical findings may be explained by differences in medication use or disease stage at the time of sampling [37].

However, maintaining long-term remission after FMT remains a challenge. Factors such as lifestyle, diet, and concurrent medications can affect gut microbiota and axSpA disease activity [37]. High dietary fibre and prebiotics may enhance FMT efficacy by promoting beneficial bacteria and increasing short-chain fatty acid production. The interaction between FMT and axSpA treatments

like NSAIDs and tumour necrosis factor inhibitors remains an area for further research [37,39].

Advances in imaging and diagnostics

Early and accurate diagnosis of axSpA is essential to prevent long-term structural damage and improve patient outcomes. To support precise classification, clinicians are encouraged to apply the Assessment of Spondyloarthritis International Society (ASAS)/European Alliance of Associations for Rheumatology (EULAR) classification criteria, mainly when axSpA is suspected based on clinical symptoms and risk factors. According to ASAS, a positive MRI is defined by the presence of:

- Bone marrow oedema in two quadrants in a single section or two consecutive sections in a single quadrant
- Erosion in two quadrants in a single section or two consecutive sections in a single quadrant
- Bone marrow oedema and erosion together in any quadrant in a single section

These criteria help identify early inflammatory changes before radiographic signs become visible [40].

Conventional radiography of the sacroiliac (SI) joints is recommended as the first-line imaging method to assess for sacroiliitis, which is a hallmark feature of axSpA. This method is widely available and can demonstrate structural changes such as joint space narrowing, sclerosis, or bone fusion—all indicative of chronic inflammation [1]. However, in the early stages of the disease, structural damage may not yet be visible on X-rays, underscoring the value of MRI in early detection.

MRI of the sacroiliac joints is particularly recommended in specific clinical scenarios, notably in younger patients or those with a short duration of symptoms, where conventional radiography may not yet reveal structural changes [40]. MRI is also recommended when clinical symptoms strongly suggest axSpA, but X-rays are inconclusive [40,41]. The key advantage of MRI lies in its sensitivity to early inflammatory changes, especially bone marrow oedema, which is considered a marker of active inflammation [42]. Beyond detecting inflammation, MRI can also reveal structural lesions, such as bone erosions, subchondral sclerosis, and fat infiltration, which support the diagnosis and help assess disease progression [42,43].

Recent advancements in imaging techniques, especially functional MRI (fMRI) and positron emission tomography (PET), have significantly improved the early diagnosis and monitoring of diseases such as axSpA [41]. The use of MRI, particularly for visualising early inflammatory changes in the sacroiliac joints and spine, has proven to be more sensitive than traditional X-rays, especially in detecting non-radiographic axial spondyloarthritis (nr-axSpA), an earlier stage of axSpA [40]. This allows for earlier diagnosis, critical for initiating treatment before irreversible structural damage occurs.

Furthermore, artificial intelligence (AI) is revolutionising the field of medical imaging. AI-powered tools, integrated with multimodal imaging, can analyse vast amounts of imaging data to enhance diagnostic precision. Deep learning algorithms, for instance, are being used to enhance PET/MRI images, improving the accuracy of low-dose scans without compromising image quality [44,45]. In axSpA, AI algorithms are being explored to track disease progression and predict treatment response, potentially enabling more tailored and effective therapeutic interventions [45].

These innovations represent a significant step forward in personalised care for patients with axSpA, allowing for earlier and more precise interventions.

Future directions in axSpA treatment

In the future treatment of axSpA, several innovative approaches are poised to transform patient care. One promising direction is personalised medicine, which aims to tailor therapeutic strategies based on individual genetic profiles, environmental exposures, and lifestyle factors. Advances in genomic technologies enable more precise interventions that can address the unique needs of each patient, improving treatment efficacy and reducing side effects [46].

Stem cell therapy also holds significant potential in axSpA management, particularly due to its potential to regenerate tissues damaged by chronic inflammation. Among the most promi-

sing approaches is the use of induced pluripotent stem cells, which can be tailored to individual patients. This personalised strategy may reduce the risk of immune rejection and enhance treatment effectiveness [47].

The study conducted by Li A et al. [48] proved that transfusions of umbilical cord mesenchymal stem cells were not only safe and well-tolerated in axSpA patients but also led to noticeable reductions in disease activity and clinical symptoms. This approach may be especially beneficial in countering the chronic inflammation and joint degeneration characteristic of axSpA.

CRISPR-based gene editing is another promising innovation with potential applications in axSpA. By precisely targeting and correcting genetic mutations linked to the disease, CRISPR technology may one day allow for interventions that prevent disease onset or reduce its severity [49,50]. Recent improvements in CRISPR systems, such as high-fidelity Cas9 variants, have significantly increased the accuracy of gene editing, minimising the risk of unintended genetic changes and bringing this approach closer to clinical application [4].

Taken together, stem cell therapy and CRISPR gene editing represent a new era of regenerative and personalised medicine in axSpA. These technologies could fundamentally change how we manage the disease.

Limitations of the study

This review is limited to English-language publications, which may exclude relevant international research. The included studies vary in design, duration, and patient populations, making comparisons difficult. Some of the discussed therapies, especially those involving the microbiome and gene editing, are still in early stages of research, and their long-term effects remain unclear. Additionally, while recent advances are promising, more robust clinical data are needed to confirm their effectiveness and safety over time.

Conclusions

In recent years, the management of axial spondyloarthritis has progressed significantly, particularly with the introduction of targeted therapies such as IL-17 and JAK inhibitors. These treatments provide effective options for patients who do not respond to conventional approaches and have improved the ability to control disease activity and limit structural damage. The growing understanding of genetic and microbiome-related mechanisms has opened new avenues for personalised care. Imaging innovations and AI-supported diagnostics have enhanced early detection and disease monitoring. While these developments mark substantial progress, their long-term safety and effectiveness still require further investigation. Continued research is essential to validate these approaches and determine how best to combine them in clinical care.

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REVIEW PAPER

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Bexagliflozin – evaluation of the clinical efficacy, safety profile and potential new applications of the SGLT2 inhibitor: a review of the literature

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ABSTRACT

Introduction. Type 2 diabetes (T2DM) is a growing global health, social, and economic problem. T2DM is often accompanied by other diseases and is associated with complications such as kidney damage and cardiovascular diseases. The 21st century has seen a breakthrough in treating T2DM, including incretins and flozins. This review focuses on bexagliflozin, a new SGLT-2 inhibitor approved by the FDA for treating T2DM. The efficacy, safety, and potential benefits of new uses are analysed.

Materials and methods. Sources were obtained using PubMed and Google Scholar, as well as Medline, with the keywords: bexagliflozin, EGT1442, new indications for bexagliflozin, and bexagliflozin trials.

Results. Unlike other SGLT-2 inhibitors, bexagliflozin has a different molecular structure, which may increase its selectivity and efficacy. Preliminary results suggest that the drug may offer additional therapeutic benefits over other drugs in this group, particularly in the treatment of hypertension and cardiac arrhythmias. Studies are currently underway to investigate its potential use in the treatment of chronic kidney disease in children and sleep apnoea.

Conclusions. Bexagliflozin shows promise as a treatment for type 2 diabetes, with additional potential benefits in treating other conditions. Although the drug is relatively new to the market, preliminary studies indicate that it may offer advantages over other SGLT-2 inhibitors in some areas. Nevertheless, further clinical trials are needed to further evaluate its efficacy compared to other therapies and its potential in a broader range of applications

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease, the main feature of which is elevated blood glucose levels due to impaired insulin production or response [1,2]. Symptoms of the disease were known in antiquity, and since the 17th century, it has been reported in Europe, Asia and South America [3]. According to the WHO, there are currently about 537 million adults struggling with T2DM, and an additional 541 million people have impaired glucose tolerance, which sig-

nificantly increases the risk of developing T2DM [4]. Moreover, the number of cases of T2DM is steadily increasing. Data show that the incidence rate has increased by 157.63% between 1990 and 2019 [5]. It is estimated that the number of adults with T2DM will reach 643 million by 2030 and 783 million by 2045 [6]. Although the peak incidence in both sexes is between the ages of 60 and 64, men are diagnosed earlier than women [5]. Moreover, it is believed that up to half of patients with T2DM may be unaware of their disease. T2DM is associated with numerous complications, both macrovascular and microvascular, which significantly affect mortality. It is estimated that as much as 12% of global health care spending goes to treating complications of the disease [7]. In 1990, the DALY (disability-adjusted life years) rate was 628.33 per 100,000 people, while in 2019 it had increased to 801.55 per 100,000 people [5]. In 2021, T2DM accounted for more than 12.2% of deaths in the 20–79 age group [2]. Over the past 30 years, the number of T2DM-related deaths has increased by 142.90% [5]. For this reason, new therapeutic options are constantly being sought [6]. A relatively new group of drugs used in the treatment of diabetes mellitus are inhibitors of the sodium-glucose cotransporter SGLT2. Their mechanism of action is through the induction of sugar metabolism, which leads to a reduction in serum glucose levels [8]. These drugs have been shown to have beneficial cardio- and nephroprotective effects [1]. In addition, SGLT2 inhibitors appear promising as adjunctive therapy for conditions such as hypertension, asthma, and chronic obstructive pulmonary disease. They may also have beneficial effects on the central nervous system, non-alcoholic steatohepatitis and the treatment of obesity [8]. In clinical practice, SGLT-2 inhibitors are used as second-line drugs in the treatment of type 2 diabetes when glycemic control with metformin is inadequate, or as first-line drugs in high-risk patients with cardiovascular disease, heart failure (HF) or chronic kidney disease [9]. It is indicated that only 8.3% of patients who could benefit are taking SGLT2 inhibitors [10]. The first drug in this group, approved by the Food and Drug Administration (FDA) in 2013, was canagliflozin [8]. Subsequent flozines, such as empagliflozin and dapagliflozin, gained approval a year later, while ertugliflozin was approved in 2017 [1]. The fifth flozin, approved in 2023 for the treatment of type 2 diabetes, is bexagliflozin [6,10]. It was registered as a drug to improve glycemic control in adults with type 2 diabetes as an adjunct to diet and exercise. However, the drug is not currently approved in Europe [11]. Although bexagliflozin belongs to the SGLT2 inhibitors, it is distinguished by its molecular structure, which translates into its high selectivity [2,3]. Its potential use in veterinary medicine has been recognised [12].

Materials and methods

In preparing the manuscript, a literature review was conducted from October 2024 to January 2025. The search for relevant sources was performed using PubMed, Google Scholar, and Medline databases. The following keywords were used in the search: bexagliflozin, EGT1442, new indications for bexagliflozin, and bexagliflozin trial. Inclusion criteria included original papers, systematic reviews, meta-analyses, and narrative reviews published in English, with a particular focus on clinical studies addressing the efficacy, safety, and new potential uses of bexagliflozin. However, works of low methodological quality and articles that did not provide relevant scientific data were excluded. The search process is summarised in **Figure 1**.

Structure and mechanism of action

Bexagliflozin, also known as EGT1442, has the IUPAC name: (2S,3R,4R,5S,6R)-[4-chloro-2-(4-((2-(cyclopropoxy)ethoxy)phenyl)methyl)-3-(phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol. Its chemical formula is $C_{24}H_{29}ClO_7$, and its molecular weight is 464.16018 g/mol [13]. The structural formula of bexagliflozin is presented in **Figure 2**. The bexagliflozin molecule consists of a glycone fragment, an aglycone fragment, a central phenyl ring and a peripheral phenyl ring. The glycone and aglycone fragments are linked by a C-glycosidic bond, which is more metabolically stable compared to an O-glycosidic bond. Bexagliflozin is a derivative of dapagliflozin, and the main difference between the two is the R2 group on the phenyl ring. Bexagliflozin contains a cyclopropyl ethoxy group, which gives it grea-

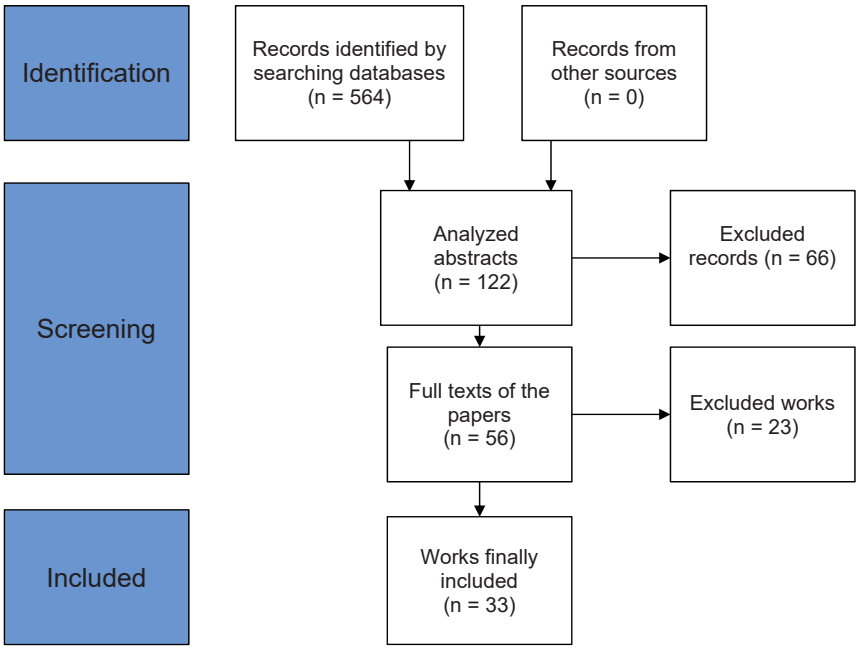

Figure 1. Scheme of review and identification of works.

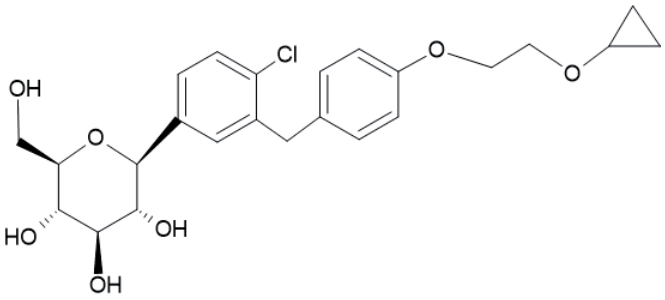

Figure 2. Structural formula of bexagliflozin.

ter SGLT2 inhibitory potency and higher selectivity to SGLT1 compared to dapagliflozin, which has an ethoxy group. Due to this unique molecular structure, bexagliflozin exhibits a more potent action and greater potency than dapagliflozin, and its selectivity against SGLT2 is almost twice as high. In addition, compared to canagliflozin, bexagliflozin has greater selectivity, even though both drugs have similar SGLT2 inhibitory potency [2]. Also, a study by Celik et al. showed that bexagliflozin binds more weakly to DPPIV compared to the other drugs tested, confirming that its efficacy in the treatment of type 2 diabetes is primarily due to SGLT2 inhibition rather than a direct effect on DPPIV [3]. Bexagliflozin belongs to the SGLT-2 inhibitor group. They are oral drugs that are absorbed from the gastrointestinal tract into the blood and then filtered through the kidneys, where they reach the glomeruli [2]. There are two types of sodium-glucose cotransporters: SGLT1 and SGLT2, which play a key role in glucose reabsorption [3]. It is estimated that SGLT2 is responsible for 90% of glucose reabsorption, while SGLT1 is responsible for the remaining 10% [2]. The mechanism of action of flozins involves inhibition of sodium-glucose cotransporter subtype 2, located in the proximal ileal tubule of the kidney. Inhibition of SGLT2 leads to inhibition of reabsorption of filtered glucose and sodium, resulting in sugaruria and natriuresis. This mechanism, by lowering blood glucose levels, reduces the need for insulin, which is particularly important in the context of impaired insulin secretion or action in type 2 diabetes mellitus (T2DM).In addition, the loss of glucose and sodium produces a diuretic effect, leading to a reduction in plasma volume,

which is essential in the treatment of heart failure or hypertension. In addition, the loss of glucose is associated with a loss of calories, which can be beneficial in the treatment of obesity [14].

Dosage

Bexagliflozin is a drug intended for oral administration [15]. It is recommended to be taken at a dose of 20 mg once a day. [16]. It should be taken in the morning, regardless of meals [17]. The maximum concentration of bexagliflozin in the blood appears within 2–4 hours after oral administration, and its half-life is about 13 hours [13]. Before initiating therapy, it is recommended to assess renal function. A dose of 30 ml/min/17.3 m2 is the same as in patients with normal renal function [17].

Effectiveness

The 12-week study evaluated the efficacy of bexagliflozin in monotherapy in adult patients, comparing it with placebo. The treatment effects in placebo-adjusted HbA1c reduction for the 5 mg, 10 mg, and 20 mg groups were shown to be -0.55%, -0.68%, and -0.80%, respectively. In addition, a dose-dependent lowering effect on fasting plasma glucose levels was observed. In terms of weight reduction, decreases of 1.44 kg, 1.59 kg, and 1.75 kg were noted for the 5 mg, 10 mg and 20 mg doses of bexagliflozin. In addition, a mean decrease of -3.83 mmHg in systolic blood pressure (SBP) and -2.04 mmHg in diastolic blood pressure (DBP) was recorded in patients taking the drug at the 20 mg dose [18]. In a prolonged 96-week study using only the 20 mg dose of the drug, a reduction in HbA1c by -0.53%, systolic blood pressure (SBP) by -4.9 mmHg, and diastolic blood pressure (DBP) by -1.43 mmHg was achieved. In addition, a weight loss of -2.41 kg was noted. Treatment with bexagliflozin for 96 weeks was shown to have a lasting effect [19]. Halvorsen et al. studied the impact of baxogliflozin compared to placebo in combination with metformin in adult patients with T2DM. After 24 weeks of treatment, they showed that bexagliflozin was more effective in reducing HbA1c by 0.7% compared to placebo plus metformin. The adjusted weight loss in the bexagliflozin group was 3.6 kg, compared with only 1.1 kg in the placebo group, a difference of 2.5 kg. In addition, there was a decrease in systolic blood pressure in the flozins group, in contrast to the placebo group, where blood pressure increased. [20] In 2024, the efficacy of bexagliflozin was compared to that of dapagliflozin. The results showed that the difference in HbA1c reduction between the bexagliflozin and dapagliflozin groups was 0.03%, in favour of dapagliflozin. The difference in mean weight change from baseline to week 24 was -0.30 kg in favour of bexagliflozin. After 24 weeks, HbA1c < 7% was achieved by 32.3% of patients in the bexagliflozin group and 31.6% in the dapagliflozin group, while HbA1c < 6.5% was achieved by 12.2% and 11.2% of patients, respectively. [21] In another study in patients taking metformin, the addition of bexagliflozin compared to glimepiride at week 96 resulted in a difference in HbA1c -2.30 mmol/mol (-0.21%), confirming the greater efficacy of bexagliflozin. It should be noted, however, that at the beginning of the study, it was glimepiride that resulted in a greater average reduction in HbA1c, but ultimately bexagliflozin proved more effective. In addition, the percentage of patients experiencing severe or documented symptomatic hypoglycemia was found to be 20.8% in the glimepiride group compared to 3.3% in the bexagliflozin group. Bexagliflozin showed better efficacy than glimepiride in reducing body weight, with the added benefit of lowering fasting glucose levels and reducing the rate of eGFR decline [22]. Another study compared the efficacy of bexagliflozin to sitagliflozin, an oral dipeptidylpeptidase (DPP-4) inhibitor drug, as an adjunct to metformin treatment. Bexagliflozin was not inferior to sitagliflozin in terms of reducing % HbA1c and provided a significantly greater reduction in fasting glucose. Among overweight and obese participants, significant weight loss was observed, with adjusted weight losses of 3.35 kg in the bexagliflozin group and 0.81 kg in the sitagliflozin group, resulting in a difference of 2.54 kg. In addition, patients in the bexagliflozin group had a decrease in SBP of 4.23 mmHg, compared to 1.90 mmHg in the sitagliflozin group [23]. It is noteworthy that the reduction in HbA1C compared to placebo in maximum bexagliflozin was higher (0.80%) than with maximum empagliflozin at 0.68%.[18] To date, no randomised controlled trial has been published that directly compared bexagliflozin with other SGLT-2 inhibitors in the treatment of type 2 diabetes. Therefore, further investigation is needed to determine whether bexagliflozin is more or less

effective than other common SGLT-2 inhibitors used in clinical practice [6]. Some researchers note that in indirect comparisons, bexagliflozin's HbA1c reduction was higher (0.80%) compared to empagliflozin (0.68%) at maximal doses. [18]

Side effects

In studies evaluating the safety of bexagliflozin versus placebo, the percentage of patients experiencing at least one adverse event was 42.3% in the bexagliflozin group and 40.3% in the placebo group. Most reported adverse events were mild to moderate [18]. In the 96-week extension study, although genital fungal infections are frequently observed with some drugs, their incidence was low, and differences from placebo were not noticeable. In addition, there was no increased risk of hypoglycemia in the bexagliflozin group compared to placebo. Severe adverse reactions occurred less frequently in the bexagliflozin group (2.8%) than in the placebo group (8.5%) [24]. In the study comparing bexagliflozin with dapagliflozin, the most common adverse reactions were urinary tract infections (6.4% in the bexagliflozin group vs. 8.4% in the dapagliflozin group) and hypoglycemia (3.5% vs. 3.9%). In addition, major cardiovascular incidents (1.48% vs. 0.99%) and falls and fractures (0.49% vs. 1.48%) were reported. The incidence of urinary ketone bodies was higher in the bexagliflozin group (9.85% vs. 4.43%) [21, 24]. In another study, bexagliflozin increased HDL and LDL cholesterol levels, but improved the LDL to HDL ratio, which is beneficial for cardiovascular health [16, 20]. In a study comparing bexagliflozin with glimepiride, 80.8% of patients (172 patients) taking bexagliflozin experienced at least one treatment-related adverse event (AE), compared with 81.2% (173 patients) in the glimepiride group. Urinary tract infections and joint pain were more frequently observed in the bexagliflozin group compared to the glimepiride group [22]. In another study comparing bexagliflozin with sitagliptin, adverse events occurred in 47.1% of patients in the bexagliflozin group and 56.0% of patients taking sitagliptin. Serious adverse events were reported in 3.7% of patients in the bexagliflozin group and 2.1% in the sitagliptin group [23]. The meta-analysis did not show that bexagliflozin increased the risk of Major Adverse Cardiovascular Events (MACE) in patients with T2DM compared to placebo or active control. [25] Another meta-analysis showed that bexagliflozin (20 mg) significantly lowered eGFR compared to other SGLT2 inhibitors, with a probability of 77.8%. In addition, the risk of urinary tract infections was higher with bexagliflozin (20 mg) than with ertugliflozin (5 mg) [26]. The C-476 study showed a higher incidence of amputation in patients treated with bexagliflozin compared to placebo [27]. The drug's effects may further be associated with fluid loss, which can lead to dehydration [17]. Registration studies of the drug showed that the most common adverse effects during bexagliflozin use were more frequent urination, fungal infections in the genital area and urinary tract infections. Such effects are also characteristic of other SGLT-2 inhibitors, due to the process of glucosuria [6].

Contraindications and interactions

Before initiating bexagliflozin therapy, it is necessary to assess renal function, as well as analyse the patient's history for factors that increase the risk of amputation. It should be noted that bexagliflozin may potentiate the hypoglycemic effects of other drugs, such as acarbose, GLP-1 receptor agonists, dipeptidylpeptidase-4 (DPP-4) inhibitors, sulfonylurea derivatives, and insulin [13,17]. In addition, bexagliflozin is contraindicated in patients with type 1 diabetes, as it may increase the risk of developing ketoacidosis. The drug is contraindicated in patients with end-stage chronic kidney disease who are receiving dialysis and in those with an estimated glomerular filtration rate (eGFR) of ≤30 ml/min/1.73 m² [6]. It should not be administered to pregnant women or nursing mothers. The use of the drug is also not recommended in patients with severe liver failure [17]. Attention should be paid to patients with type 2 diabetes who have been given SGLT2 inhibitors (including bexagliflozin), who have been reported to develop ketoacidosis, a serious, life-threatening condition requiring emergency hospitalisation. Adult patients with type 2 diabetes treated with bexagliflozin who show clinical signs of dehydration and severe metabolic acidosis (with associated symptoms such as nausea, vomiting, abdominal pain, general malaise, or shortness of breath) should be tested for ketoacidosis, regardless of blood glucose levels. It has been observed that bexagliflozin-associated ketoacidosis, although rare, can occur even at glucose levels below 250 mg/dl [6].

New research directions and applications of bexagliflozin

In animals

It is the first gliflozin registered for the treatment of diabetes in animals, specifically in cats [12]. It has shown glucose-lowering potential in dogs, suggesting its potential use in other animals as well [28].

In humans

Due to the relatively recent FDA approval of bexagliflozin and its short time on the market, available trial data on this drug are still limited [2]. In 2021, a study on the use of bexagliflozin for the treatment of spontaneous hypertension was completed with promising results [29]. A meta-analysis showed that bexagliflozin may have borderline tachycardia-reducing effects [30]. The results of these studies are included in **Table 1**. In 2027, a trial is scheduled to be completed to evaluate the impact of bexagliflozin on the severity of sleep apnoea in overweight or obese adults, compared with placebo [31]. The potential is seen for the use of bexagliflozin in pediatric patients for the treatment of type 2 diabetes between the ages of 10 and 17, with a projected completion date of 2031 [27]. It is described that bexagliflozin is the cheapest SGLT2 inhibitor available on the market in the US [32]. Its introduction may increase competition among antidiabetic therapies, which is likely to translate into lower prices for other drugs in this group [33]. However, it is noted that future cost-effectiveness analyses will be needed to determine whether bexagliflozin is more cost-effective than other SGLT2 inhibitors for the treatment of type 2 diabetes [6].

Conclusions

Bexagliflozin is the fifth flozin registered for the treatment of type 2 diabetes in the United States, marking an essential step in the development of treatments for the disease. As SGLT-2 inhibitors, flozines are playing an increasingly important role in the treatment of type 2 diabetes, not only for their ability to lower blood glucose levels, but also for their beneficial effects on other aspects of patients' health. Numerous clinical studies confirm that bexagliflozin effectively lowers glycated haemoglobin (HbA1c), a key indicator of long-term glycemic control. As a result, the drug shows high efficacy in the treatment of type 2 diabetes, especially in patients for whom previous therapies have not yielded satisfactory results. A critical aspect of bexagliflozin is its good safety profile. Studies indicate that the incidence of side effects, such as urinary or genital tract infections, is comparable to other drugs in the flozin group. These effects are well known and are related to the mechanism of action of the

Table 1. Summary of bexagliflozin studies in indications other than type 2 diabetes.

Name of study/Authors	Methodology	Results
THR-1442-C-603 [29]	The study evaluated the Effect of bexagliflozin 20 mg on the change in SBP and DBP values after 24 and 36 wks of cumulative exposure in 673 participants compared to placebo.	At the 24th week, a decrease in SBP of 9,262 mmHg and a decrease in DBP of 4,044 mmHg were observed. At the 36th week of treatment, there was a decrease in SBP of 12,454 mmHg and a decrease in DBP of 5,566 mmHg.
Xu et al. [30]	A study of the relationship between SGLT2 inhibitors and cardiac disorders conducted on a group of 35,432 patients using these drugs.	Bexagliflozin may have a borderline reducing effect on the incidence of ventricular tachycardia (RR 0.25; 95% CI 0.06–1.00; P = 0.05). Bexagliflozin did not clearly affect the incidence of cardiac arrest (RR 2.99; 95% CI 0.77–11.60; P = 0.11).
ADIPOSA [31]	The study will include overweight/obese adults (BMI 25–40 kg/m²) and moderate/severe OSA, evaluating whether bexagliflozin (20 mg/day) reduces AHI compared to placebo, as well as its effects on visceral fat/neck volume, closing pressure, fluid shift, and clinical indicators of OSA severity and sleep deprivation.	The study is scheduled to be completed in 2027.

AHI – apnea hypopnea index, SBP – Systolic Blood Pressure, DBP – Diastolic Blood Pressure, OSA – obstructive sleep apnoea, SGLT2 – Sodium-Glucose Co-Transporter 2.

flozines, which increase the excretion of glucose in the urine, which can promote the development of infections. However, it should be kept in mind that bexagliflozin is a relatively new drug on the market, and thus long-term studies evaluating its efficacy and safety, especially in the context of treating cardiovascular disease, are still lacking. Previous studies of bexagliflozin in the treatment of hypertension and cardiac arrhythmias indicate that the drug may have broader applications. If its multi-target effects are confirmed, it could reduce polypharmacy, increasing the safety of therapy and patient comfort. However, further studies are needed to determine in which patient groups it will provide the most significant benefit. Also hopeful are the planned studies on the use of bexagliflozin for the treatment of chronic kidney disease in children. If the results of these studies prove promising, it could open up new therapeutic options for young patients in whom currently available treatment options are limited. It is also worth noting a unique aspect of bexagliflozin – it is the first flozin registered not only for the treatment of diabetes in humans but also in animals. This shows how drugs developed for humans can find application in veterinary medicine, which can help improve animal health.

Acknowledgements

List of abbreviations: AHI: Apnea Hypopnea Index;; DBP: Diastolic Blood Pressure; FDA: Food and Drug Administration; HbA1c: Hemoglobin A1c; OSA: Obstructive Sleep Apnea; SBP: Systolic Blood Pressure; SGLT2: Sodium-Glucose Co-Transporter 2; T2DM: Type 2 Diabetes Mellitus.

Availability of Data and Materials
All data generated or analysed during this study are included in the published article.

Author's Contribution
JM (Józef Muszyński): Conceptualisation, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Supervision, Project Administration, Writing – Review & Editing. The author read and approved the final content.

Conflict of interest statement
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REVIEW PAPER

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The efficacy and safety of adamgammadex for reversing rocuronium-induced deep neuromuscular blockage. A systematic review and network meta-analysis

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ABSTRACT

Aim. We aim to evaluate the efficacy and safety of adamgammadex, a new modified γ-cyclodextrin, in reversing rocuronium-induced neuromuscular blockade compared with sugammadex or placebo.
Material and methods. We comprehensively searched three electronic databases (PubMed, Scopus, and ScienceDirect) from inception until February 2024 to detect all randomised controlled trials comparing adam-

gammadex versus sugammadex or placebo. STATA software 17 and RevMan version 5.4 were used for network and pairwise meta-analyses, respectively. The study protocol was prospectively registered in PROSPERO (CRD42024516623).
Results. Five randomised controlled trials comprising 530 patients were included in our study. There was a statistically significant difference between adamgammadex and sugammadex compared to placebo in the recovery time of neuromuscular function. A dose-response relationship was observed except for adamgammadex 7 mg/kg, which ranked first. Sugammadex was found to be more effective than a similar dose of adamgammadex. There was a non-significant difference between adamgammadex and sugammadex compared to placebo in the incidence of adverse events.
Conclusions. Adamgammadex or sugammadex can be a safe and effective therapeutic option in reversing rocuronium-induced neuromuscular blockade. More clinical trials with larger sample sizes should be conducted to obtain better evidence regarding these two drugs' most effective and safe doses.

Introduction

Neuromuscular blocking agents (NMBAs) were introduced in anesthesia in 1942 [1]. Their use facilitates tracheal intubation and mechanical ventilation, enhancing surgical intervention quality [2,3]. However, a residual persistence in the neuromuscular blocking effect beyond the end of surgery is a common problem. This may prolong the recovery time and lead to various adverse events, including respiratory complications, airway obstruction, and upper airway dysfunction [4,5]. Therefore, postoperative neuromuscular monitoring is recommended. Additionally, novel therapeutic agents were suggested to ensure adequate recovery of neuromuscular function and the early detection of the need to administer relaxant binding agents such as adamgammadex sodium or sugammadex [6,7].

The traditional neuromuscular block antagonists (neostigmine and edrophonium) are anticholinesterase drugs and have limited efficacy in reversing profound levels of NMB. Moreover, their non-selective action on the muscarinic acetylcholine receptors can lead to various adverse effects due to the interaction of the drug with the muscarinic receptors in other tissues [8]. Consequently, these medications are usually administered with atropine, which may cause untoward events, such as tachycardia, dry mouth, and blurred vision [9]. Thus, more research was directed toward other selective relaxant medications such as adamgammadex sodium and sugammadex.

Sugammadex, the selective relaxant binding agent (SRBA), effectively and quickly restores neuromuscular function from NMBA [10]. However, the concerns about sugammadex associated with hypersensitivity reactions and anaphylaxis restrict its approval in some countries [11]. Furthermore, this SRBA increases the risk of postoperative bleeding [12].

The new medication Adamgammadex sodium, a modified γ-cyclodextrin derivative, has a similar mechanism of action to sugammadex and shows, in pre-clinical animal studies, a similar efficacy to sugammadex but with fewer potential side effects [13,14]. New clinical trials have been done that evaluate the safety and efficacy of Adamgammadex in humans by comparing it with sugammadex or a placebo.

Aim

In this systematic review and network meta-analysis (NMA), we aim to evaluate the efficacy and safety of Adamgammadex or Sugammadex in reversing rocuronium-induced deep neuromuscular blockade in patients undergoing surgery.

Material and methods

We conducted our systematic review and network meta-analysis strictly adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guideline and the extension statement for network meta-analysis [16]. The study protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number CRD42024516623.

Eligibility criteria

Our inclusion criteria were randomised controlled trials that evaluated the efficacy or safety of Adamgammadex or Sugammadex in reversing rocuronium-induced neuromuscular blockage in healthy adults or patients undergoing surgery with 1–3 American Society of Anesthesiologists (ASA) physical status. We excluded review articles, case reports, conference abstracts, animal studies, observational studies, case series, and data analysis based on these publications. Additionally, we excluded articles if there was no available English full-text version.

Literature search

A comprehensive search was conducted across PubMed, Scopus, and Science Direct to find pertinent published RCTs from inception until February 22, 2024. This date reflects when the literature search was conducted and completed, ensuring consistency and reproducibility. The following search terms were used:

((Adamgammadex) OR (cyclodextrin-based selective reversal agent) OR cyclodextrin)) AND ((rocuronium) OR (neuromuscular blockade) OR (caused deep neuromuscular blockade)).

Manual research was carried out using backward citation analysis on Google Scholar to find all pertinent papers, and the studies' references were screened for possible articles to include.

Screening of the literature

Titles and abstracts from retrieved citations were imported into Rayyan, a web-based tool designed to help researchers organise and manage systematic reviews [17]. EndNote, a reference management software, was used to identify and exclude duplicate citations. Then, two independent authors blindly assessed the retrieved citations in two stages: the first involved checking all extracted publication titles and abstracts, and the second involved examining full-text screening of all eligible abstracts and assessing their suitability for conducting meta-analysis. A third author helped to resolve any conflict.

Data extraction

Two independent reviewers extracted data from the eligible studies into a data extraction Google sheet. Discrepancies were cross-checked and addressed. For each included randomised controlled trial, the following data were extracted if present: population characteristics (age, sex, BMI, weight, and height), study characteristics (Study design, country, number of centers, total participants, follow-up duration, main inclusion criteria, primary outcomes, and conclusion), primary outcomes which assessed the start of adamgammadex drug to the recovery of Train-of-Four ratio (TOFr) 0.9 and the percentage (%) of patients with TOFr ≥0.9.In addition to secondary outcomes, which summarised TOFr 0.7, TOFr 0.8, and percentage (%) of patients with (TOFr ≥0.7 and ≥0.8) and adverse effects, which included (drug-related severe adverse effects, serious adverse effects, anaphylactic reaction, bradycardia, and urinary tract infection).

Quality assessment

Using the Cochrane Collaboration's Risk of Bias tool (version 2, RoB2) [18], two blinded authors assessed the quality of the included studies. The randomisation process, deviation from the intended interventions, missing outcome data, outcome assessment, and choice of the reported result are the five primary categories that make up the composite score used by this tool. The investigators' conclusions are classified as "low risk," "some concerns," or "high risk" of bias for each of these topics. A third investigator reanalysed the disagreements and resolved them. The RoB2 tool summary and graph were produced using the Robvis [19].

Synthesis of results

Network meta-analysis was performed using STATA software 17 for Mac (StataCorp, 2021) [20], and pairwise meta-analyses were conducted using ReviewManager software (RevMan version 5.4) [21]. The network plots were used to represent the different interventions included in each outcome. Each intervention is represented as a circle, and the lines connecting them represent the randomised comparisons between these interventions. The nodal size represents the sample size included in each intervention group. The TOFr (0.9,0.7,0.8) outcomes were used to evaluate the efficacy outcomes, which were then presented as mean difference and 95% confidence interval. Dichotomous outcomes, such as adverse events, were pooled as risk ratio (RR) with its confidence interval.

Continuous data were pooled as Cohen's d with its 95% CI, whereas dichotomous data were pooled as odds ratio (OR) with its 95% confidence interval (CI). The Wan et al. approach was used to transform data from the median (or interquartile range) to the mean and standard deviation (SD) in any study that reported the data. The pooled effect size for all outcomes was determined using the Der Simonian Laird random effects model, which gives weight to a small number of studies at the expense of larger studies. This allowed for the provision of pooled estimates with a wider standard error to account for any inconsistent effect sizes.

Assessment of heterogeneity

Visual inspection of the forest plots was used to assess statistical heterogeneity between trials. Higgins and Thompson I^2 and a chi-square test (CoPlotse Q test) were used to measure it. The equation used is $I^2 = ((Q - df)/Q) \times 100\%$ [23]. Heterogeneity was classified as low, moderate, or high if the I^2 was less than 25%, between 25% and 75%, or greater than 75%. It was considered significant if the p-value of the chi-square test was less than 0.1, as noted in [23].

Publication bias and funnel plots

For the reporting bias assessment, we arranged to assess the publication bias using the funnel plot method. However, the evaluation was not statistically feasible due to the limited number of included studies. According to Egger et al., bias assessment requires at least ten pooled studies [24].

Results

Characteristics of the included studies

Our literature search retrieved a total of 2031 records from online medical databases. We removed 67 duplicates, and the remaining studies underwent title and abstract screening. One thousand nine hundred fifty-three papers were

Table 1. Summary of the studies evaluating adamgammadex in reversing neuromuscular blockage.

Study ID	Follow up (days)	Main inclusion criteria	Primary outcomes	Conclusion
Zhao et al. 2024 (RCT, China, 7 centers) n = 80 [26]	2	18–64-year-old patients, grade 1 or 2 ASA, underwent elective surgery under general anesthesia with rocuronium	ToF 0.9 ratio.	Adamgammadex 7, 8, 9 mg/kg > sugammadex in TOF recovery, tolerance, and low incidence of adverse events
Jiang et al. 2022 (RCT, China, multicenter) n = 52 [27]	1	Patients aged 18–64 years with grade 1 or 2 (ASA), if muscle relaxation was necessary to be used in surgery more than for intubation	ToF 0.9 ratio.	Adamgammadex was safe, effective, and tolerable
Zhao et al 2021 (RCT, China, 1 center) n = 36 [28]	7	Patients aged 18–64 years, ASA 1–2, (BMI) <30 kg m^2, weight 50 kg for men and 45 kg for women underwent elective surgery under general anesthesia using rocuronium to facilitate tracheal intubation and maintain muscle relaxation	ToF 0.9 ratio.	Adamgammadex enabled rapid TOF recovery and good tolerance
Jiang et al. 2020† (RCT, China, 1 center) n = 52 [29]	8	Male and female subjects aged 18–40 with (BMI) of 19–26 kg/m^2 and weight of 50–90 kg for males and 45–85 kg for females	Adverse events.	Adamgammadex may be a novel and safe option
Zhang et al. 2023 (RCT, China, 15 centers) n = 326 [25]	1	Patients aged 18-65 years had an ASA physical status 1-3, had freely given written informed consent, were scheduled to undergo elective surgery with a tracheal tube or laryngeal mask airway, and were expected to receive rocuronium during the surgical procedure	The proportion of subjects with a ToF ratio returning to 0.9 within 5 min. Recovery time to ToF 0.9.	Adamgammadex was non-inferior to sugammadex with a possible lower incidence of adverse drug reactions compared with sugammadex

TOF 0.9 – Recovery time of the TOF ratio to 0.9; ASA – American Society of Anesthesiologists; TOF – Train-Of-Four; BMI – Body Mass Index
† Shown in figures as Jiang et al. 2019

excluded in title and abstract screening. The remaining studies underwent full-text screening, after which we included five records. A detailed description of the selection process is shown in **Supplementary Figure 1.**

Five randomised controlled trials comprising 530 patients were included in our study, [25–29]. Most of the studies included patients scheduled for elective surgery and expected to receive rocuronium during the surgical procedure to facilitate intubation and muscle relaxation. It is worth noting that the Jiang et al. 2020 study was conducted on healthy volunteers, and the Jiang et al. 2022 study included patients if rocuronium was necessary for the surgery rather than the intubation. Among the included studies, four intervention groups were included with rocuronium doses of 4 mg/kg and 8 mg/kg. Three studies included a rocuronium dose of 2 mg/kg, and two included a rocuronium dose of 6 mg/kg. Rocuronium doses of 7, 9, 16, 24, or 32 were only used in one study. Moreover, some studies used sugammadex as an intervention with a 2 or 4 mg/kg dose. A detailed description of the baseline characteristics and a summary of the included studies are shown in **Tables 1** and **2**, respectively.

Risk of bias assessment

Based on the Cochrane risk of bias assessment tool 2 (ROB-2), all included papers had a low risk of bias. The risk of bias graph and summary of the quality assessment domains are shown in **Figure 1**. The network plots are shown in the **Supplementary Figures 2** and **3**.

The recovery time (minutes) of the TOF ratio to 0.9 (Tof 0.9)

A summary of the characteristics of the network meta-analysis is shown in **Supplementary Table 1**. Our network meta-analysis found that TOF 0.9 was significantly faster in adamgammadex 2 mg/kg (MD = -36.25, 95% CI = [-44.54: -27.95]), 4 mg/

Table 2. Baseline characteristics of patients.

Study ID	Groups	Dose (mg/kg)	Age (Years), Mean (SD)	Sex (male) N (%)	Weight (kg), Mean (SD)	Height (cm), Mean (SD)	BMI (kg/m^2), Mean (SD)
Zhao et al. 2024 [26]	Adamgammadex	7	41.8 (11.17)	5 (25%)	66.28 (12.08)	165 (10.10)	24.28 (3.12)
		8	43.6 (11.36)	9 (45%)	65.23 (8.91)	166.2 (8.92)	23.61 (2.35)
		9	40.3 (13.73)	9 (45%)	62.88 (12.96)	165.3 (10.06)	22.88 (3.55)
	Sugammadex	4	49.7 (11.34)	10 (50%)	62.75 (8.93)	163.6 (7.63)	23.38 (1.94)
Jiang et al. 2022 [27]	Adamgammadex	2	38.5 (10.9)	5 (50%)	63 (12)	161 (9)	24.4 (4)
		4	46.4 (10.6)	6 (55%)	68 (11)	162 (8)	25.8 (3)
		6	41.7 (11.1)	4 (33%)	61 (6)	162 (5)	23.1 (2.7)
		8	38.9 (10.1)	3 (30%)	64 (11)	161 (10)	24.8 (3.1)
	Placebo	NA	32.7 (7.7)	6 (67%)	60 (16)	164 (9)	22 (3.6)
Zhao et al. 2021 [28]	Adamgammadex	2	47.5 (13.63)	3 (50%)	64 (10)	163.3 (8.76)	23.8 (2.32)
		4	45 (13.45)	1 (16.6%)	64.17 (11.37)	163.2 (6.31)	24 (2.61)
		6	40.2 (14.47)	3 (50%)	66.5 (11.91)	168.3 (6.38)	23.5 (3.21)
		8	35.8 (11.82)	3 (50%)	67.67 (13.49)	166.3 (9.85)	24.5 (2.81)
		10	39.5 (11.67)	4 (66.6%)	69 (17.7)	168.2 (9.45)	23.8 (3.87)
	Sugammadex	4	52.2 (10.4)	5 (83.3%)	67.75 (5.19)	166.2 (6.52)	24.8 (2.04)
Jiang et al. 2020† [29]	Adamgammadex	0.5	27 (2.5)	2 (50%)	59 (5.9)	166 (3)	NA
		2	21.6 (1.5)	4 (50%)	57 (8.3)	164 (6)	NA
		4	23.1 (3.6)	4 (50%)	55.9 (10.8)	161 (8)	NA
		8	25.4 (3.9)	4 (50%)	59.4 (9.9)	165 (11)	NA
		16	24.9 (1.7)	4 (50%)	60.8 (13.1)	165 (10)	NA
		24	25 (1.5)	4 (50%)	61.6 (8.6)	166 (9)	NA
		32	23.9 (2.6)	4 (50%)	60.6 (9.4)	164 (9)	NA
	NA	NA	NA	NA	NA	NA	NA
Zhang et al. 2023 [25]	Adamgammadex	4	42 (3)	60 (38.7%)	65 (10)	163 (8)	24 (3)
	Sugammadex	4	37 (3.33)	46 (29.7%)	62 (10)	162 (8)	24 (3)

BMI – Body Mass Index; SD – standard deviation; N – number; NA – not available
† Shown in figures as Jiang et al. 2019

Figure 1. Risk of bias graph and Summary of the Rob-2 domains.

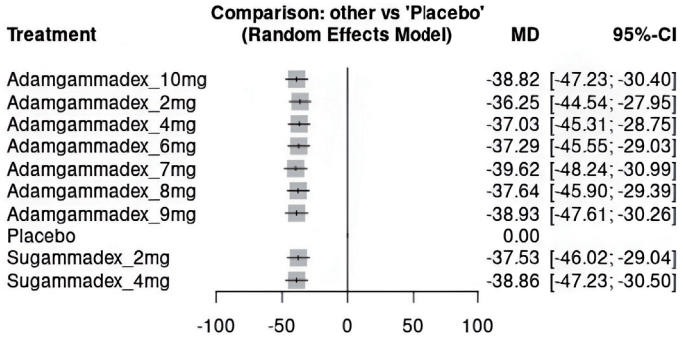

Figure 2. Train-of-Four ratio 0.9 comparison with placebo.

kg (MD = -37.03, 95% CI = [-45.31: -28.75]), 6 mg/kg (MD = -37.29, 95% CI = [-45.55: -29.03]), 7 mg/kg (MD = -39.62, 95% CI = [-48.24: -30.99]), 8 mg/kg (MD = -37.64, 95% CI = [-45.90: -29.39]), 9 mg/kg (MD = -38.93, 95% CI = [-47.61: -30.26]), 10 mg/kg (MD = -38.82, 95% CI = [-47.23: -30.40]) compared to placebo. Moreover, both included Sugammadex doses (2 mg/kg, 4 mg/kg), which led to significantly faster Tof recovery than placebo (see **Figure 2**). The pairwise comparison revealed that sugammadex 2 mg/kg led to a quicker recovery of ToF when compared to adamgammadex 4 mg/kg (MD = -0.50, 95% CI = [-0.53: -0.47]). Moreover, there was a significant difference in favor of sugammadex 4 mg/kg when compared to adamgammadex 6 mg/kg (MD = 0.88, 95% CI = [0.15: 1.61]) and adamgammadex 8 mg/kg (MD = -4.87, 95% CI = [-8.16: -1.58]). Adamgammadex 4 mg/kg, 8 mg/kg, and 10 mg/kg had a significantly faster recovery of ToF when compared to adamgammadex 2 mg/kg (see **Figure 3**).

To confirm the effectiveness of these interventions on ToF 0.9, a cumulative ranking curve was used to rank the different interventions. The ranking showed a dose-response relationship, except for adamgammadex 7 mg/kg, which achieved the highest ranking. Regarding sugammadex, the 2 mg/kg dose was positioned between the adamgammadex 6 mg/kg and 8 mg/kg groups. In contrast, the sugammadex 4 mg/kg dose was positioned between the adamgammadex 10 mg/kg and 7 mg/kg groups, as shown in **Supplementary Figure 4**.

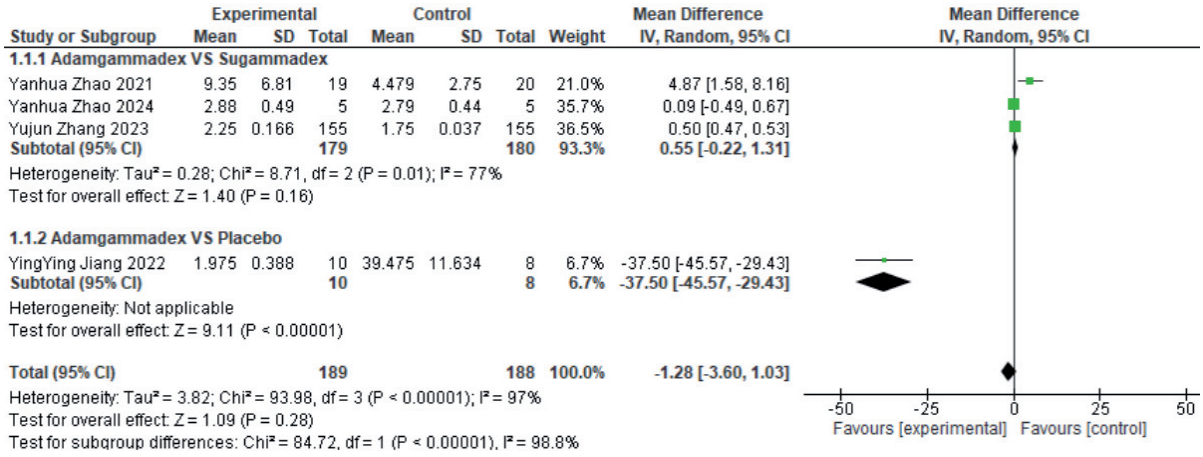

Figure 3. Train-of-Four ratio 0.9, individual study result.

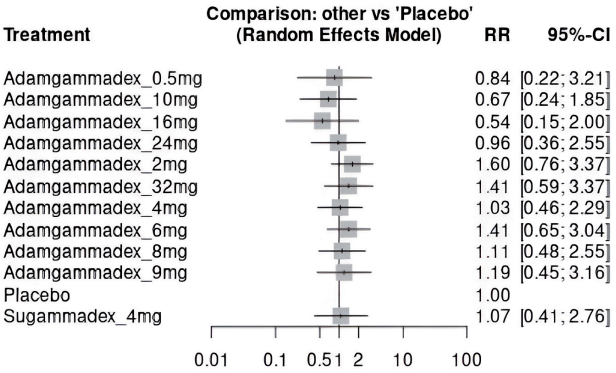

Figure 4. Adverse events comparison with placebo.

Direct pairwise meta-analysis showed no significant difference between adamgammadex and sugammadex (see **Supplementary Figure 5**).

The recovery time (minutes) of the TOF ratio to 0.7 (ToF 0.7)

Our direct pairwise meta-analysis showed a non-significant difference regarding ToF 0.7. Based on the intervention time, subgrouping showed a statistically significant difference between adamgammadex and placebo in favour of adamgammadex (MD = -23.85, 95% CI = -28.40: -19.30). However, there was no statistically significant difference between adamgammadex and sugammadex (MD = -3.53, 95% CI = -11.59: 4.52). The test for subgroup differences showed substantial results (I^2 = 94.6%, P < 0.0001) (see **Supplementary Figure 6**).

Adverse events

The network meta-analysis showed a non-significant difference in adverse effects among any intervention group compared to placebo, indicating that adamgammadex or sugammadex could be a safe intervention for inducing muscle relaxation in elective surgeries (see **Figure 4**). A summary of the individual study results is shown in **Supplementary Figure 7**. The ranking of risk ratios of the different intervention groups and placebo is shown in **Supplementary Table 3**. Our direct pairwise comparison showed no significant difference between adamgammadex and sugammadex in the incidence of adverse events. No heterogeneity was observed in any of the subgroups (see **Supplementary Figure 8**).

Discussion

Significance of the study

Our findings indicate that adamgammadex and sugammadex significantly expedite the recovery of neuromuscular function, as measured by ToF 0.9. The analysis revealed that higher doses

generally resulted in faster recovery of muscular function, except for the adamgammadex 7 mg/kg dose, which exhibited the highest efficacy. Furthermore, our study determined that the 4 mg/kg dose of sugammadex did not show a statistically significant difference in efficacy compared to the 7 mg/kg dose of adamgammadex. Additionally, the 2 mg/kg dose of sugammadex was associated with a significantly faster recovery of neuromuscular function compared to an equivalent dose of adamgammadex.

Explanation of our findings

Neuromuscular blocking agents such as rocuronium are widely used during surgical procedures to facilitate tracheal intubation and mechanical ventilation and optimise surgical conditions [30]. However, a high percentage of patients may experience persistent residual neuromuscular blockade postoperatively, which may put the patients at an increased risk of postoperative complications such as hypoxia, airway obstruction, aspiration, or prolonged hospital stay [31,32]. Thus, selective relaxant binding agents such as Sugammadex and Adamgammadex were suggested as a therapeutic option to reverse the rocuronium-induced neuromuscular blockade [10]. Literature indicates that sugammadex can be safe and effective. However, it was reported that it may be associated with a risk of bleeding due to its anticoagulant effect and a risk of hypersensitivity reaction, up to anaphylaxis [33,34].

On the other hand, adamgammadex is a newly synthesised selective relaxant-binding agent. Structural modifications of the core of sugammadex were synthesised. Pores studies found that adamgammadex reverses the neuromuscular blocking effect of rocuronium in a concentration-dependent manner. Moreover, it was suggested to have a lower risk of bleeding or hypersensitivity [9,13]. We conducted this network meta-analysis to assess the difference in efficacy and safety of different doses of adamgammadex or sugammadex in reversing the rocuronium-induced neuromuscular blockade.

All of the included studies assessed neuromuscular function recovery using the train of four measure, which is the simple count of muscle twitches resulting from neuromuscular stimulation. The ToF ratio is the ratio between the amplitude of the fourth and the first twitch. Adequate recovery is considered when the TOF ratio is ≥0.9 [13].

Our study found that adamgammadex and sugammadex significantly accelerated recovery from neuromuscular blockade compared to placebo. The cumulative ranking curve revealed a dose-response relationship consistent across all included clinical trials [35]. This dose-response effect may be attributed to the competitive inhibition mechanism of adamgammadex and sugammadex against rocuronium. Although the cumulative ranking curve indicated a dose-response relationship, the 7 mg/kg dose of adamgammadex ranked higher than the 8, 9, and 10 mg/kg doses. However, a statistically significant difference was only observed between the 7 mg/kg and 8 mg/kg doses of adamgammadex. No statistically significant difference was found between the 7 mg/kg dose of adamgammadex and the 4 mg/kg of sugammadex. The lack of significance in many cases could be due to the small number of included trials, which might have limited the ability to detect significant differences between the interventions. Zhao et al. supported our findings by reporting no intergroup differences between the 7, 8, and 9 mg/kg doses of adamgammadex. They also noted that adamgammadex led to a slightly longer recovery time than sugammadex, although the results did not reach statistical significance [29].

Implications of these findings for clinical practice

The development of adamgammadex appears mainly targeted toward the Chinese market, and it remains unclear whether this drug will become popular enough globally to replace sugammadex [36]. Clinically, adamgammadex was expected to perform similarly to sugammadex [36]. Our study supports this idea, showing that adamgammadex and sugammadex are safe therapeutic options for reversing rocuronium-induced neuromuscular blockade during surgery. Both drugs provided rapid recovery of neuromuscular function, with no significant difference in adverse events compared to placebo.

Strengths and limitations

This is the first meta-analysis to investigate the efficacy and safety of adamgammadex and sugammadex for reversing neuromuscular blockade, primarily during surgery. However, this study is not without limitations. First, the limited number of included studies may lead to imprecision

and an inability to detect statistically significant differences among the various doses. Second, there was heterogeneity in the study populations; four included patients undergoing elective surgery, while one involved healthy volunteers. This variability could affect patient characteristics or baseline neuromuscular function, influencing the efficacy and risk of adverse events. Third, most included studies focused on elective surgery patients, introducing variability in surgery duration, potential complications, and anaesthetic techniques, which could impact the efficacy and timing of adamgammadex or sugammadex administration. This also limits the generalizability of our results to emergency surgeries or other types of elective surgeries not assessed in our study. Fourth, all included studies were conducted in China, which may limit the generalizability of our findings to populations with different demographics and health conditions. Finally, due to the limited number of included studies, we could not perform a publication bias assessment, potentially affecting the robustness of our meta-analysis.

Recommendations for future research and clinical practice

Further well-conducted clinical trials are needed to establish the evidence regarding the use of Adamgammadex or Sugammadex for rocuronium-induced neuromuscular blockade. This will lead to a deeper understanding of these patients' most effective and safe doses.

Conclusion

Our study indicates that Adamgammadex/Sugammadex can be a safe and effective intervention in reversing the neuromuscular blockade caused by rocuronium during surgical procedures. Although a dose-response relationship is noted, few studies have drawn a solid conclusion. Further studies using multiple doses should be conducted to establish this effect.

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Authors' contributions
AA: conceptualisation and methodology. AA, OY, AZ, and TIEG: investigation and data curation. AE: formal analysis. AA, NIH, AZ, and TIEG: Writing – Original Draft. NIH and MA: Supervision. AA and MA: Project administration. ZO, MA, and YN: Writing – Review & Editing. All authors read and approved the final content.

Ethical consideration
This article is based on previously conducted studies and contains no new studies with human participants.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

Data Availability
All data will be available from the first or corresponding author upon reasonable request.

Conflict of interest statement
The authors declare no conflict of interest.

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There are no sources of funding to declare.

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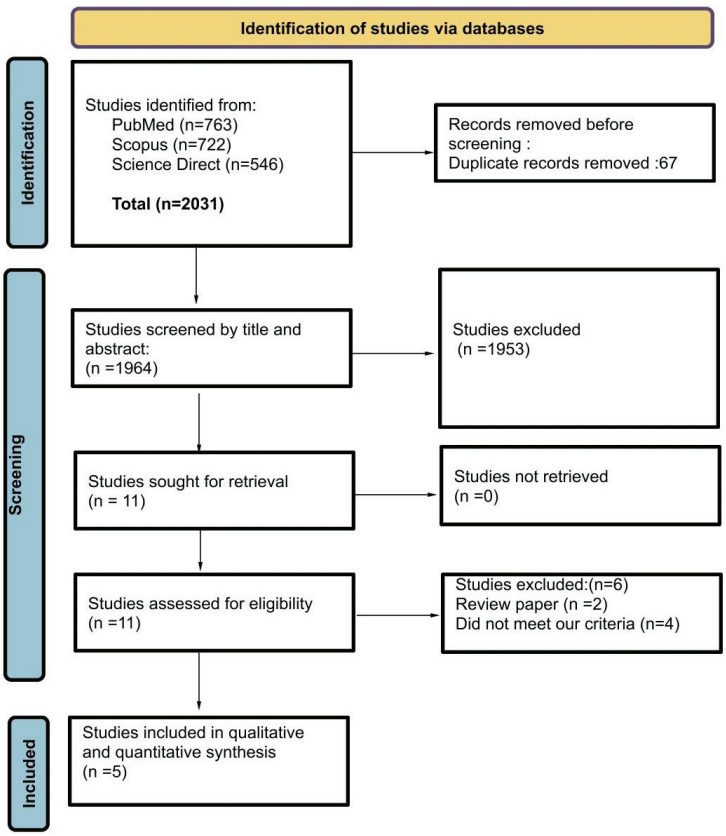
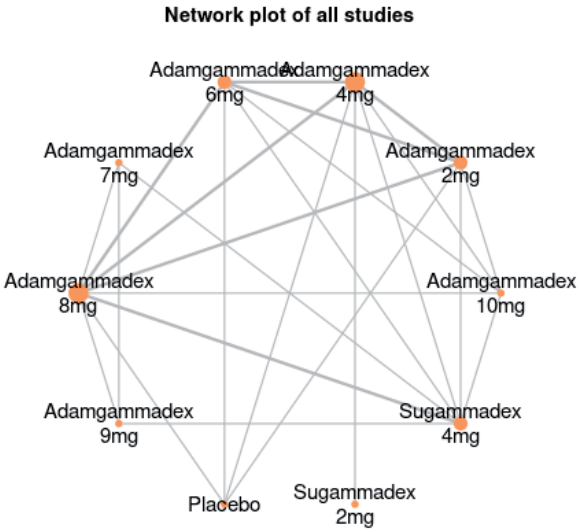
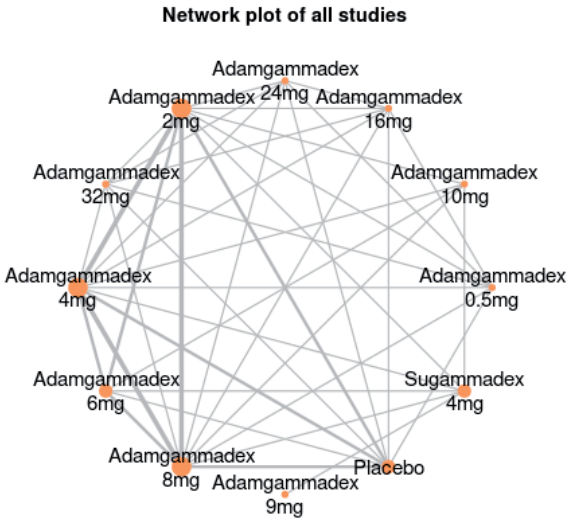


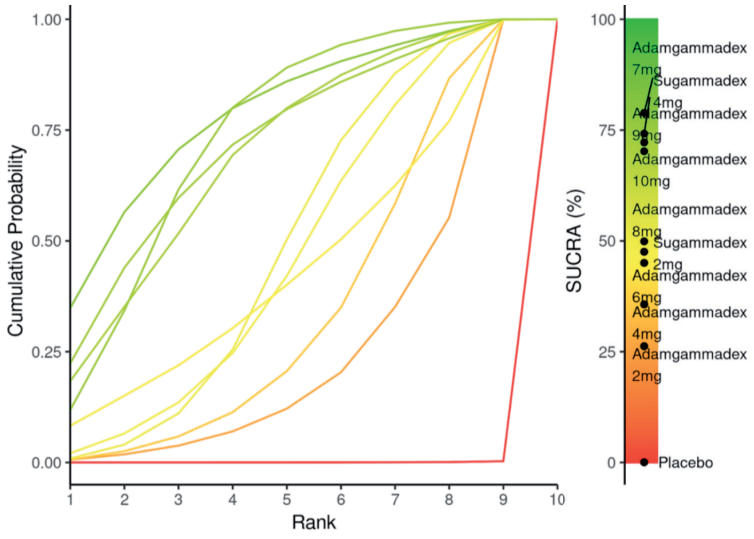
Supplementary Figure 1. Prisma.



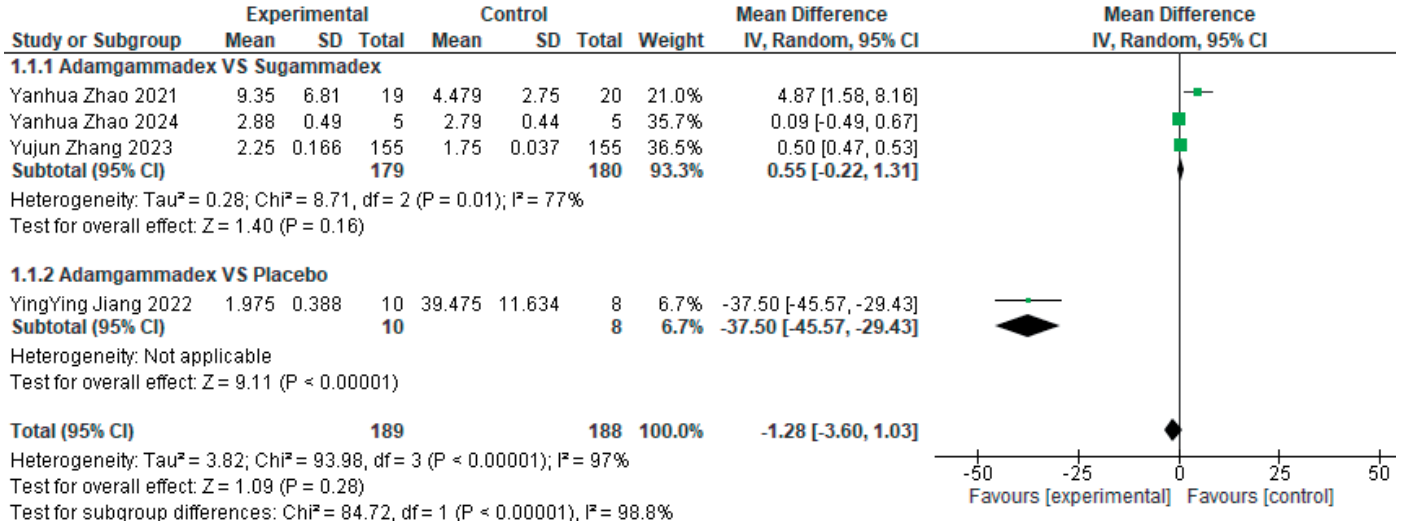
Supplementary Figure 2. Network plot of all studies reporting ToF 0.9.



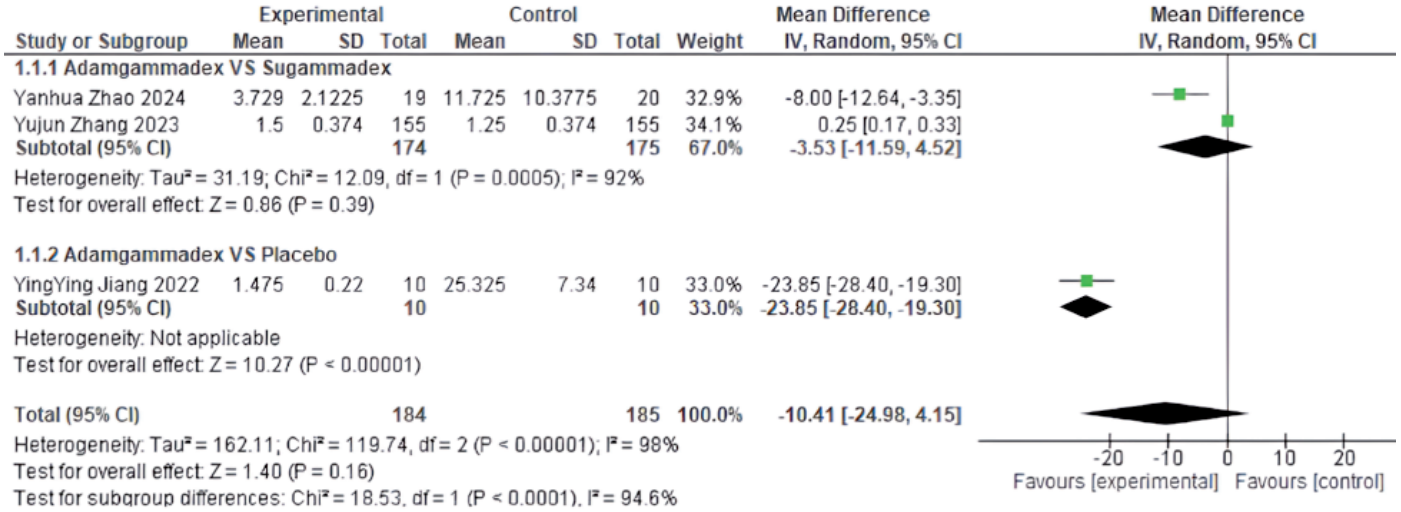
Supplementary Figure 3. Network plot of all studies reporting Adverse events.



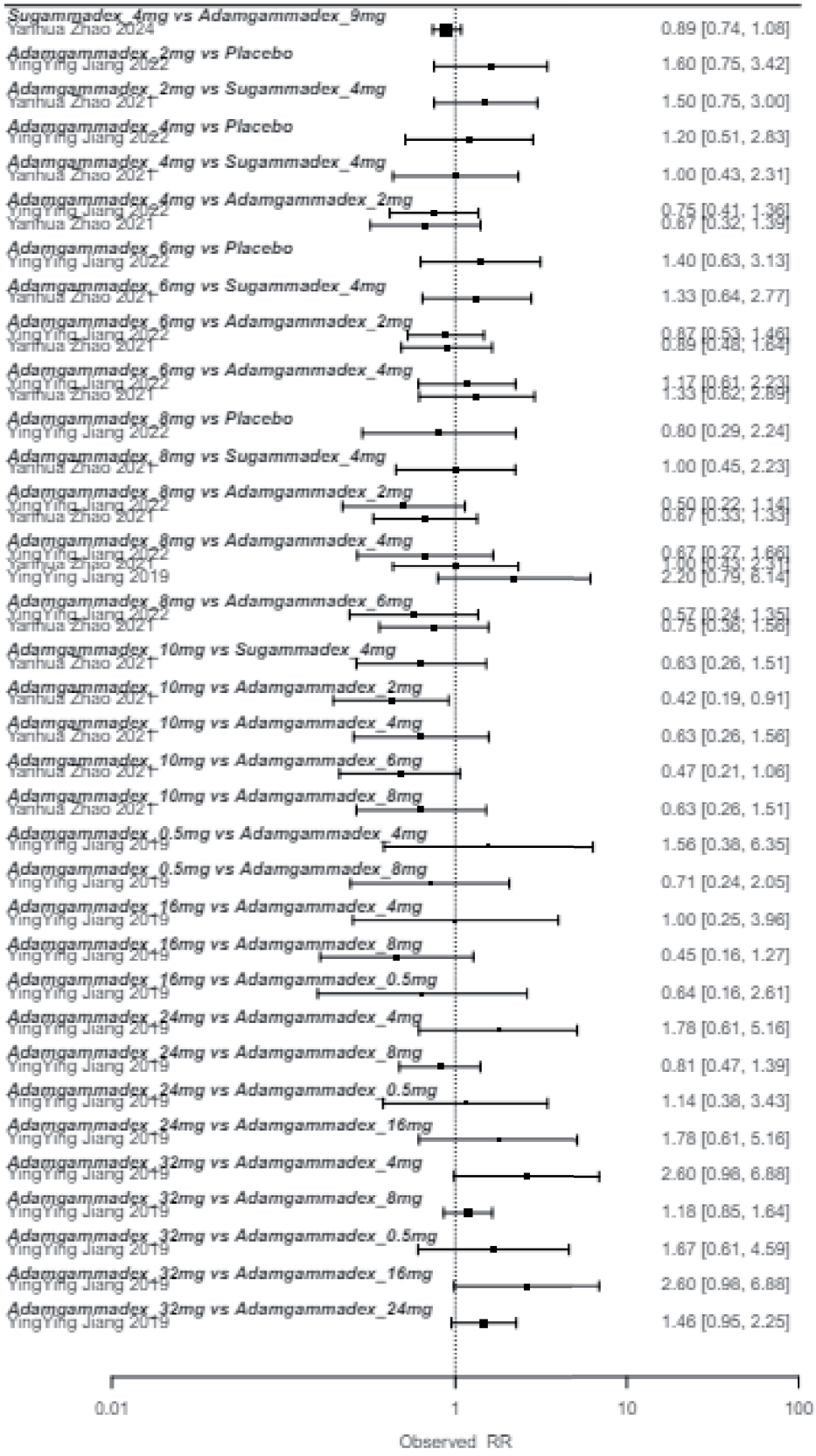
Supplementary Figure 4. Cumulative ranking of ToF 0.9.



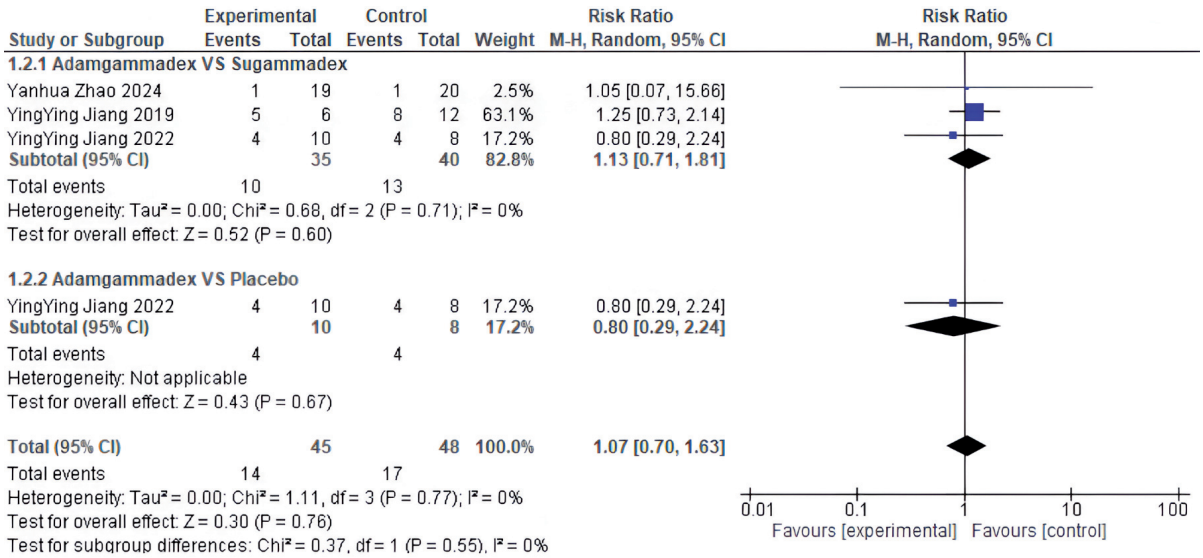
Supplementary Figure 5. Direct pairwise meta-analysis of ToF 0.9.



Supplementary Figure 6. Forest plot of ToF 0.7.



Supplementary Figure 7. Individual study results for adverse events.



Supplementary Figure 8. Direct pairwise meta analysis adverse events.

Supplementary Table 1. Summary characteristics of all studies reporting ToF 0.9.

Characteristic	Value
Number of Interventions	10
Number of Studies	4
Total Number of Patients in Network	464
Total Possible Pairwise Comparisons	45
Total Number of Pairwise Comparisons With Direct Data	25
Is the network connected?	TRUE
Number of Two-arm Studies	1
Number of Multi-Arms Studies	3
Average Outcome	3.498

Supplementary Table 2. Ranking Tof 0.9.

Adamgammadex_7mg	0.06 [-2.39; 2.52]	.	-0.68 [-3.22; 1.86]	-4.81 [-8.54; -1.07]	.	.	.	.	.
-0.75 [-3.09; 1.59]	Sugammadex_4mg	0.44 [-1.61; 2.49]	-0.75 [-3.36; 1.87]	-1.10 [-2.84; 0.64]	.	-0.88 [-2.89; 1.13]	-5.32 [-11.64; 1.00]	-10.52 [-21.32; 0.28]	.
-0.80 [-3.75; 2.16]	-0.04 [-2.04; 1.95]	Adamgammadex_10mg	.	-0.53 [-2.59; 1.53]	.	-1.32 [-3.42; 0.78]	-5.76 [-12.11; 0.59]	-10.96 [-21.77; -0.15]	.
-0.68 [-3.22; 1.86]	0.07 [-2.43; 2.57]	0.11 [-2.98; 3.20]	Adamgammadex_9mg	-4.12 [-7.97; -0.28]	.	.	.	.	.
-1.97 [-4.61; 0.66]	-1.22 [-2.86; 0.42]	-1.18 [-3.07; 0.72]	-1.29 [-4.07; 1.49]	Adamgammadex_8mg	.	-0.59 [-1.98; 0.81]	-0.70 [-2.54; 1.15]	-1.40 [-3.35; 0.54]	-37.50 [-45.78; -29.22]
-2.09 [-5.68; 1.50]	-1.34 [-4.25; 1.57]	-1.29 [-4.33; 1.74]	-1.41 [-5.11; 2.29]	-0.12 [-2.69; 2.45]	Sugammadex_2mg	.	-0.50 [-2.37; 1.37]	.	.
-2.33 [-5.10; 0.45]	-1.57 [-3.33; 0.19]	-1.53 [-3.48; 0.42]	-1.64 [-4.56; 1.27]	-0.35 [-1.73; 1.03]	-0.24 [-2.82; 2.35]	Adamgammadex_6mg	-0.27 [-2.15; 1.60]	-1.00 [-2.97; 0.98]	-37.10 [-45.39; -28.81]
-2.59 [-5.65; 0.47]	-1.84 [-4.06; 0.38]	-1.79 [-4.19; 0.60]	-1.91 [-5.10; 1.28]	-0.62 [-2.37; 1.14]	-0.50 [-2.37; 1.37]	-0.26 [-2.04; 1.51]	Adamgammadex_4mg	-0.94 [-2.91; 1.04]	-37.23 [-45.51; -28.94]
-3.37 [-6.49; -0.25]	-2.62 [-4.92; -0.32]	-2.57 [-5.04; -0.11]	-2.69 [-5.93; 0.55]	-1.40 [-3.24; 0.44]	-1.28 [-4.00; 1.44]	-1.04 [-2.90; 0.81]	-0.78 [-2.75; 1.19]	Adamgammadex_2mg	-36.40 [-44.70; -28.10]
-39.62 [-48.24; -30.99]	-38.86 [-47.23; -30.50]	-38.82 [-47.23; -30.40]	-38.93 [-47.61; -30.26]	-37.64 [-45.90; -29.39]	-37.53 [-46.02; -29.04]	-37.29 [-45.55; -29.03]	-37.03 [-45.31; -28.75]	-36.25 [-44.54; -27.95]	Placebo

Supplementary Table 3. Ranking Adverse events.

Adamgammadex_16mg	.	0.64 [0.16; 2.61]	0.56 [0.19; 1.63]	1.00 [0.25; 3.96]	.	.	0.45 [0.16; 1.27]	.	.	0.38 [0.15; 1.02]	.
0.80 [0.22; 2.90]	Adamgammadex_10mg	.	.	0.63 [0.26; 1.56]	.	0.63 [0.26; 1.51]	0.63 [0.26; 1.51]	.	0.47 [0.21; 1.06]	.	0.42 [0.19; 0.91]
0.64 [0.16; 2.61]	0.80 [0.21; 2.96]	Adamgammadex_0.5mg	0.88 [0.29; 2.64]	1.56 [0.38; 6.35]	.	.	0.71 [0.24; 2.05]	.	.	0.60 [0.22; 1.65]	.
0.56 [0.19; 1.63]	0.70 [0.27; 1.79]	0.88 [0.29; 2.64]	Adamgammadex_24mg	1.78 [0.61; 5.16]	.	.	0.81 [0.47; 1.39]	.	.	0.68 [0.44; 1.05]	.
0.53 [0.17; 1.61]	0.65 [0.30; 1.43]	0.82 [0.26; 2.61]	0.93 [0.46; 1.90]	Adamgammadex_4mg	1.20 [0.51; 2.83]	1.00 [0.43; 2.31]	0.93 [0.55; 1.58]	.	0.81 [0.49; 1.33]	0.38 [0.15; 1.02]	0.72 [0.45; 1.14]
0.54 [0.15; 2.00]	0.67 [0.24; 1.85]	0.84 [0.22; 3.21]	0.96 [0.36; 2.55]	1.03 [0.46; 2.29]	Placebo	.	1.25 [0.45; 3.49]	.	0.71 [0.32; 1.59]	.	0.62 [0.29; 1.34]
0.51 [0.15; 1.75]	0.63 [0.26; 1.51]	0.79 [0.22; 2.81]	0.90 [0.38; 2.17]	0.97 [0.48; 1.95]	0.94 [0.36; 2.43]	Sugammadex_4mg	1.00 [0.45; 2.23]	0.89 [0.74; 1.08]	0.75 [0.36; 1.56]	.	0.67 [0.33; 1.33]
0.49 [0.18; 1.36]	0.61 [0.27; 1.35]	0.76 [0.26; 2.20]	0.87 [0.51; 1.49]	0.93 [0.55; 1.57]	0.90 [0.39; 2.08]	0.96 [0.47; 1.97]	Adamgammadex_8mg	.	0.67 [0.38; 1.17]	0.85 [0.61; 1.17]	0.59 [0.35; 1.00]
0.45 [0.13; 1.58]	0.56 [0.23; 1.37]	0.71 [0.20; 2.55]	0.81 [0.33; 1.98]	0.86 [0.42; 1.79]	0.84 [0.32; 2.21]	0.89 [0.74; 1.08]	0.93 [0.44; 1.94]	Adamgammadex_9mg	.	.	.
0.38 [0.12; 1.19]	0.48 [0.23; 1.01]	0.60 [0.19; 1.93]	0.68 [0.33; 1.41]	0.73 [0.45; 1.18]	0.71 [0.33; 1.53]	0.76 [0.39; 1.48]	0.79 [0.47; 1.32]	0.85 [0.42; 1.70]	Adamgammadex_6mg	.	0.88 [0.60; 1.30]
0.38 [0.15; 1.02]	0.48 [0.21; 1.10]	0.60 [0.22; 1.65]	0.68 [0.44; 1.05]	0.73 [0.42; 1.28]	0.71 [0.30; 1.70]	0.76 [0.35; 1.62]	0.79 [0.57; 1.08]	0.85 [0.39; 1.86]	1.00 [0.56; 1.79]	Adamgammadex_32mg	.
0.34 [0.11; 1.04]	0.42 [0.20; 0.88]	0.53 [0.17; 1.67]	0.60 [0.30; 1.22]	0.64 [0.41; 1.00]	0.63 [0.30; 1.32]	0.67 [0.35; 1.28]	0.69 [0.42; 1.13]	0.75 [0.38; 1.47]	0.88 [0.60; 1.30]	0.88 [0.50; 1.53]	Adamgammadex_2mg

REVIEW PAPER

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Does a healthy lifestyle reduce the risk of obesity in type 1 diabetes?

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ABSTRACT

A healthy lifestyle is recommended for every person with type 1 diabetes. Unfortunately, the incidence of type 1 diabetes is steadily increasing. Many studies confirm that maintaining a balanced diet or incorporating physical activity helps to maintain good health over the long term. It helps to balance the diabetes metabolically and to prevent the development of excessive body weight. Making appropriate lifestyle modifications, as recommended by researchers and associations, will undoubtedly help with this. This study aims to analyse the impact of a healthy lifestyle, including a balanced diet, sleep hygiene, psychological conditions, insulin therapy and physical activity, on metabolic control and the prevention of excess body weight in patients with type 1 diabetes. The introduction and alignment of the described components in patients with type 1 diabetes contribute to better metabolic control of the disease and reduce the risk of excessive body weight.

Introduction

Since 1975, the global prevalence of obesity has almost tripled, and in 2019, it was estimated that comorbidities such as diabetes contributed to five million deaths [1]. Since 1980, the incidence of diabetes has quadrupled, becoming one of the leading causes of premature death. In the US, type 1 diabetes accounts for approximately 5.6% of all adult-onset diabetes cases. Although historically associated with thin people, type 1 diabetes is now shaped by factors beyond autoimmunity. The number of people with type 1 diabetes is projected to increase globally from 3.7 million in 2021 to 13.5–17.4 million in 2040. In the context of dynamic socio-economic and nutritional changes, accelerated pancreatic β-cell damage due to obesity is becoming increasingly apparent [2]. Everyone's lifestyle largely determines their health.

Changing lifestyle and, most importantly, aspects of it, such as diet, physical activity, sleep quality and mental state, is the first and most crucial step in the management of diabetes [3]. Unfortunately, excessive body weight, defined as overweight or obesity, is increasingly seen in

people with type 1 diabetes [4]. The present work is a narrative review. Search engines such as PubMed, Google Scholar, and Wiley Online Library for the period June 2024 – August 2024) were analysed. The search terms used were mainly: „diabetes type 1", „obesity", „overweight" and the search terms respectively under the search terms: „diet", „physical activity", „sleep", „psychological condition". The results of studies indicate that people, either with type 1 diabetes or obesity, do not engage in health behaviour more intensively than healthy individuals.

Furthermore, a strong need for social approval was associated with the level of health behaviour in the study groups, which may suggest that the already average level of health behaviour is due to a desire to present oneself in a more positive light [3]. Nevertheless, special attention should be paid to the educational aspect so that an increasing number of type 1 diabetes recipients take care not only of metabolic compensation but also of their body weight. A healthy lifestyle can help to maintain a healthy body weight and reduce the risk of obesity. This study aims to analyse the impact of a healthy lifestyle, including a balanced diet, sleep hygiene, psychological conditions, insulin therapy and physical activity, on metabolic control and prevention of excess body weight in patients with type 1 diabetes. These factors will be discussed in this study.

Balanced diet

The word 'diet' derives from Classical Greek and means 'diaita' or 'lifestyle'. Already at this stage, it can be deduced that it is one of the most important elements of a healthy lifestyle, i.e., one that does not negatively affect the body's functions. Diet is primarily about nutrition, which plays a key role in the therapeutic process and also reduces the risk of obesity. Every patient should take care to control metabolic alignment through lifestyle changes, including eating habits. The challenge is to develop an appropriate plan to support individuals to make lifestyle changes [5]. Adherence to recommended dietary recommendations can improve tissue insulin sensitivity and glycaemic control, thereby improving lifestyle and quality of life. Unfortunately, it appears that adherence to a rational pattern of proper nutrition is probably one of the most challenging elements of diabetes management [6]. It appears that excessive body weight, until recently mainly associated with type 2 diabetes, is increasingly observed in people with type 1 diabetes. DuBose et al. evaluated a population of children and adolescents with type 1 diabetes from countries such as Germany, Austria, and the USA. The study group had a higher body mass index (BMI) than reference values. Twelve per cent were diagnosed as obese and 24 per cent were overweight [7]. On the other hand, Minges et al., in their study, confirm that BMI values above the norms for normal weight occur in up to one in three children diagnosed with type 1 diabetes [8]. At this point, it should be emphasised that there is no single dietary pattern that all people with type 1 diabetes should follow. Each patient should have an individualised nutrition plan. Of course, it is recommended to emphasise the importance of eating raw vegetables, choosing whole grain products and eliminating the consumption of refined sugar. According to the American Diabetes Association, individualisation of dietary recommendations is recommended to take into account metabolic status, clinical goals, personal preferences, and socio-cultural considerations [9]. Individuals with type 1 diabetes and coexisting obesity may benefit from diet plans with reduced calories, lower total carbohydrate and glycaemic index, and higher fibre and lean protein [10]. Although low-carbohydrate (<130 g carbohydrate/day) and ketogenic (<55 g carbohydrate/day) diets are becoming increasingly popular and advertised as the gold standard in weight-loss therapy, there is limited evidence for their use in type 1 diabetes, as some concerns have been raised mainly about the risk of hypoglycaemia and ketoacidosis [11]. The ketogenic diet is a way of eating characterised not only by a low carbohydrate intake, but also by an increased intake of fats and proteins. Due to the low carbohydrate intake, the body has to change and find new sources for peripheral tissues and the brain, leading to the breakdown of fatty acids in the liver and the formation of ketone bodies, which is unfavourable for diabetics [12]. The ACTION research group evaluated in young adults with type 1 diabetes and overweight or obesity the effects of a hypocaloric low-carbohydrate diet, a hypocaloric low-fat diet and a Mediterranean diet without calorie restriction

on body weight and glycaemia. A three-month diet, regardless of macronutrient distribution or calorie restriction, resulted in weight loss while improving or maintaining HbA1c levels without increasing the risk of hypoglycaemia in adults with type 1 diabetes [13]. Although the Mediterranean diet is considered the 'gold standard' for dietary patterns, there is insufficient evidence for its effectiveness in type 1 diabetes [14]. A study by Mottalib et al. involving patients with type 1 diabetes and metabolic syndrome compared the effects of a Mediterranean diet without calorie restriction with a low-fat diet. The results proved to be similarly beneficial in terms of waist circumference, anthropometric, and metabolic outcomes in both trials [14]. It should be particularly noted that the above studies mention the Mediterranean diet as a diet without caloric restriction, which is significantly needed in the development of excessive body weight as well as in the maintenance of normovolemia. There is evidence to suggest that the Palaeolithic diet has a positive effect on monitoring glucose homeostasis, but other studies have not confirmed these results. This diet includes eating vegetables, fruit, meat, fish, eggs, and nuts, and avoiding dairy products, oils, and legumes. It is often classified as a low-carbohydrate diet, low in sodium and rich in fat, potassium and antioxidants. However, the paleo diet and the diet recommended by the American Diabetes Association share similar effects. The Palaeolithic diet can affect the body and the organism, especially with type 2 diabetes, as it affects HbA1c values or anthropometric parameters. However, the results of recent studies evaluating the effect of the Palaeolithic diet on glucose homeostasis are inconclusive. Therefore, well-designed long-term studies are needed to confirm the efficacy of the Palaeolithic diet in diabetic patients, especially in type 1 diabetes [15]. According to the recommendations of the Diabetes Poland (PTD), the proportion of carbohydrates in the diet should be about 45% of daily energy requirements, fats 25–40%, and protein 15–20% [16]. Adequate carbohydrate intake in the patient's diet is one of the first steps towards a rational diet, which can prevent excessive weight gain in the future. Healthy eating habits can include refrigerating starchy products after cooking, as this results in the retrogradation of starch, which becomes an unabsorbable product in the human digestive tract due to the reduction in available carbohydrates. When starch is cooled, amylose molecules and long amylopectin chains form double helices and lose their ability to bind water. The crystallised form of starch may be resistant to enzymatic degradation in the small intestine, thus reducing the digestible starch concentration in cooked starch products [17,18]. A Study by Stróżyk et al. confirms the relevance of the occurrence of such a phenomenon. Consumption of rice that has undergone a cooling process has resulted in a lower increase in postprandial blood glucose levels in people with type 1 diabetes [19]. The phenomenon may be beneficial for people with diabetes, as the conversion of starch into an unabsorbable form may contribute to lower postprandial glucose values and less glycaemic variability. In addition, the presence of resistant starch lowers the glycaemic index of a given meal. It is the diet based on the principles of a low glycaemic index (GI) that is considered the most beneficial for health and is not only recommended for diabetic patients. People with type 1 diabetes who eat rationally and follow a low glycaemic index diet have better glycaemic control. In addition, a low GI diet may have a beneficial effect on lipid concentrations [20]. Higher BMI in people with type 1 diabetes has also been shown to be directly associated with higher LDL-C and non-HDL-C values [21]. A healthy lifestyle also includes regularity of meal intake. Studies indicate that adolescents who did not eat breakfast compared to peers who ate breakfast daily had a higher risk of excessive body weight. Interestingly, a similar phenomenon was observed about dinner consumption. Those who ate dinner regularly throughout the week had a lower risk of being overweight than those who ate dinner irregularly [22,23]. Unfortunately, unhealthy and potentially dangerous practices of wanting to balance body weight or following widely available fad diets, such as skipping insulin doses, excessive fasting, stimulating vomiting, and using laxatives in type 1 diabetes, are common strategies [24]. A study by Lawrence et al. found that among adolescents who had ever tried to lose weight, healthy weight loss practices, i.e., diet and exercise, were the most common. In contrast, unhealthy practices (fasting, use of diet aids, vomiting or use of laxatives, and skipping insulin doses) were less common. All unhealthy weight

loss practices, except fasting, were more common in women than in men. Diets, fasting and use of dietary aids were more common in adolescents with type 2 diabetes than in those with type 1 diabetes [24]. Unhealthy weight loss practices are more common in overweight or obese women and may be influenced by depression and their perception of their body shape [25]. Analysing the studies, it is clear that there is insufficient data to show an association between carbohydrate-restricted diets and ketogenic diets with weight gain or loss among people with type 1 diabetes. Nutrition education by specialised treatment teams is important to enable weight reduction with metabolic compensation.

Physical activity

High levels of sedentary lifestyles and low levels of physical activity are associated with the development of obesity, with an increased risk of premature mortality and the development of some chronic diseases. Incorporating moderate to high-intensity physical activity can reduce the mortality risk associated with excessive sedentary lifestyles. Understanding the relationship of these behaviours can help practitioners determine whether to prioritise interventions targeting sedentary lifestyle, physical activity or both components [26]. The introduction of physical activity is important for weight reduction not only in healthy people, but also among individuals with type 1 diabetes, who are often overweight [27]. The most important thing is to change the lifestyle, and this cannot be done without including physical activity. Regular physical exercise is a key element in the prevention and treatment of obesity. In people with type 1 diabetes, participation in sports was a barrier in the past; today, the opposite is true. A typical barrier to weight control in type 1 diabetes is a reluctance to engage in exercise for fear of hypoglycaemia, which may occur during, after or overnight after exercise [28]. Adequate patient education regarding insulin dosing and carbohydrate adjustment to maintain stable blood glucose levels during exercise should alleviate patients' fear of hypoglycaemia [29]. Unfortunately, the fear of a sudden drop in blood glucose during exercise can lead to avoidance of exercise, which negatively affects overall health. Among the causes of fear of hypoglycaemia, we can include the experience of hypoglycaemic episodes in the past and the fear of their recurrence, or unfamiliarity with how to prevent and manage hypoglycaemia during exercise. However, there are strategies to cope with the fear of hypoglycaemia, such as appropriate education on diet, glycaemic monitoring, adjusting the intensity and type of exercise to individual needs and abilities, and psychological support. Regular exercise in people with type 1 diabetes not only prevents the development of excessive body weight, but also improves overall fitness, and allows, with skilful insulin adjustment, to improve glycaemic outcomes [30]. The positive factors following the introduction of physical activity are many more. Regular physical activity leading to weight loss has a positive effect on the cardiovascular system, reducing visceral adipose tissue [31]. It is essential to have good glucose control before exercising, as both too low and too high values can lead to serious health consequences. If glucose levels are too low, there is a risk of hypoglycaemia, which can result in weakness, dizziness and even unconsciousness. In the case of hyperglycaemia, the body may not be able to use glucose effectively as an energy source, leading to increased fat burning and production of ketone bodies. In extreme cases, this can lead to ketoacidosis – a life-threatening condition that manifests as dehydration, abdominal pain, nausea and confusion. This is why it is a good idea to monitor your glycaemia before training and balance it appropriately to make exercise safe and effective. A group of people with type 1 diabetes are at risk of high blood pressure, triacylglycerol and LDL cholesterol levels and low HDL levels. These factors are associated with an increased risk of vascular disease [32]. In some cases, there is a reduction in apolipoprotein B, which is proatherogenic and associated with premature mortality in type 1 diabetes [33]. Physical activity also increases levels of the anti-atherogenic apolipoprotein. The consensus in research is that these benefits are independent of changes in glycaemic control and body weight, and that they are most pronounced in individuals with an unfavourable lipid profile [34]. Unfortunately, with the development of overweight and obesity comes the development of lipid disorders and other cardiovascular diseases, which is why

it is so important to incorporate physical activity in patients with excess body weight and type 1 diabetes to prevent all these adverse complications, including microvascular complications [35]. For microvascular complications, it is important to note that studies confirm that there is a correlation between the presence of complications impairing the ability to undertake physical activity and not physical activity reducing diabetes complications [36]. Physical activity and exercise recommendations in people with type 1 diabetes and coexisting obesity should be tailored to the specific needs of the individual, including the challenges of blood glucose control during exercise and the presence of diabetes-related complications [28]. Given the prevalence of overweight and obesity among adolescents with type 1 or type 2 diabetes, health professionals caring for adolescents with diabetes need to pay particular attention to prevention [24]. In their study, Semiz et al. evaluated the effectiveness of a diabetes camp for adolescents diagnosed with type 1 diabetes to balance insulin dose with activity level and diet. Using a pretest-posttest design without a comparison group, the intervention delivered during the 10-day camp used social physical activity and food education programmes. Twenty-eight adolescents participated in the study, eight of whom were overweight or obese. The average weight loss was 0.9 kg after the first 10 days of the camp [37]. However, weight loss can be achieved with regular exercise, especially if there is a small reduction in total daily insulin doses [38]. Regular exercise facilitates weight loss, thereby increasing insulin sensitivity, which in turn helps to maintain normal blood glucose levels [31]. It is important to appropriately adjust the duration and type of physical exercise so that it is most beneficial in every measure. According to Diabetes Poland, people with type 1 diabetes without established clinically significant chronic diabetic complications can undertake any type of physical exercise, including maximum intensity. Obese people with diagnosed type 1 diabetes are recommended to do 200–300 minutes of exercise per week, leading to an energy deficit of 500–750 kcal/day [16]. To achieve the expected results in terms of weight reduction and metabolic control of diabetes, key factors such as the type of physical activity (aerobic vs. resistance or mixed), preprandial status (fasting vs. non-fasting activity), active insulin levels, blood glucose levels at the start of the activity, glucose trends preceding the activity, the composition of the last meal or snack, and the intensity and duration of the activity should be considered [39]. Physical activity is selected individually according to the patient's needs and capabilities. This can be summarised as walking is good for everyone, but sport is good for a select few. Exercise can benefit everyone, regardless of condition. Physical activity should be varied, not according to the type of diabetes, but according to the age and physical strength of the patient, the anti-diabetic treatment used, and the presence of diabetes and related diseases.

Insulin therapy

Insulin therapy is a key component of the treatment of type 1 diabetes. It improves glycaemic control, but can also lead to weight gain. Different insulin regimes, which can include multiple insulin injections, insulin pump therapy and the use of varying insulin analogues, can affect this aspect in various ways. Studies analysing the impact of continuous subcutaneous insulin infusion compared to multiple injections show differences in their effect on body weight, namely better glycaemic control and less weight gain. A meta-analysis of studies showed that the use of insulin pumps is associated with more stable glucose levels and a reduced risk of hypoglycaemia. Intensive insulin therapy can lead to weight gain, with some studies suggesting that insulin pumps may mitigate this effect by adjusting the insulin dose more precisely to the body's needs (40). Contemporary research indicates that the choice of insulin type can affect a patient's body weight. For example, some basal insulins, such as insulin degludec, may be associated with less weight gain compared to other long-acting insulins. A meta-analysis of randomised clinical trials showed that insulin degludec may be associated with less weight gain compared to insulin glargine U100. This mechanism may be due to the more stable action of insulin degludec, which reduces the risk of hypoglycaemia and thus the need for additional calorie intake. Studies on the faster-acting insulin FIASP indicate its potential benefit in controlling glucose levels without excessive impact on body weight, although its long-term

impact requires further study (41,42). Considering glycaemic monitoring, regularly checking blood glucose levels allows insulin doses to be adjusted and hypoglycaemia to be avoided, which can reduce the need for additional calorie intake. Modern insulin regimes, such as insulin pumps and new insulin analogues, can help to improve glycaemic control and reduce weight gain. However, therapy must be appropriately tailored to the individual patient's needs, taking into account nutritional education and physical activity.

Sleep hygiene

Sleep is part of a healthy lifestyle, as important as a balanced diet and physical activity. Although everyone devotes roughly a third of their life to it, the importance of sleep is often ignored. According to the National Sleep Foundation, the optimal amount of sleep for an adult is between seven and nine hours per night. Sufficient and uninterrupted sleep allows people to rest, concentrate and be productive. It also affects health. In addition, insufficient sleep can affect glycaemic control in adolescents with type 1 diabetes [43]. Evidence is rapidly accumulating indicating that chronic partial sleep loss may increase the risk of obesity and diabetes. Laboratory studies in healthy volunteers have shown that experimental sleep restriction is associated with adverse effects on glucose homeostasis. Insulin sensitivity decreases rapidly and significantly without adequate compensation for beta-cell function, resulting in an increased risk of diabetes. Prospective epidemiological studies conducted on both children and adults are consistent with a causal role of short sleep in increased diabetes risk. Sleep restriction is also associated with dysregulation of neuroendocrine control of appetite, with a decrease in the satiety factor, leptin, and an increase in the hunger-stimulating hormone, ghrelin. Sleep loss may therefore alter the ability of leptin and ghrelin to accurately signal caloric requirements, acting together to produce an internal misperception of insufficient energy availability [44].

Overweight and obesity in adolescents with type 1 diabetes are now increasingly common and are associated with health consequences. Overweight in adolescents diagnosed with type 1 diabetes is associated with infrequent naps, longer time spent in front of a screen and skipping breakfast and dinner [45]. Regarding the first factor, some studies support this theory. Estrada et al. examined the sleep process to determine its relationship with obesity, diabetes and insulin resistance. Using a patient self-report questionnaire, the authors found that regular naps were significantly and inversely associated with overweight, including high BMI, high body fat percentage and greater waist circumference. A lower prevalence of obesity was associated with regular naps in adolescents. The high prevalence of insufficient sleep in young people with type 1 diabetes and their relatives detected in this study may have significant health implications [46]. Diet therapy remains an essential component of behavioural treatment.

Psychological conditioning

The World Health Organisation (WHO) psychological dimension is one of the core areas that concern quality of life. It concerns both positive and negative feelings. Unfortunately, the prevalence of depression is three times higher in people with type 1 diabetes compared to the general population [47]. Psychological assessment and cognitive-behavioural therapy, including the setting of specific, achievable and relevant goals, as well as self-monitoring of food intake and exercise, and education, should be included in the routine clinical management of obesity in type 1 diabetes [48]. Many authors also note the presence of reduced tissue sensitivity to insulin during adolescence. This can, in a person with type 1 diabetes, lead to uncontrolled increases in insulin doses, resulting in significant weight gain, one of the most critical risk factors for the development of abnormal eating behaviour [49,50]. Jones et al. found that skipping insulin doses was the most common weight loss strategy used by patients [51]. Uncontrolled appetite resulting from hypoglycaemic episodes and inappropriate insulin dosing is common in young people with diabetes [52]. Other risk factors related to the nature of the disease include frequent weight control, high concentration of food (especially carbohydrates), dietary restrictions or accurate calculation of carbohydrate content in meals [53]. In addition to socio-cultural factors, psychological behaviours make people with type 1 diabetes just as prone to

inappropriate eating behaviours as healthy individuals. These are related to the proper management of the disease, in which attention to diet and body is an integral part of treatment [54,55].

Conclusions

- Overweight and obesity are growing problems among people with type 1 diabetes, which can negatively affect the course of the disease and increase the risk of complications.
- A key element in the prevention of excess body weight is patient education, which should be implemented as early as the diagnosis of diabetes.
- A healthy lifestyle, including an appropriate diet, regular physical activity, quality of sleep and a good psychological approach, significantly improves the quality of life of patients.
- The effectiveness of preventive and therapeutic interventions depends on complete awareness of the patient and consistent adherence to the recommendations of the treatment team.

Recommendations for clinical practice:

- Early patient education – educational programmes on healthy lifestyles should be implemented as early as the diagnosis of type 1 diabetes.
- Individualisation of recommendations – dietary strategies and physical activity plans should be tailored to the patient's age, lifestyle and preferences.
- Continuous weight monitoring – regular monitoring of weight and metabolic parameters should be an integral part of diabetes care.
- Support from a multidisciplinary team – the patient should have access to diabetes educators, dietitians and physical activity specialists to help maintain a healthy lifestyle.
- Patient motivation and engagement are essential to building patient awareness of the long-term benefits of adhering to health recommendations.

Summary

There is a growing problem of overweight and obesity in people with type 1 diabetes. Preventive measures, consisting mainly of education, should be implemented at the stage of diagnosis of type 1 diabetes. Every person with type 1 diabetes, at any stage of the disease, should make a special effort to maintain a normal weight. A healthy lifestyle does not lead to a full recovery of type 1 diabetes, but it can provide a comfortable, long and fulfilling life for the patient. A healthy lifestyle can improve metabolic control and reduce the risk of complications, but it will not restore pancreatic function or cause beta-cell regeneration on its own. Curing type 1 diabetes would require stopping the autoimmune process and restoring insulin production, which is not currently possible with available treatments. However, it requires full awareness and adherence to the recommendations made by the treatment team. All the factors listed are closely related and closely associated with weight among people with type 1 diabetes. The most substantial support in the literature is, of course, diet and physical activity, but there is a compelling need to study new things. Nevertheless, the long-term effects of specific diets, the optimisation of physical activity programmes and the effectiveness of psychological interventions require further research.

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REVIEW PAPER

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Chemokines and inflammation in COPD: implications for targeted therapy

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ABSTRACT

A severe global health issue, chronic obstructive pulmonary disease (COPD) is characterised by recurrent respiratory symptoms and restricted breathing because of long-term lung inflammation. A class of minuscule cytokines known as chemokines is essential for immune cell recruitment and activation, which sustains the inflammatory response in COPD. This study thoroughly examines the origins, modes of action and effects of chemokines on developing COPD-related inflammation. We detail the involvement of key chemokines, such as CXCL9, CXCL10, and CXCL11, in COPD pathophysiology. These chemokines are integral in attracting neutrophils, macrophages, and T lymphocytes to the lungs, leading to chronic inflammation, airway remodelling, and emphysema. Increased levels of these chemokines correlate with increased disease severity and frequency of exacerbations. The review additionally examines the possibility of using chemokine pathway targeting as a treatment approach. Current COPD treatments primarily address symptoms without adequately controlling underlying inflammation. By inhibiting chemokine signalling, it may be possible to reduce inflammation, slow disease progression, and improve patient outcomes. We discuss various therapeutic approaches, including developing chemokine receptor inhibitors, biologics such as monoclonal antibodies, drug repurposing, and combination therapies with existing treatments.

Furthermore, we review ongoing and completed clinical trials investigating chemokine-targeted therapies in COPD, highlighting their efficacy and safety. This review also emphasises the need for further research to optimise these therapies and identify biomarkers for monitoring treatment response. In conclusion, chemokines are pivotal in the inflammatory processes of COPD. Targeting chemokine pathways presents a promising avenue for developing more effective treatments, which could significantly enhance patient care and disease management.

Introduction

A prevalent and chronic respiratory condition known as chronic obstructive pulmonary disease (COPD) is marked by continuous airflow restriction and long-term respiratory symptoms such as coughing up phlegm, producing mucus, and dyspnea. It mainly results from prolonged exposure

to toxic gases and particles, mostly from tobacco smoking, but also from occupational and environmental contaminants. COPD encompasses chronic bronchitis and emphysema, conditions that often coexist and lead to significant lung damage and impaired pulmonary function [1]. The impact of COPD extends beyond the respiratory system, affecting the overall quality of life and daily functioning of individuals. It is a primary global source of morbidity and mortality, placing a significant strain on healthcare systems because of the need for long-term care, frequent hospital stays, and related comorbidities such as metabolic and cardiovascular illnesses. The progressive nature of COPD results in a gradual decline in lung function, increased risk of acute exacerbations, and a higher mortality rate, contributing to a significant socioeconomic burden [2]

Chronic inflammation is a significant contributing element to the pathophysiology of chronic obstructive pulmonary disease (COPD). Chronic exposure to gases and particles in the air, particularly cigarette smoke, inflames the airways and lung parenchyma. This reaction is characterised by the activation and recruitment of several immune cells, including neutrophils, T lymphocytes, and macrophages, which release inflammatory mediators, including chemokines and cytokines [2]. These mediators cause continuous tissue remodelling and injury by sustaining the inflammatory cycle. Airflow restriction and poor gas exchange are caused by structural alterations to the lung tissue and airways brought on by the chronic inflammatory process associated with COPD. These changes include fibrosis, mucus hypersecretion, and the breakdown of alveolar walls. Prolonged inflammation not only causes damage to lung tissue but also seeps into the bloodstream, exacerbating coexisting illnesses, including muscular atrophy and cardiovascular disease [3]. Comprehending the pivotal function of inflammation in the pathophysiology of COPD is imperative to discern therapeutic targets capable of modulating the inflammatory response, diminishing tissue damage, and ameliorating patient outcomes.

Chemokines are micro-signalling proteins that are essential for controlling inflammation and the immune system. In reaction to inflammatory stimuli like infection or tissue damage, leukocytes, endothelial cells, and fibroblasts are among the cell types that release them. By attaching to particular receptors on immune cell surfaces and guiding them toward areas of infection or inflammation, chemokines control the movement and activation of immune cells. Leukocyte recruitment to the site of damage is crucial for the removal of pathogens and the start of tissue repair processes [4]. Besides their function in immune cell trafficking, chemokines also influence leukocyte activation, adhesion, and survival in the inflammatory response. They facilitate the extravasation of immune cells from the bloodstream into the tissue by controlling the contacts between the immune cells and the endothelium. Additionally, pro-inflammatory cytokines and other mediators can be released in response to chemokines, intensifying the inflammatory response and encouraging tissue damage [5].

This study aims to clarify how chemokines contribute to inflammation associated with COPD and to discuss the possibility that targeted chemokine medicinal products may help COPD patients achieve better treatment outcomes. This review also examines the processes by which chemokines contribute to inflammation in COPD by a thorough analysis of recent research findings, including their role in immune cell recruitment, tissue damage, and disease progression [6]. The evaluation also assesses the safety and effectiveness of targeted chemokine treatments in preclinical and clinical settings, providing information about their potential as cutting-edge COPD treatment modalities.

Role of chemokines in COPD inflammation

Key chemokines involved in COPD (e.g., CXCL9, CXCL10, CXCL11)

A group of miniature signalling proteins known as chemokines is involved in directing immune cells to areas of inflammation or injury, where they take part in host defence, tissue repair, and immune surveillance. Chemokines regulate the migration, activation, and adherence of target cells by binding to specific receptors on their surface. Numerous chemokines are significant mediators of inflammation in the lung parenchyma and airways in the context of Chronic Obstructive Pulmonary Disease (COPD). It has been determined

that CXCL9, CXCL10, and CXCL11 play a significant role in the pathogenesis of COPD [7]. These chemokines, which belong to the CXC chemokine subfamily, are connected by the CXCR3 receptor, which is expressed on a range of immune cells, including natural killer cells, T lymphocytes, and dendritic cells.

When triggered with interferon-gamma (IFN-γ), activated macrophages and dendritic cells are the primary producers of CXCL9, also known as monokine produced by interferon-gamma (MIG). CXCL9 functions as a chemoattractant for T lymphocytes, namely T helper 1 (Th1) cells, which express CXCR3 at high levels. Patients with COPD have elevated CXCL9 levels in their lung tissue and airways, and these levels are linked to the severity of the condition and airflow restriction [8]. Th1 cells are drawn to the lungs by CXCL9, where they produce pro-inflammatory cytokines and cause tissue damage and persistent inflammation. Moreover, CXCL9 signalling has been implicated in airway remodelling by promoting fibroblast proliferation and extracellular matrix deposition, which exacerbates lung function decline in COPD. Studies have shown that CXCL9 expression in bronchial epithelial cells is significantly upregulated in response to oxidative stress and cigarette smoke exposure, both key environmental triggers in COPD pathogenesis. This suggests a feedback loop in which chronic exposure to irritants perpetuates inflammation through CXCL9-mediated immune recruitment and tissue remodelling [51].

Comparably, IFN-γ induces the production of CXCL10, sometimes referred to as interferon-gamma-induced protein 10 (IP-10), which is produced by a variety of cell types, including dendritic cells, macrophages, and epithelial cells. Th1 cells, natural killer cells, and monocytes are all drawn to inflammatory areas by CXCL10, which functions as a chemoattractant [9]. Increased amounts of CXCL10 are found in the airways and lung tissue of COPD patients. This protein is involved in immune cell recruitment and activation, which intensifies inflammation and tissue damage. Recent evidence suggests that CXCL10 contributes to COPD exacerbations by amplifying neutrophilic inflammation. Unlike CXCL9, which primarily attracts T cells, CXCL10 has been found to promote neutrophil chemotaxis via CXCR3-dependent and independent pathways. This is particularly relevant in acute exacerbations of COPD (AECOPD), where neutrophilic inflammation dominates, leading to increased mucus hypersecretion, airway obstruction, and bacterial colonisation. Targeting CXCL10 with neutralising antibodies has been proposed as a strategy to mitigate AECOPD severity, although clinical trials are still in early stages [52].

Similar to CXCL9 and CXCL10, CXCL11, also known as interferon-inducible T cell alpha chemoattractant (I-TAC), has a similar structure and function. It is initiated by multiple cell types, including endothelial, dendritic, and macrophage cells, and is triggered by IFN-γ. **Table 1** provides a comparative summary of the roles of CXCL9, CXCL10, and CXCL11 in COPD pathogenesis, highlighting their cellular sources, receptors, immune targets, and pathogenic contributions. CXCL11 exhibits a higher binding affinity for CXCR3 compared to CXCL9 and CXCL10, making it a potent chemoattractant for activated T cells in COPD. In addition to its role in Th1-mediated inflammation, CXCL11 has been linked to fibrosis

Table 1. Key differences between CXCL9, CXCL10, and CXCL11 in COPD pathogenesis.

Chemokine	Alternative name	Primary cellular sources	Primary receptor	Key immune cell targets	Pathogenic roles in COPD
CXCL9	Monokine Induced by IFN-γ (MIG)	Macrophages, dendritic cells, epithelial cells	CXCR3	Th1 cells, NK cells	T-cell recruitment, chronic inflammation, airway remodeling
CXCL10	Interferon-Induced Protein 10 (IP-10)	Macrophages, dendritic cells, epithelial cells, fibroblasts	CXCR3	Th1 cells, NK cells, monocytes, neutrophils	Amplifies neutrophilic inflammation, contributes to AECOPD, promotes mucus hypersecretion
CXCL11	Interferon-Inducible T Cell Alpha Chemoattractant (I-TAC)	Endothelial cells, macrophages, dendritic cells	CXCR3	Th1 cells, NK cells, fibroblasts	Enhances T-cell activation, promotes fibrosis, contributes to lung function decline

in COPD by enhancing myofibroblast differentiation and collagen deposition. This dual role in both inflammation and tissue remodelling suggests that CXCL11 inhibition could be a promising approach for reducing lung function decline in COPD patients [53].

Impact of chemokines on chronic inflammation and lung tissue damage

Chemokines have a significant role in the pathophysiology of several inflammatory lung diseases, including pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease (COPD). They have a substantial effect on lung tissue destruction and chronic inflammation. The persistent inflammation in the lungs is caused by the dysregulation of chemokine signalling pathways, which results in tissue damage, remodelling, and compromised lung function. Chemokines play a significant role in lung tissue damage and chronic inflammation by directing the recruitment and activation of immune cells in the lung microenvironment [10]. Chemokines are chemoattractants that guide immune cells, including dendritic cells, neutrophils, macrophages, and T lymphocytes, to regions of inflammation or injury. Once enlisted, these immune cells release pro-inflammatory cytokines, chemotactic factors, and reactive oxygen species, which further damage tissue and initiate the inflammatory cascade. [11].

Chemokines like CCL11 (eotaxin) and CCL5 (regulated on activation, normal T cell produced and released, RANTES), for instance, play a role in drawing eosinophils to the airways in asthma, where they exacerbate hyperresponsiveness and inflammation in the airways. Similar to this, in COPD, chemokines such as CXCL1 (growth-related oncogene-alpha) and CXCL8 (interleukin-8) are essential for attracting neutrophils to the lungs, where they produce proteases and reactive oxygen species, which cause tissue damage and emphysema [12]. Furthermore, by promoting angiogenesis, fibrosis, and airway remodelling, chemokines exacerbate chronic inflammation and lung tissue damage. Chemokines like B lymphocyte chemoattractant (CXCL13) and stromal cell-derived factor-1 (CXCL12) are involved in controlling the migration and activation of myofibroblasts and fibroblasts, leading to the deposition of extracellular matrix proteins and the formation of fibrotic lesions in the lungs. Furthermore, chemokines such as macrophage-derived CCL22 and pulmonary and activation-regulated CCL18 facilitate the recruitment of regulatory T cells and fibrocytes, which support tissue remodelling and fibrosis [13].

Chemokines have global impacts on lung tissue damage and chronic inflammation in addition to local tissue microenvironmental effects. Chemokines secreted by the lungs can reach the bloodstream, resulting in the development of extrapulmonary lung disease symptoms such as metabolic abnormalities, skeletal muscle dysfunction, and systemic inflammation. In patients with inflammatory lung disorders, systemic inflammation adds to the overall illness burden by exacerbating lung tissue destruction and impairing lung function. In brief, in inflammatory lung disorders, chemokines are essential mediators of lung tissue damage and persistent inflammation [14].

Chemokines' role in the pathophysiology of COPD

Vast role of CXCL9 in COPD

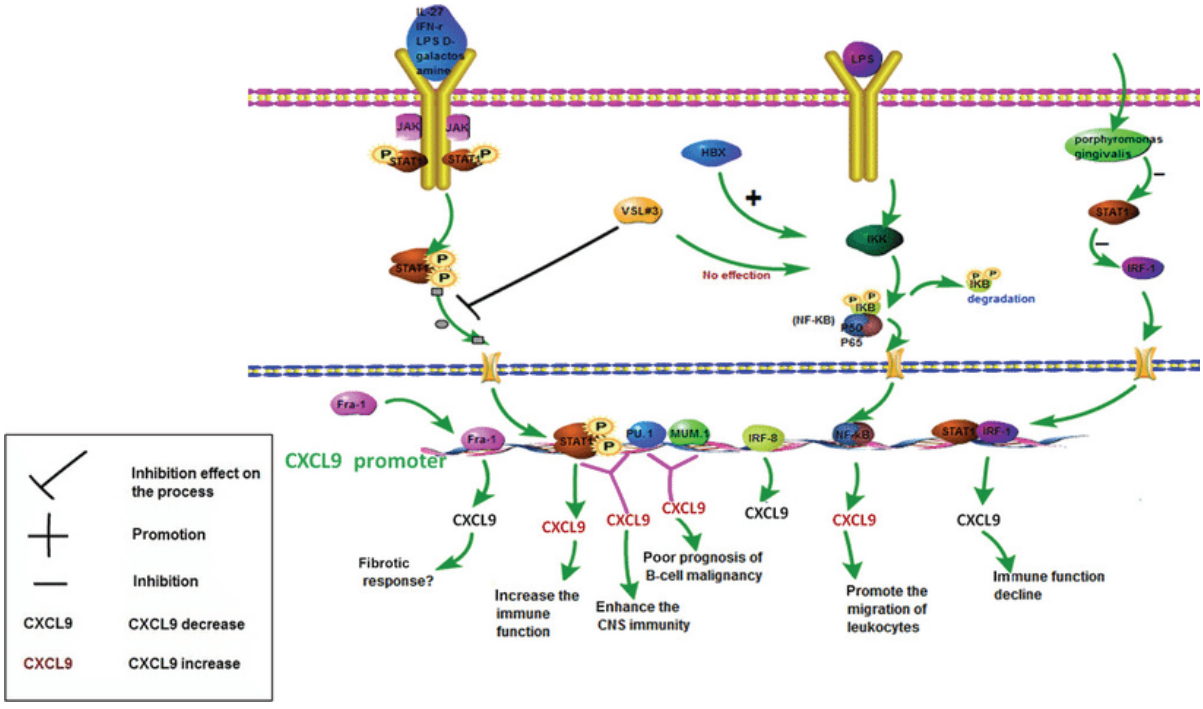
CXCL9, also known as monokine generated by interferon-gamma (MIG), is a chemokine that plays a significant role in the pathogenesis of COPD, or chronic obstructive pulmonary disease. Numerous cell types and inflammatory mediators are involved in tightly regulated mechanisms that produce and regulate it within the lung microenvironment. The primary mechanism that triggers the production of CXCL9 is interferon-gamma (IFN-γ), an essential pro-inflammatory cytokine released by natural killer cells and activated T lymphocytes in response to inflammation or infection. In response to IFN-γ activation, the lungs' macrophages, dendritic cells, and epithelial cells all increase the expression of CXCL9 mRNA and protein [9]. As a result, CXCL9 is released into the surrounding tissue milieu, where it chemoattracts immune cells bearing the expression of its receptor, CXCR3. The degree of inflammation, the presence of other cytokines and chemokines, and the state of immune cell activation in the lungs are a few of the factors that influence the regulation of CXCL9 production in COPD. Patients with COPD who have elevated levels of pro-inflamma-

tory cytokines such as TNF-α and interleukin-1 beta (IL-1β), as well as IFN-γ, are shown to produce more CXCL9 (see **Figure 1**). Moreover, oxidative stress and the activation of the nuclear factor-kappa B (NF-κB) pathway in response to inhaling cigarette smoke increase the production of CXCL9 in COPD [15].

When CXCL9 emerges, it attaches to its receptor, CXCR3, which is expressed on several immune cells, including natural killer cells, T lymphocytes, and dendritic cells, initiating its function. Intracellular signalling cascades triggered by CXCR3 activation are responsible for cell movement, activation, and effector activities. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K) are examples of these cascades. The interaction of CXCL9 and CXCR3 in COPD is critical for managing the lungs' recruitment and activation of immune cells (see **Figure 1**). For T lymphocytes that express CXCR3, especially T helper 1 (Th1) cells, CXCL9 acts as a chemoattractant. Th1 cells are implicated in the promotion of tissue damage and chronic inflammation in COPD patients [16].

Contribution of other chemokines to COPD progression

In addition to CXCL9, several other chemokines influence the course of Chronic Obstructive Pulmonary Disease (COPD). These chemokines have a crucial role in the recruitment and activation of various immune cells in the lungs, which results in the remodelling of airways, persistent inflammation, and the onset of emphysema. Key immune cells implicated in the pathophysiology of COPD include neutrophils, macrophages, and T lymphocytes. A variety of chemokines mediate their migration to the lungs. Interleukin-8, or CXCL8 (Figure 1), is a strong chemoattractant for neutrophils that is markedly increased in COPD patients' airways, which helps to draw and activate neutrophils in the lungs. Proteases, reactive oxygen species, and cytokines are released by neutrophils, which cause tissue damage, mucus hypersecretion, and airway blockage—all of which advance COPD [17].



IFN-γ, IL-27, D-galactosamine, and so on may all trigger CXCL9 expression via JAK/STAT1, PU.1, MUM1, NF-kB, Fra-1 (direct binding to the CXCL9 promoter), and Egr-1 (uncertain). Additionally, CXCL9 demonstrated a critical involvement in immunological function, including leukocyte, B-cell, and T-cell chemotaxis. JAK, Janus-activated kinase; PU.1, Myeloid Transcription Factor PU.1; MUM1, Multiple Myeloma Oncogene 1; Egr-1, Early Growth Response-1; Fra-1, Fos-related antigen 1; and STAT1, signal transducer and activator of transcription.

Figure 1. Role and Regulation of CXCL9 In COPD (Ding et al., 2016).

Another significant immune system cell group implicated in the pathogenesis of COPD is macrophages. The recruitment and activation of macrophages is caused by chemokines, such as CCL2 (monocyte chemoattractant protein-1) and CCL5 (regulated on activation, normal T cell generated and released, RANTES). Macrophages have a variety of functions in COPD, such as tissue remodelling, pro-inflammatory mediator synthesis, and phagocytosis of pathogens and debris. In COPD, dysregulated macrophage activation is a factor in tissue damage, persistent inflammation, and compromised lung function [18]. The development of emphysema in patients with COPD is linked to T lymphocytes, namely CD8+ cytotoxic T cells. Chemokines that control the recruitment and activation of these cells include CXCL10 (interferon-gamma-induced protein 10) and CXCL11 (interferon-inducible T cell alpha chemoattractant). The increase in airspace that is symptomatic of emphysema and the breach of the alveolar wall are caused by the cytotoxic and pro-inflammatory cytokines generated by CD8+ T cells [19].

Therapeutic targeting of chemokines in COPD

Rationale for targeting chemokines in COPD treatment

Chemokines play a critical role in regulating lung inflammation that is implicated in the pathogenesis of Chronic Obstructive Pulmonary Disease (COPD), which is why treating the condition with an emphasis on chemokines is relevant. Chemokines are essential for immune cell recruitment, activation, and trafficking in the lung microenvironment. They thereby exacerbate tissue damage, persistent inflammation, and diminished lung function in COPD patients. Chemokines are targeted in the therapy of COPD primarily because they are critical for immune cell recruitment to the lungs. As chemoattractants, chemokines guide immune cells to regions of inflammation or injury in the parenchyma and airways of the lung [20]. These cells include neutrophils, macrophages, dendritic cells, and T lymphocytes. These immune cells produce proteases and pro-inflammatory mediators, which cause tissue damage, excessive mucus secretion, and blockage of airways—all of which are factors in the pathophysiology of COPD [21].

Chemokine targeting can be used to modify immune cell trafficking and lower lung inflammation, which can slow down the course of COPD and improve clinical outcomes for patients. Targeting chemokines in COPD treatment has been approached from several angles, including gene therapy, monoclonal antibodies, and small-molecule inhibitors. One method that shows promise for addressing chemokine signalling pathways in COPD is the use of small-molecule inhibitors [22]. By specifically blocking the connection between chemokines and their receptors, these inhibitors stop immune cells from recruiting and activating in the lungs. Targeting chemokine receptors, such as CXCR2 and CXCR3, several small-molecule inhibitors have shown promise in preclinical studies and could be helpful in COPD treatment trials. Another potential method for treating COPD that targets chemokines is the use of monoclonal antibodies. By attaching themselves to particular chemokines or chemokine receptors, these antibodies prevent immune cells from interacting with them and obstruct subsequent signalling pathways. In preclinical models of COPD, for instance, monoclonal antibodies that target CXCL8 and its receptors have demonstrated effectiveness in lowering inflammation and enhancing lung function [23]. A promising strategy for regulating chemokine expression in COPD is gene therapy. Gene constructs expressing chemokine inhibitors or decoy receptors can be introduced into the lung to control chemokine levels locally and reduce lung parenchymal and airway inflammation. Gene therapy has the potential to deliver therapeutic molecules locally and sustainably, reducing systemic side effects and increasing treatment efficacy for COPD patients.

Overview of current therapeutic strategies for COPD

The hallmarks of Chronic Obstructive Pulmonary Disease (COPD), a chronic lung condition, are airflow limitation, persistent inflammation, and structural changes in the lung parenchyma and airways. Despite advances in our understanding of the biology of COPD, the disease still has a significant worldwide influence on public health and has few therapeutic choices. Reducing symptoms, decreasing the frequency of exacerba-

tions, improving quality of life, and delaying the progression of the condition are the objectives of current COPD treatment strategies. These strategies integrate non-pharmacological and pharmaceutical interventions that are tailored to each patient's needs in an interdisciplinary way [24]. Reduced airway inflammation, bronchospasm, and mucus hypersecretion are the main goals of pharmacological therapies for COPD because these conditions play a significant role in the onset and progression of the disease. Bronchodilators, such as beta2-agonists and anticholinergics, which lower smooth muscle tone and improve airflow dynamics in the airways to ease symptoms, are first-line therapy for COPD symptoms. Long-acting bronchodilators offer continuous bronchodilation when taken in conjunction with inhalation devices; these drugs are recommended for maintenance therapy in patients with COPD who have recurrent symptoms [25]. In addition to bronchodilators, anti-inflammatory drugs such as inhaled corticosteroids (ICS) are frequently used to treat COPD, especially in those with a history of exacerbations and eosinophilic inflammation. ICS reduce airway inflammation, lessens the likelihood of aggravation, and inhibits the production of pro-inflammatory cytokines and chemokines, all of which have anti-inflammatory effects, for COPD patients with moderate to severe disease. Combination therapy, which combines long-acting bronchodilators with ICS, is frequently recommended to maximise symptom control and prevent exacerbations [26].

The pharmacological medicines known as phosphodiesterase-4 (PDE4) inhibitors are an additional class that has been approved for the management of COPD. PDE4 inhibitors, particularly roflumilast, work by preventing immune cells from breaking down cyclic adenosine monophosphate (cAMP), which lowers the release of pro-inflammatory mediators and lessens airway inflammation. Patients with substantial airflow limitation and a history of exacerbations despite receiving optimum bronchodilator therapy are recommended to use roflumilast. Non-pharmacological therapies, which include pulmonary rehabilitation, supplementary oxygen therapy, and lifestyle adjustments, are essential parts of managing COPD [27]. For COPD patients, quitting smoking is the single most effective intervention for slowing the development of the disease and lowering death. Programs for pulmonary rehabilitation, which include education, exercise training, and psychological support, help individuals with COPD improve their quality of life, exercise capacity, and dyspnea. For COPD patients with severe hypoxemia, supplemental oxygen therapy is advised to reduce symptoms and enhance exercise tolerance [28].

Drug development and repurposing efforts

Development of chemokine receptor inhibitors

The identification of chemokine receptor inhibitors represents a significant breakthrough in drug development and repurposing, particularly for chronic inflammatory diseases such as Chronic Obstructive Pulmonary Disease (COPD). A viable treatment approach to control the inflammatory environment and lessen illness symptoms is to target these receptors. The development of chemokine receptor inhibitors was justified by their capacity to specifically inhibit the interaction between chemokines and their receptors, obstructing subsequent signalling cascades that result in the recruitment and activation of immune cells [29]. Due to their involvement in inflammation associated with COPD, several chemokine receptors, including CXCR2, CXCR3, and CCR5, have been identified as possible targets. For example, CXCR2 is a receptor for chemokines like CXCL1 and CXCL8, which are essential players in the migration of neutrophils to the lungs. By blocking CXCR2, tissue injury and inflammation can be lessened by decreasing neutrophil influx and the subsequent release of proteases and ROS [30].

Chemokine receptor inhibitor development has moved through several phases, including preclinical research and clinical trials. These inhibitors are effective in lowering inflammation and enhancing lung function in preclinical models of COPD. In animal studies, for instance, CXCR2 inhibitors have demonstrated encouraging outcomes by reducing neutrophil infiltration and reducing airway hyperresponsiveness. Clinical trials to assess the safety and effectiveness of chemokine receptor inhibitors in humans have been made possible by these results. Sev-

eral chemokine receptor inhibitors are presently being evaluated clinically for inflammatory illnesses such as COPD [9]. A prominent example is the CXCR2 antagonist AZD5069, which has been studied in clinical trials for COPD. Early-phase trials indicate that AZD5069 is well-tolerated and may be used as a treatment agent for COPD since it successfully lowers lung neutrophilic inflammation. Other CXCR2 inhibitors, including MK-7123, have shown promise in clinical studies, indicating that targeting this receptor may have therapeutic benefits [31].

Apart from creating new chemokine receptor inhibitors, attempts are still on to repurpose current medications for the treatment of COPD. By finding new therapeutic applications for already-approved medications, drug repurposing helps to cut costs and speed up the development of new medicines. For example, Maraviroc, which was first created as a CCR5 antagonist for the treatment of HIV, has demonstrated promise in controlling immune responses and lowering inflammation in COPD patients [32]. One example of how current medications can be used to address unmet medical needs in chronic inflammatory illnesses is the repurposing of Maraviroc for COPD. Chemokine receptor inhibitor development and repurposing also require resolving several issues, including maximising drug delivery, reducing off-target effects, and guaranteeing long-term safety. To increase the local concentration of chemokine receptor inhibitors in the lungs and minimise systemic exposure, inhalation administration devices are being investigated as a potential means of optimising therapeutic efficacy. Furthermore, current studies seek to increase these inhibitors' selectivity to lessen side effects and improve their safety profile [33].

Drug repurposing

Drug repurposing is a cutting-edge method of drug research that involves developing new therapeutic uses for pharmaceuticals that have already received approval. This method has numerous advantages in this regard. Safe and efficient treatments for long-term illnesses such as Chronic Obstructive Pulmonary Disease (COPD) are always in demand. Drug repurposing lowers the time, expense, and risk associated with conventional drug development pathways by making use of the established pharmacological profiles, safety information, and manufacturing procedures of already approved medications [34]. Because COPD is a complicated illness involving immunological responses, tissue remodelling, and intricate inflammatory processes, there is justification for drug repurposing in its therapy. With numerous medications now on the market and a wealth of clinical experience behind them, researchers can narrow down those whose mechanisms of action have the potential to impact the pathophysiology of COPD positively. This strategy may open up new therapeutic avenues that weren't previously thought of while these medications were being developed [34].

A noteworthy instance of repurposing drugs for COPD is the application of inhibitors of phosphodiesterase-4 (PDE4). PDE4 inhibitors have proven to help reduce COPD exacerbations and inflammation. They were initially created to treat conditions including rheumatoid arthritis and asthma because of their anti-inflammatory and immunomodulatory properties. PDE4 inhibitor rolumilast is now a licensed treatment for COPD, with an emphasis on individuals with severe disease and a history of recurrent exacerbations [35]. The efficacy of roflumilast highlights the possibility of repurposing medications with well-understood mechanisms of action to fill gaps in COPD treatment. Repurposing statins, which are typically utilised for their ability to decrease cholesterol in cardiovascular illnesses, is another example. Due to their anti-inflammatory and immunomodulatory qualities, statins have sparked research into possible COPD benefits. Due to their impact on systemic inflammation, clinical trials have revealed that statins may improve overall outcomes and lessen the frequency of COPD exacerbations. Statins represent the promise of repurposing well-established medications to take advantage of their pleiotropic effects in treating chronic inflammatory illnesses like COPD, despite the inconsistent outcomes and need for additional research [36].

Azithromycin and other macrolide antibiotics have been repurposed for the treatment of COPD. In addition to their antibacterial properties, macrolides have immunomodulatory and anti-inflammatory properties. It has been demonstrated that long-term low-dose azithromycin treatment lowers the incidence of exacerbations in COPD patients. This effect is probably due to alterations

in inflammatory pathways and a decrease in bacterial colonisation of the airways. Azithromycin's newfound use in the treatment of COPD has given patients another therapeutic option, especially those who have a chronic bronchitis phenotype and a history of exacerbations [37]. Profiting from advances in disease biology is another benefit of drug repurposing. For example, biologics that were first created for other inflammatory diseases are now being investigated for COPD. Treatment for COPD has shifted from asthma to monoclonal antibodies that target cytokines linked to eosinophilic inflammation, such as interleukin-5 (IL-5) or interleukin-13 (IL-13). Mepolizumab and benralizumab, for example, were first licensed for the treatment of severe eosinophilic asthma. Currently, clinical trials are being conducted to evaluate the effectiveness of these drugs in COPD patients who exhibit eosinophilic phenotypes. This illustrates how knowledge from one illness can influence treatment approaches in another [38].

The repurposing of drugs is not without difficulties. Significant obstacles include those related to intellectual property, regulations, and the requirement for strong clinical data to support new indications. Furthermore, the dosage, mode of administration, and any adverse effects of the repurposed medicine need to be carefully considered in light of the latest therapeutic application. Despite these difficulties, medication repurposing is a tempting way to increase the range of treatments available for COPD and other chronic illnesses due to the time and money savings as well as the possibility of significant clinical advantages [34].

Clinical trials and future research

Clinical trials provide the foundation for developing new medicines and establishing their safety and efficacy in the treatment of illnesses like chronic obstructive pulmonary disease (COPD). Numerous clinical trials have looked into the possibility of different chemokine inhibitors to address the immunological dysregulation and chronic inflammation that are hallmarks of COPD, either as monotherapy or in conjunction with traditional therapies. These trials have been conducted in the last few years. Targeting chemokine pathways is becoming increasingly popular, according to an overview of recent clinical trials. Trials involving CXCR2 inhibitors, including danirixin and navarixin, have demonstrated encouraging outcomes. The goal of these inhibitors is to prevent neutrophil recruitment, which is a significant cause of inflammation associated with COPD [39]. Research has indicated that these medications can enhance lung function and lower inflammatory markers in sputum, while their effects on the frequency of exacerbations have varied. While some trials found no discernible benefits when compared to placebo, others showed a decrease in the frequency of exacerbations. These contradictory findings demonstrate the complexity of COPD and the need for more improvement in medication targeting and patient selection [40].

Clinical trials have also focused on monoclonal antibodies that are directed against chemokines or their receptors. For example, COPD patients who experience frequent exacerbations have been screened for the anti-CXCL8 antibody, which neutralises the chemokine responsible for neutrophil recruitment. Early-stage studies demonstrated that this antibody could enhance specific clinical outcomes and lower lung neutrophil levels. Large-scale trials are yet required to validate these advantages and ascertain the medicines' long-term safety profile [41]. Combination therapies using chemokine inhibitors and conventional COPD medications have been investigated in addition to monotherapy. Studies involving CXCR2 inhibitors in combination with long-acting bronchodilators and inhaled corticosteroids (ICS) have demonstrated that the addition of a chemokine inhibitor can further enhance the anti-inflammatory benefits of conventional therapies. By treating the underlying inflammation as well as the symptoms of COPD, these combination therapies seek to offer a more thorough method of controlling the condition. According to preliminary findings, these combinations are more successful than standard medications alone at improving lung function and lowering exacerbation rates [42].

Many important factors must be taken into account while designing clinical trials for COPD medicines to ensure reliable and significant outcomes. The gold standard for determining the effectiveness and safety of novel drugs is still randomised controlled trials (RCTs). Usually,

these studies have several arms, such as a placebo group and one or more treatment groups that receive various dosages of the experimental medication. The use of substitute controls provides a clear comparison and an accounting for the placebo effect, which is necessary to evaluate the actual impact of the new medication [43]. Patient selection is a crucial component of COPD clinical trial design. With diverse phenotypes, including differing degrees of neutrophilic and eosinophilic inflammation, COPD is a heterogeneous illness. Using biomarkers, like sputum neutrophil levels or blood eosinophil counts, to stratify patients can improve clinical trial precision by identifying patient subgroups more likely to react to particular treatments. This tailored strategy can lower trial result variability and raise the chance of finding meaningful treatment benefits [44].

Further research needs

Further COPD research is required to meet several essential criteria and further our understanding of this complex illness and its management. Primarily, additional investigation is needed to comprehend the fundamental mechanisms of COPD pathogenesis, particularly concerning the role of chemokines and their interactions with immune cells. Clarifying the precise pathways via which chemokines cause lung tissue damage and persistent inflammation will lay the groundwork for the development of more specialised and potent treatments. More investigation is required to find trustworthy biomarkers that can forecast the course of the disease, the likelihood of an exacerbation, and the effectiveness of therapy in COPD patients, in addition to mechanistic research [6]. Though they are somewhat utilised now, biomarkers like sputum neutrophil counts, blood eosinophil counts, and inflammatory cytokines still need to be validated and standardised for usage in various patient populations and therapeutic contexts. Strong biomarkers will enable customised medicine strategies, enabling medical professionals to customise patient regimens according to unique patient profiles [45].

Clinical research ought to concentrate on improving treatment approaches, such as chemokine inhibitors and combination therapy dosage and duration. Comprehensive long-term follow-up randomised controlled trials (RCTs) are necessary to validate the safety and effectiveness of new treatments and determine how best to use them to treat various COPD phenotypes. Research on comparative efficacy can help clarify the relative advantages of multiple treatment modalities and direct clinical judgment. Studies that examine the long-term effects of COPD therapy beyond symptom management and the reduction of exacerbations are also necessary [46].

Collaboration between researchers, doctors, pharmaceutical companies, and regulatory bodies is necessary to address these research demands. To expedite the development of more potent COPD treatments, multidisciplinary strategies that incorporate basic science, translational research, and clinical trials will be essential. Additionally, encouraging global cooperation and data-sharing programs can make it possible to include bigger and more diverse study populations, which will improve the generalizability of research findings and hasten the integration of new knowledge into clinical practice [47]. Ultimately, consistent funding support and prioritised research efforts to meet the various hurdles presented by this crippling disease are necessary to advance research on COPD. The field can significantly improve patient outcomes and lessen the worldwide burden of COPD by giving priority to these research requirements [48].

Conclusions

The numerous functions that chemokines play in the pathogenesis of chronic obstructive pulmonary disease (COPD) and their potential as targets for treatment are examined in this research. Chemokines are essential for immune cell recruitment, the maintenance of chronic inflammation, and the unique anatomical abnormalities in the lungs associated with COPD. Numerous studies have been conducted on important chemokines, such as CXCL8, CXCL9, CXCL10, and CXCL11, demonstrating their diverse functions in controlling inflammatory responses and influencing disease progression [22]. Chemokine targeting as a therapeutic approach is a viable way to reduce inflammation associated with COPD and enhance clinical results. Preclinical research and clinical

trials are providing new information about the effectiveness of biologics and chemokine inhibitors in lowering exacerbation rates, improving lung function, and even altering disease course.

Furthermore, combination medicines that include chemokine inhibitors and conventional COPD medications have demonstrated synergistic results, providing a multimodal approach to managing this complex disease [49]. Even though there has been significant progress, there are still several obstacles to overcome and areas that need more research. Subsequent investigations ought to clarify the exact processes by which chemokines contribute to the pathophysiology of COPD, verify biomarkers that forecast treatment outcomes, and refine therapy approaches via novel medication development and personalised medicine methods. Long-term research is also required to evaluate the sustainability of treatment effects and any potential safety issues related to continued usage of chemokine-targeted medicines [50]. By bridging these knowledge gaps and utilising multidisciplinary teamwork, the field can progress toward more efficient, customised COPD treatments. Translating scientific findings into tangible improvements in patient care and outcomes will require sustained investment in translational research, strong clinical trials, and data-driven insights. Ultimately, there is hope for reducing the impact of COPD on individuals, healthcare institutions, and society at large through the continued exploration of novel strategies to target chemokines.

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Conflicts of Interest
The authors declare that they have no conflicts of interest in this work.

Ethical Statement
This study does not contain any studies with human or animal subjects performed by any of the authors.

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