NEW APPROACHES IN HEALTH SCIENCES

THEORY, METHOD, AND PRACTICE

Editor: Prof. Dr. Yeltekin DEMİREL

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Chapter 1

Origanum majorana (Marjoram) as a Spice– Identification, Authentication, Pharmacology, Safety, Stability and Clinical Applications

Gökhan DEGE[1], Dursun Alper YILMAZ[2]

ABSTRACT

Origanum majorana L. (marjoram) is a widely used medicinal and culinary herb valued for its rich phytochemical profile, diverse pharmacological actions, and broad industrial applications. This chapter provides a comprehensive overview of the plant's chemical composition, authentication methods, biological activities, clinical evidence, safety profile, and stability characteristics. Advances in chromatographic, spectroscopic, and molecular techniques have enhanced the identification and quality control of marjoram, enabling the detection of adulteration and supporting its standardization in the pharmaceutical and spice trade. Phytochemical analyses reveal abundant flavonoids, phenolic acids, and terpenoids—particularly sabinene hydrate, terpinen-4-ol, and γ-terpinene—responsible for its antioxidant, antimicrobial, anti-inflammatory, cardiometabolic, neuroprotective, and anticancer properties. Findings from in vitro and in vivo models demonstrate significant antidiabetic, cardioprotective, immunomodulatory, and gastroprotective mechanisms, while clinical trials further confirm benefits in glycemic regulation, lipid modulation, blood pressure reduction, pain relief, cognitive enhancement, and anxiety reduction. Toxicological assessments indicate a high safety margin with minimal adverse effects, although heavy metal contamination remains a concern that necessitates rigorous quality control. Furthermore, factors such as storage conditions, drying methods, and nanoencapsulation strategies critically influence the stability and bioavailability of marjoram extracts and essential oils. By integrating phytochemical, pharmacological, technological, and clinical perspectives, this chapter highlights O. majorana as a promising natural

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therapeutic agent with significant potential for evidence-based applications in modern medicine, functional foods, and nutraceutical development.

Keywords: Origanum majorana; marjoram; phytochemistry; essential oils; pharmacological activities; antioxidant; antidiabetic; neuroprotective.

1. Introduction

Plant-derived natural products are widely preferred in various fields, including healthcare, cosmetics, and the food industry [1]. *Origanum majorana* (Marjoram) is a popular aromatic plant that is a member of the Lamiaceae family and is well-known for its many uses in medicine, cooking, and preservation. Traditionally used in Mediterranean, Middle Eastern, and European cuisines, marjoram adds a unique taste to numerous recipes and acts as a natural preservative because of its potent antimicrobial characteristics [2]. Beyond its culinary uses, *O. majorana* has been acknowledged in traditional medicine for its healing properties, including antispasmodic, anti-inflammatory, analgesic, and antioxidant effects [3].

Over the past three decades, many scientific research has focused on the phytochemical, analytical characteristics, and pharmacological of *O. majorana*. Improvements in spectroscopy, chromatography, and molecular authentication methods have improved the identification and quality control of its essential oils and bioactive components. Researchers have also found its pharmacological activities, such as neuroprotection, pain management, and digestive disorders [4]. Furthermore, studies on its stability and safety profile have helped optimize its uses in the pharmaceutical, food, and cosmetic industries.

With the increasing interest in natural product-based therapeutics, *O. majorana* continues to attract attention as a herb with therapeutic properties. This chapter provides a comprehensive scientific review of *O. majorana* as a spice, medicinal herb, and bioactive ingredient.

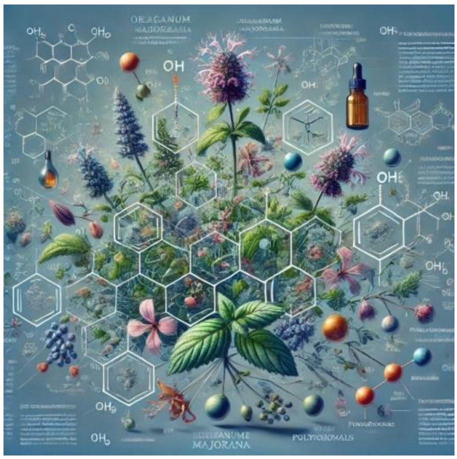
Fig. 1. A Botanical Drawing of *O. Majorana*.

2. Identification, Authentication, and Source of *O. majorana*

2.1 Chemical Composition and Phytochemical Analysis

The identification, authentication, and sourcing of *O. majorana* as spice have been examined in analytical sciences, chemistry, and pharmacology. The medicinal plant is rich in flavonoids, phenolic acids, essential oils, and terpenes, similar to *Rosa damascena* of the Rosaceae family and *Ficus carica* of the Moraceae family [5-7].

Fig. 2. Scientific Diagram of Marjoram's Chemical Composition.



Gas chromatography-mass spectrometry (GC-MS) analysis has detected many bioactive compounds with pharmacologic effects, such as linalool, phytols, and squalene [8]. Liquid chromatography-mass spectrometry (LC-MS/MS) has identified several polyphenolic compounds with antioxidant activities, such as caffeic acid, gallic acid, and chlorogenic acid [9].

Essential oils can be extracted from medicinal herbs through various production methods, such as steam distillation, cold pressing, and solvent extraction [10]. Phytochemical studies have shown that terpenes, alcohols, ketones, and aldehydes are abundant in the volatile aromas of potential phytotherapeutic agents [11,12]. Notably, monoterpenes and sesquiterpenes are abundant in the essential oil of *O. majorana*. The major constituents are sabinene hydrate, terpinen-4-ol, *γ*-terpinene, and p-cymene, with chemical variations influenced by cultivation conditions and geographical origin [13].

The phytochemical study on the ethanolic and aqueous extracts of *O. Majorana* revealed many chemical components, including alkaloids, triterpenoids, anthraquinones, steroids, flavonoids, tannins, phenols, and saponins. The plant's therapeutic properties may be associated with the

phytochemical compounds [14]. Flavonoids play an imperative role in human health [15]. In this context, the presence of catechin, quercetin, and rutin suggests potential neuroprotective and anti-inflammatory benefits [16].

2.2 Authentication and Identification Techniques

The authenticity of *O. majorana* is crucial for quality control in the pharmaceutical and spice industries. *O. majorana* can be discriminated from species within the *Origanum* genus using microsatellite markers (SSRs) combined with high resolution melting (HRM) analysis [17]. Similarly, *O. majorana* can be differentiated from *Origanum vulgare* and *Origanum onites* through microsatellite markers [18].

Rapid chemical fingerprinting of *O. majorana* essential oils is obtained with electrospray ionization mass spectrometry (ESI-MS), which provides a powerful tool for quality control and adulteration detection [19]. Inter-simple sequence repeats (ISSR) molecular markers are the preferred method for assessing the genetic uniformity of *O. majorana* [20].

2.3 Geographic and Environmental Influences on Chemical Composition

The chemical composition of *O. majorana* alters according to environmental conditions, including altitude, soil composition, and climate. A study on Greek oregano genotypes showed that regional differences in cultivation significantly influenced thymol and carvacrol levels [21]. Another study reported positive correlations between altitude and carvacrol and thymol concentrations [22].

Fig. 3. Cartoon-Style Artwork of Marjoram in a Kitchen.

3. Quality Control and Adulteration in Marjoram Spices

3.1 Common Adulterants and Their Detection

Botanical substitutions with closely related herbs, such as *Thymus* species or *Origanum vulgare* (common oregano), are commonly used in adulteration in *O. majorana*. The other forms of adulteration techniques are the addition of the filler, such as sawdust, starch, or ground leaves from non-medicinal plants. In some cases, artificial colors and synthetic chemicals are introduced to enhance appearance and aroma [23].

One study utilizing high-performance liquid chromatography (HPLC) found inconsistencies in commercial marjoram samples, revealing significant levels of flavonoid adulteration and substitutions with *O. vulgare* [24]. DNA metabarcoding methods confirmed species mislabeling in several spice products, indicating a need for rigorous regulatory monitoring [25].

3.2 Analytical Techniques for Quality Control and Authentication

Several analytical methods have been employed to verify the authenticity of *O. majorana*, including:

Fig.4.4. Fun Cartoon of Marjoram and Other Herbs.

3.2.1 Chromatographic Methods

Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) are widely used to detect marjoram adulterants.

GC-MS Analysis: Studies have shown that marjoram essential oil contains key markers like carvacrol, thymol, and γ-terpinene, which differentiate it from adulterated samples [26].

HPLC with UV detection: Chromatographic profiles of marjoram samples confirmed that distinct flavonoid markers could be used to detect substitution with cheaper herbs [27].

3.2.2 Spectroscopic Methods

Spectroscopic techniques offer non-destructive, rapid, and precise means of assessing marjoram quality.

Near-Infrared Spectroscopy (NIRS): This technique has been applied to detect starch and non-volatile adulterants in powdered marjoram samples [28].

Fourier Transform Infrared Spectroscopy (FT-IR): Used for rapid identification of adulteration, particularly in assessing essential oil purity [29].

A review on spectroscopy applications concluded that infrared spectroscopy combined with chemometric modeling could efficiently detect adulterants in spices, including marjoram [30].

3.2.3 Molecular Techniques for Species Authentication

DNA-based molecular methods have proven effective for detecting botanical adulteration in marjoram.

DNA Barcoding: DNA analysis using ITS2 and psbA-trnH markers successfully identified adulteration in spice samples by differentiating *O. majorana* from closely related species [31].

High-Resolution Melting (HRM) Analysis: This method enables the identification of genetic differences between *O. majorana* and its adulterants, improving spice authentication [32].

3.2.4 Non-Destructive and Emerging Technologies

Innovative and rapid testing methods have gained importance in ensuring the authenticity and safety of marjoram in the spice trade.

Electronic Nose and Artificial Intelligence: E-nose systems, when combined with machine learning algorithms, have successfully differentiated genuine *O. majorana* from adulterated samples based on volatile compound analysis [33].

Ion Mobility Spectrometry (IMS): IMS is used to detect volatile organic compounds in marjoram and can distinguish between genuine and adulterated marjoram samples with high precision [34].

Portable X-ray Fluorescence (pXRF) Analysis: This method detects heavy metal adulteration, particularly lead chromate contamination in spices, including marjoram [35].

4. Pharmacological Activity and Mechanisms of Action

Numerous studies have revealed that ethnomedicinal herbs possess therapeutic potential in treating cardiovascular, gastrointestinal, and respiratory disorders [36,37]. Moreover, medicinal plants may serve as alternative herbal sources that provide potent bioactive compounds, minimal undesirable side effects, low cost, and easy availability [38,39]. In this regard, over the past three decades, much research has elucidated the pharmacological effects and marjoram's complex mechanisms of action. This medicinal herb exerts biological effects, including antidiabetic, anticancer, antimicrobial, analgesic, antioxidant, anticonvulsant, cardioprotective, and immunomodulatory effects. The following sections provide detailed findings from in vitro, in vivo, and clinical studies.

4.1 Antidiabetic and Cardioprotective Effects

Glycemic Control and Lipid Metabolism: Research conducted on diabetic animal models indicates that ethanolic extracts of *O. majorana* significantly reduce glycated hemoglobin (HbA1c) levels and fasting blood glucose. However, these extracts improve lipid profiles by lowering triglycerides, total cholesterol, and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL). These effects are related to polyphenols like quercetin and rutin, which enhance glucose uptake and insulin sensitivity [40].

Enzyme Inhibition and Postprandial Glucose Regulation: *O. majorana* has an inhibitory effect on digestive enzymes, such as α-amylase and α-glucosidase.

The plant reduces carbohydrate breakdown and thus prevents postprandial hyperglycemia [41].

Cardiovascular Protection and Blood Pressure Regulation: A clinical study performed on hypertensive patients demonstrated that marjoram extract significantly lowered systolic and diastolic blood pressure. The hypotensive effect may be related to endothelium-dependent vasorelaxation. This effect is mediated by increased nitric oxide (NO) production and inhibition of voltage-gated calcium channels, resulting in vascular smooth muscle relaxation [41].

4.2 Antimicrobial and Immunomodulatory Activity

Antibacterial and Antifungal Effects: The methanol extract of *O. majorana* exhibits broad-spectrum antimicrobial activity against pathogenic strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Mechanistic studies suggest that the extract disrupts bacterial cell

membranes, increasing permeability and triggering lysis through oxidative damage and ion leakage [42, 43].

Immune System Modulation: *O. majorana* enhances immune responses by modulating key signaling pathways. It activates NF-κB and MAPK cascades, leading to increased secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase (iNOS), which collectively strengthen innate immune defenses [44].

4.3 Anticancer Activity

Colon Cancer: In vitro studies on human colon cancer cells (HT-29) reveal that *O. majorana* induces apoptosis via the intrinsic mitochondrial pathway. The extract causes DNA fragmentation, cell cycle arrest at the G2/M phase, and caspase-9 activation, ultimately triggering cell death [45].

Breast Cancer: In breast cancer cell lines (MCF7), marjoram extract promotes mitochondrial fusion, leading to the loss of mitochondrial membrane potential and apoptosis. It also upregulates caspase-7 activity and downregulates NF-κB signaling, impairing metastatic potential by reducing the expression of matrix metalloproteinases (MMPs) and NO production, both critical for tumor invasion [46, 47].

4.4 Antioxidant and Neuroprotective Effects

Oxidative Stress Reduction: Rich in polyphenols, flavonoids, and tannins, *O. majorana* exhibits potent antioxidant properties. It scavenges reactive oxygen species (ROS), reduces lipid peroxidation, and restores antioxidant enzyme levels, such as superoxide dismutase (SOD) and catalase, mitigating oxidative stress-linked diseases [48].

Neuroprotection: Preclinical studies suggest that *O. majorana* may protect against neurodegenerative conditions like Alzheimer's disease by lowering brain oxidative stress biomarkers, reducing acetylcholinesterase activity, and preserving neuronal integrity [9].

4.5 Analgesic and Anticonvulsant Activity

Pain Relief: *O. majorana* extracts display significant antinociceptive effects in animal models, reducing pain perception in thermal and chemical-induced pain assays. The analgesic effect was concerned with flavonoid-mediated inhibition of cyclooxygenase (COX) enzymes and transient receptor potential (TRP) channels [48].

Seizure Management: Ursolic acid and flavonoids contributed to *O. majorana*'s anticonvulsant effect. The bioactive compounds delay seizure onset, modulate GABAergic neurotransmission, and reduce seizure duration in pentylenetetrazole (PTZ)-induced seizure models [49].

4.6 Gastrointestinal and Anti-Diarrheal Effects

Gut Health and Ion Transport: In models of infectious diarrhea, *O. majorana* extract balances electrolyte transport and lowers stool frequency by improving intestinal sodium absorption and inhibiting chloride secretion. The anti-diarrheal effect may partly be associated with the inhibiting cystic fibrosis transmembrane conductance regulator (CFTR) channels [23].

Smooth Muscle Relaxation: The extract exhibits spasmodic effects via muscarinic receptor antagonism and calcium channel blockage and, helps relieve
gastrointestinal cramping [50].

In summary, *O. majorana* is a medicinal plant with many pharmacological properties. *O. majorana* positions a promising drug candidate. Further, many clinical studies are needed to elucidate optimal therapeutic dosages and formulations.

5. Clinical Studies on *O. majorana*

Extensive clinical studies have confirmed the medicinal benefits of *O. majorana* for human health. These trials investigated its activities on cardiovascular function, microbial infections, metabolic health, and pain management. The following sections present clinical findings and highlight the action mechanisms.

5.1 Metabolic and Cardiovascular Benefitsü

Diabetes Management: A randomized controlled trial examined the effects of marjoram tea consumption in patients with type 2 diabetes. After 8 weeks, fasting blood glucose, HbA1c, and insulin resistance markers decreased in patients. The hypoglycemic effect was attributed to the inhibition of α-glucosidase and α-amylase, alongside enhanced GLUT4 translocation and insulin receptor signaling [51].

Lipid Profile Improvement: In patients with metabolic syndrome, marjoram supplementation led to a notable decrease in total cholesterol, LDL, and triglycerides, while increasing HDL. These changes were associated with marjoram's antioxidant properties, which reduced lipid peroxidation and modulated hepatic lipid metabolism genes [52].

Blood Pressure Regulation: A clinical trial on hypertensive patients showed that daily consumption of *O. majorana* extract for 12 weeks significantly lowered systolic and diastolic blood pressure. The antihypertensive effects were linked to vascular smooth muscle relaxation via increased nitric oxide bioavailability and inhibition of L-type calcium channels, reducing peripheral resistance [53].

5.2 Antimicrobial and Gastrointestinal Effects

5.2.1 Antifungal and Antiviral Effects

Antifungal Activity: In patients with recurrent fungal infections, topical application of marjoram essential oil for 6 weeks reduced *Candida albicans* colonization and infection recurrence. The oil's antifungal action was associated with its ability to disrupt fungal cell membranes, impair ergosterol synthesis, and induce oxidative stress within fungal cells [54].

Antiviral Benefits: An investigation into marjoram oil inhalation for upper respiratory viral infections found significant symptom relief in treated patients, including reduced nasal congestion, cough frequency, and sore throat severity. The essential oil showed virucidal properties by modulating host inflammatory responses and interfering with viral envelope integrity [55].

5.2.2 Digestive Health and Gut Function

Gastroprotective Effects: In patients with non-ulcer dyspepsia, marjoram consumption diminished gastric acid secretion, alleviated symptoms like bloating and epigastric pain, and improved mucosal healing. The gastroprotective effects involved inhibiting H+/K+-ATPase activity and inflammatory cytokines (TNF-α, IL-1β) and increasing mucosal antioxidant defenses [56].

5.3 Pain Management and Mood Enhancement

5.3.1 Analgesic and Antinociceptive Effects

Chronic Pain Relief: The topical administration of marjoram essential oil decreased pain intensity and improved joint mobility in patients with chronic musculoskeletal pain. The analgesic effects were related to the inhibition of COX and the modulation of TRPV1 receptors [57].

Migraine Management: Marjoram aromatherapy reduced headache severity, duration, and associated nausea in migraine patients. The effects may be associated with the modulation of serotonin signaling and the reduction of neurogenic inflammation [58].

5.3.2 Neuroprotective and Anxiolytic Effects

Cognitive Enhancement: Marjoram administration significantly improved memory and attention in patients with mild cognitive impairment. The effects were related to increased acetylcholine levels, reduced oxidative stress, and upregulation of brain-derived neurotrophic factor (BDNF) [59].

Anxiolytic and Stress-Reducing Effects: Marjoram aromatherapy significantly diminished anxiety scores and cortisol levels. The anxiolytic properties may be associated with GABA-A receptors [60].

In summary, clinical studies on *O. majorana* highlight its diverse therapeutic potential. *O. majorana* regulates metabolic, neurological, cardiovascular, and immune processes. Therefore, it can be used in various clinical applications.

6. Safety and Toxicology Studies

The possible hazards of consuming *O. majorana* have been assessed by thorough safety and toxicology investigations. According to preclinical and clinical research findings, there is a high safety profile with few side effects, even at comparatively high dosages. The following section summarizes key findings regarding toxicity, heavy metal contamination, and regulatory considerations.

6.1 Acute and Chronic Toxicity

Human and preclinical research shows that marjoram is well tolerated at therapeutic dosages. Doses up to 800 mg/day of marjoram extract have been deemed safe in clinical trials, with no severe side effects reported. Key findings include:

Genotoxicity and Carcinogenicity: Long-term studies on rodent models revealed no significant genotoxic or carcinogenic effects, even at high doses. Marjoram's antioxidant compounds, such as rosmarinic acid and quercetin, may help protect against DNA damage by scavenging free radicals and reducing oxidative stress [61].

Hepatoprotective and Nephroprotective Effects: Toxicology studies in toxin-exposed animal models demonstrated that marjoram extracts could mitigate liver and kidney damage. Marjoram reduced hepatic enzyme levels (ALT, AST) and preserved renal function by inhibiting lipid peroxidation, modulating inflammatory pathways, and enhancing glutathione production [62,63].

Reproductive and Neurotoxicity: Reproductive safety studies in animal models found no adverse effects on fertility, fetal development, or offspring health. Additionally, chronic administration of marjoram showed no neurotoxic

effects; instead, it appeared to exert neuroprotective properties through modulation of GABAergic signaling and reduction of neuroinflammation [64].

6.2 Heavy Metal Contamination and Regulatory Concerns

Like other medicinal plants, marjoram's safety can be influenced by environmental factors, particularly soil contamination. Heavy metal accumulation poses a potential risk, especially in regions with high industrial activity or polluted agricultural areas.

Heavy Metal Accumulation: A biogeochemical study found that marjoram grown in contaminated soils could accumulate toxic metals, including lead (Pb), cadmium (Cd), and chromium (Cr), which may pose long-term health risks if consumed regularly [65]. The extent of metal uptake depended on soil pH, organic matter content, and proximity to pollution sources.

Regulatory Recommendations: Regulatory bodies advise sourcing marjoram from certified organic farms or regions with low environmental contamination to minimize heavy metal exposure. Good Agricultural and Collection Practices (GACP) are recommended to ensure the safety and quality of medicinal herbs [66].

Screening and Quality Control: pXRF has emerged as a promising technique for rapidly screening marjoram samples for heavy metal residues. pXRF allows for non-destructive, on-site analysis, facilitating routine quality control and helping manufacturers comply with safety standards [67].

To summarize briefly, *O. majorana* demonstrates a favorable safety profile with minimal toxicity under recommended therapeutic doses. However, quality control measures, including proper cultivation practices and heavy metal screening, are essential to ensure consumer safety. Future research should continue exploring long-term safety and potential herb-drug interactions to guide clinical applications.

7. Stability and Shelf-Life of Marjoram

Understanding the factors that influence the stability and shelf-life of *O. majorana* products is crucial for preserving their pharmacological properties and ensuring product efficacy. Research has highlighted the effects of storage conditions, encapsulation techniques, and post-harvest processing on the chemical stability and bioactivity of marjoram extracts and essential oils.

7.1 Essential Oil Stability Under Storage Conditions

The chemical stability of marjoram essential oil is highly dependent on environmental factors such as temperature, light, and oxygen exposure:

Temperature and Oxygen Exposure: Low-temperature storage (-20°C to 4°C) significantly prolongs the stability of essential oils by slowing down oxidative and hydrolytic reactions. On the other hand, high temperatures cause bioactive terpenes to degrade more quickly, which reduces their antimicrobial and antioxidant effectiveness [68].

Photodegradation and Volatile Loss: Light exposure, remarkably UV light, causes important terpenoids like p-cymene, thymol, and carvacrol to photodegrade. Chemical indicators of oil deterioration, such as 2-undecanone and p-cresol, are produced as a result of this process [69]. It has been suggested that vacuum-sealed storage and amber glass containers help prevent deterioration caused by light and oxygen.

7.2 Nanoencapsulation for Stability Enhancement

To improve the stability, bioavailability, and therapeutic effectiveness of marjoram essential oil, nanoencapsulation has shown promise:

Chitosan-Based Nanoemulsion: A chitosan nanoemulsion system was created in a study to encapsulate marjoram essential oil, greatly increasing its stability in a range of humidity and temperature conditions. The oil's chemical profile was maintained and, its antifungal efficacy against *Aspergillus flavus* was prolonged by encapsulation, which slowed the rate of terpene oxidation [70].

Controlled Release and Bioavailability: The therapeutic potential of the oil is increased by nanoencapsulation, which stabilizes volatile compounds and permits controlled release, extending the antimicrobial action. This approach is especially useful for topical applications, pharmaceutical formulations, and food preservation [71].

7.3 Post-Harvest Drying and Processing Effects

The phytochemical composition and bioactivity of *O. majorana* are significantly impacted by post-harvest handling methods:

Drying Techniques: In contrast to sun drying, which can result in a considerable loss of terpenes due to heat exposure and UV degradation, shade drying has been demonstrated to preserve higher quantities of volatile oils and polyphenolic chemicals [58].

Salinity Stress and Harvesting Stage: Salinity stress is one example of an environmental condition that can affect the accumulation of secondary metabolites during agriculture. Research suggests that marjoram harvested in the late vegetative stage has enhanced quantities of flavonoids and phenolic antioxidants, maximizing its potential for medicinal use [57, 58].

Nanoencapsulation, optimal post-harvest procedures, and careful consideration of storage conditions are all necessary to maintain the stability of *O. majorana* products. These factors are crucial for maintaining the plant's bioactivity and prolonged effectiveness in both medicinal and commercial uses.

8. Conclusion

Over the past three decades, research has established *O. majorana* as a valuable plant having pharmacological, antibacterial, and preservation qualities that can be used in both medicine and cooking. The plant has a wide range of therapeutic potential because of its rich phytochemical composition, which includes polyphenols, flavonoids, and essential oils. Developments have dramatically improved its quality control in chromatography, molecular authentication, and post-harvest storage methods, guaranteeing improved stability and uniformity of its bioactive ingredients.

O. majorana's importance in metabolic health, specifically in treating diabetes, hyperlipidemia, and disorders linked to oxidative stress, is strongly supported by clinical and preclinical research. It is a potential option for pain management due to its analgesic and anti-inflammatory qualities, especially for ailments like arthritis and muscle soreness. Furthermore, its spasmolytic and carminative properties promote gastrointestinal health by facilitating digestion and reducing symptoms of bloating, indigestion, and irritable bowel syndrome.

Despite these encouraging findings, there are still gaps in the extensive confirmation of its medical applications. Future studies should concentrate on performing extensive clinical trials to verify its therapeutic efficacy in human populations, investigating innovative delivery systems like nanoencapsulation to enhance bioavailability, and refining post-harvest processing methods to optimize the retention of bioactive compounds. Furthermore, multidisciplinary studies combining clinical medicine, molecular biology, and pharmacognosy should further clarify its action methods and incorporate them into the modern pharmacotherapy and functional food sectors.

O. majorana could emerge as a botanical resource in the development of natural health products and evidence-based herbal medicines by addressing these research priorities.

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Chapter 2

Mechanistic Pathways of Cognitıve Recovery in Glioma Patients

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ABSTRACT

Gliomas are among the most prevalent and aggressive primary brain tumors and are frequently associated with substantial cognitive impairments due to tumor localization and multimodal treatments, including surgery, chemotherapy, and radiotherapy. These deficits significantly reduce functional independence and quality of life. However, the neurobiological mechanisms that underlie cognitive decline and potential recovery remain insufficiently understood. This chapter provides a comprehensive, translational overview of the biological and neuroplastic mechanisms that underpin cognitive rehabilitation in patients with glioma, integrating molecular, neuroimaging, and clinical insights. A narrative synthesis of recent peer-reviewed literature was conducted, emphasizing glioma-related cognitive dysfunction, neuroinflammation, white matter disruption, and genetic alterations such as IDH and TP53 mutations. Evidence on mechanisms of experience-dependent neuroplasticity, neurotrophic factor modulation and functional neuroimaging-supported rehabilitation protocols was critically examined. Cognitive rehabilitation induces measurable improvements in attention, memory, and executive functions through both restorative and compensatory mechanisms. Functional neuroimaging studies consistently demonstrate cortical reorganization, interhemispheric recruitment, and network-level plasticity following structured interventions. Exercise and targeted cognitive training are associated with increased neurotrophic factor expression, enhanced synaptogenesis, and better neuropsychological performance. Molecular subtypes appear to influence recovery potential, with IDH-mutant gliomas exhibiting more favorable neuroplastic profiles compared with wild-type tumors. Emerging evidence supports the integration of neuroplasticity-driven frameworks into cognitive rehabilitation for glioma patients. Future research

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should prioritize biomarker-guided, personalized rehabilitation protocols, integration of digital therapeutics, and rigorously designed prospective clinical trials to establish long-term efficacy and optimize patient-centered outcomes.

Keywords: Glioma; cognitive rehabilitation; neuroplasticity; IDH mutation; translational neuro-oncology; neuroimaging**.**

1. Introduction

Gliomas, particularly high-grade variants such as glioblastoma, are among the most aggressive primary brain tumors and are frequently associated with early and pervasive cognitive impairments (Morshed et al., 2020; Ek et al., 2024). These deficits-commonly involving attention, memory, executive functioning, and processing speed-often manifest before the initiation of oncological treatments and are closely linked to tumor localization, particularly within the frontal and temporal lobes (Habets et al., 2014; van Kessel et al., 2022).

Standard treatment modalities further exacerbate these impairments. Surgical resection, while essential for tumor debulking and survival, carries inherent risks to eloquent cortical regions responsible for higher-order cognitive processing (Rijnen et al., 2019). Radiotherapy and chemotherapy, especially temozolomide, contribute to neuroinflammation, white matter disruption, and hippocampal injury, accelerating neurocognitive decline over time (Iqbal & Ashraf, 2024; Ghadimi et al., 2025). Although tumor burden reduction through treatment may temporarily stabilize cognitive function, longitudinal follow-up often reveals progressive deterioration, particularly in survivors (De Roeck et al., 2022).

Despite the clinical significance of these deficits, cognitive rehabilitation remains underutilized in neuro-oncology care pathways. Current rehabilitation strategies often rely on generalized, non-personalized protocols that fail to account for the heterogeneity in tumor biology, network disruption, and neuroplastic potential (Weyer-Jamora et al., 2020). This lack of precision limits both efficacy and scalability in clinical practice.

The concept of neuroplasticity offers a promising avenue for advancing personalized interventions. Neuroplastic mechanisms, including synaptic remodeling, neurotrophic modulation, and network-level reorganization, provide a biological framework for functional recovery (Iqbal & Ashraf, 2024). Recent studies integrating neuroimaging, molecular profiling, and cognitive assessment are beginning to elucidate how adaptive changes in brain networks drive resilience and recovery in glioma patients (Ghadimi et al., 2025; Reyes et al., 2024).

Furthermore, technological advances are transforming the landscape of cognitive rehabilitation. Multimodal approaches that combine advanced neuroimaging, molecular data, and artificial intelligence (AI)-assisted platforms hold promise for precision-based cognitive rehabilitation. Such frameworks can adapt therapy in real-time, tailoring intensity and modality to the individual patient's tumor profile and functional connectivity, thereby enhancing the potential for clinically meaningful outcomes.

This chapter synthesizes and critically analyzes recent peer-reviewed studies published between 2009 and 2025 that explore cognitive dysfunction, neuroplasticity, and rehabilitation in glioma patients. Priority was given to clinical trials, neuroimaging-supported studies, and translational research integrating molecular or neurobiological mechanisms. The discussion emphasizes evidence relevant to precision rehabilitation approaches, while mechanistic and exploratory studies are incorporated to contextualize clinical findings (Table 1,2).

Table 1. Summary of Cognitive Rehabilitation Studies in Glioma Patients

Year	Study Design	Level of Evidence	Participants (Age Range / Diagnosis)	N (Sample Size)	Intervention / Approach	Instructor-to-Participant Ratio	Frequency / Duration	Key Outcomes
2009	Randomized controlled trial (Gehring et al.)	1B	Patients with low-grade and anaplastic gliomas	140	Computer-based attention training + compensatory strategy training	1:1	Twice weekly / 6 weeks	Significant improvements in attention and verbal memory; reduced fatigue.
2015	Prospective study (Maschio et al.)	2B	Glioma patients with tumor-related epilepsy	60	Structured cognitive rehabilitation	1:3	Three sessions per week / 8 weeks	Notable improvements in verbal and visuospatial memory.
2021	Prospective observational study (van der Linden et al.)	2B	Glioma patients using the tablet-based ReMind rehabilitation app	52	Digital cognitive training (self-administered)	Self-administered	Daily / 6 weeks	High adherence; limited objective cognitive gains.
2024	Retrospective cohort study (Wu et al.)	3B	Postoperative glioma patients	90	Memory therapy combined with standard care	1:5	Twice weekly / 3 months	Significant improvements in memory scores and quality of life; reduced

								anxiety and fatigue.
2023	Clinical pilot study (Zheng et al.)	2B	Glioma patients with white matter disruption	30	tDCS (transcranial direct current stimulation) combined with cognitive exercise program	1:2	Three sessions per week / 6 weeks	Enhanced fronto-parietal network connectivity and improved executive functions.
2025	Pilot study with digital platforms (Innani et al.)	2C	Patients with high-grade gliomas	20	Digital twin model + AI-guided personalized rehabilitation	1:1	Personalized / Variable duration	High adherence; improvements in attention and memory scores; long-term follow-up needed.

Table 2. Mechanistic and Translational Evidence Supporting Cognitive Rehabilitation in Glioma

Domain	Mechanism / Insight	Supporting Evidence	Clinical Implications
Neuroplasticity and Cortical Reorganization	Perilesional and contralesional reorganization; interhemispheric recruitment of language and executive networks.	Duffau (2017); Poologaindran et al. (2021); Ekert et al. (2024).	Justifies early, intensive, and task-specific rehabilitation to harness adaptive reorganization.
Synaptic Remodeling and Neurotrophic Modulation	Synaptogenesis, dendritic spine growth, and long-term potentiation (LTP) mediated by BDNF, IGF-1, and VEGF.	Sleiman & Chao (2015); Budde et al. (2016); Ben-Zeev et al. (2022).	Suggests exercise and activity-based interventions enhance rehabilitation efficacy.
Functional and Structural Neuroimaging	fMRI shows restoration of connectivity within DMN and frontoparietal control systems; DTI demonstrates improved white matter integrity post-rehabilitation.	Rodríguez-García et al. (2023); Zheng et al. (2023).	Imaging biomarkers can guide and personalize rehabilitation protocols.
Neuroinflammation and White Matter Integrity	Inflammation (IL-6, TNF-α, ICAM-1) and therapy-related demyelination exacerbate cognitive decline; anti-inflammatory	Saggu et al. (2016); Guedes et al. (2024); Liang et al. (2021).	Anti-inflammatory and remyelination-focused strategies may synergize with cognitive therapy.

	effects linked to exercise.		
AI and Connectomic Mapping	Machine-learning platforms integrate imaging and molecular data to optimize rehabilitation planning.	Davatzikos et al. (2020); Akbari et al. (2024).	Supports precision rehabilitation frameworks for real-time therapy adaptation.
Digital and Closed-loop Systems	Digital twins and adaptive platforms simulate patient responses and adjust therapy dynamically.	Innani et al. (2024); Califano (2015).	Enhances personalization, scalability, and engagement in rehabilitation programs.

2. Treatment-Related Cognitive Sequelae

Gliomas inherently disrupt large-scale cognitive networks, yet standard therapeutic modalities—including surgery, radiotherapy, and chemotherapy—further exacerbate this burden. Surgical resection remains the cornerstone of glioma management, aiming for maximal safe tumor removal to prolong survival. However, despite technological advances such as intraoperative mapping and awake craniotomy, interventions near eloquent cortical and subcortical regions still risk impairing language, executive, and visuospatial functions. These sequelae are most pronounced in high-grade gliomas, where aggressive resections are prioritized to optimize oncological outcomes (Wang et al., 2019; Klein et al., 2012).

Adjuvant therapies compound these effects. Radiotherapy induces diffuse white matter injury, hippocampal atrophy, and chronic neuroinflammation, producing delayed deficits in attention, memory, and processing speed (Schlömer et al., 2022). Chemotherapeutic agents such as temozolomide contribute to mitochondrial dysfunction and impaired neurogenesis, further amplifying cognitive decline (Cascella et al., 2018). The cumulative effects of these modalities create a multifactorial pattern of neurotoxicity that persists long after treatment completion.

Despite the high prevalence of cognitive sequelae, rehabilitation efforts remain fragmented and underutilized. Most interventions adopt generic training tasks without accounting for individual variability in tumor biology, neural network disruption, or baseline neuroplastic potential (Iqbal & Ashraf, 2024). Consequently, cognitive dysfunctions are often underdiagnosed and poorly addressed in routine clinical care (Jamora et al., 2022).

Translational research now highlights recovery mechanisms such as network reorganization and neurotrophic modulation. However, these insights have yet to be systematically integrated into clinical protocols (Cayuela et al., 2025).

Bridging this translational gap will require precision-based rehabilitation approaches that combine neuroimaging biomarkers, molecular profiling, and individualized therapy design—ensuring cognitive care becomes a standard component of neuro-oncological practice (Tran, 2003).

3. Mechanisms of Cognitıve Impairment in Gliomas

Cognitive dysfunction in glioma patients arises from a multifactorial disruption of brain integrity, involving structural invasion, inflammatory cascades, and tumor-driven molecular alterations. Beyond the direct effects of mass effect and cerebral edema, gliomas profoundly affect cortical and subcortical circuits, resulting in deficits in attention, memory, and executive function (van Kessel et al., 2021).

One of the primary mechanisms is white matter infiltration. Gliomas invade and disrupt critical fiber tracts, impairing the structural and functional connectivity required for efficient cognitive processing. Advanced neuroimaging studies have shown that disruptions in the default mode network, attention networks, and language-related tracts strongly correlate with domain-specific cognitive impairments in glioma patients (Friedrich et al., 2023). Moreover, rich-club disorganization-the breakdown of highly interconnected hub regions-has been observed particularly in frontal and temporal tumors and is associated with significant impairments in memory, attention, and processing speed (Liu et al., 2020).

Neuroinflammation is another major contributor to cognitive decline. Elevated circulating levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and intercellular adhesion molecule-1 (ICAM-1) have been documented in glioma patients with cognitive dysfunction, independent of IDH mutation status (Slattery et al., 2025). These inflammatory mediators impair synaptic signaling, disrupt vascular integrity, and contribute to neuronal and glial dysfunction, exacerbating cognitive deficits (Guedes et al., 2024).

At the molecular level, genetic and epigenetic features of gliomas also influence cognitive outcomes. IDH-mutant gliomas are generally associated with better-preserved cognitive performance and higher neuroplastic potential, whereas IDH-wildtype tumors and more aggressive molecular phenotypes, such as those characterized by elevated neuroligin-3 (NLGN3) expression and reduced brain-derived neurotrophic factor (BDNF) signaling, are linked to poorer cognitive trajectories (van Kessel et al., 2021; Zhang et al., 2024). These molecular differences not only determine tumor growth dynamics but also

modulate synaptic remodeling and plasticity, both of which are critical for cognitive resilience and recovery potential.

Taken together, the interplay of network disruption, neuroinflammatory signaling, and molecular heterogeneity generates a complex landscape of cognitive impairment in glioma patients. Understanding these mechanisms provides the foundation for developing precision-based rehabilitation strategies tailored to the neurobiological profile of each patient.

4. Neuroınflammatıon-Demyelinatıon

Gliomas foster a chronic pro-inflammatory microenvironment that substantially contributes to cognitive decline. This inflammatory state is characterized by the release of cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6), alongside activation of microglia and astrocytes (Iqbal & Ashraf, 2024; Saggu et al., 2016). Sustained inflammation disrupts neuronal signaling, reduces synaptic plasticity, and promotes excitotoxicity, ultimately impairing cognitive function.

One major downstream consequence of this neuroinflammatory cascade is demyelination, which reduces axonal conduction velocity and compromises the integrity of white matter tracts essential for attention, memory, and processing speed. Longitudinal neuroimaging studies have demonstrated that diffuse myelin loss and reduced structural connectivity strongly correlate with progressive cognitive deterioration in glioma patients (Kövari et al., 2004).

Therapeutic interventions can further exacerbate these effects. Radiotherapy and alkylating chemotherapies such as temozolomide induce mitochondrial stress, oligodendrocyte toxicity, and impaired neurogenesis, accelerating the breakdown of white matter architecture (Matsos et al., 2017; Taraboletti & Fornace, 2019). Over time, astrocytic NF-κB activation leads to chronic gliosis and axonal degeneration (Saggu et al., 2016).

Collectively, inflammation-driven demyelination forms a critical mechanistic bridge between tumor biology, treatment toxicity, and cognitive impairment. These findings underscore the potential of anti-inflammatory and remyelination-focused strategies—not only to mitigate decline but also to enhance responsiveness to cognitive rehabilitation.

5. Genetic Mutatıons and Cognitive Profıles

The molecular landscape of gliomas plays a pivotal role in shaping cognitive outcomes, reflecting the interplay between tumor biology, network disruption, and neural resilience. Among the most clinically relevant markers are isocitrate dehydrogenase (IDH1/2) mutations, which are associated with slower tumor

growth, better cellular differentiation, and more preserved functional connectivity. These biological characteristics translate into more favorable neurocognitive trajectories and improved postoperative cognitive recovery compared with IDH-wildtype tumors (Lee & Jang, 2017; Mukasa et al., 2012).

In contrast, IDH-wildtype gliomas, particularly those exhibiting high-grade molecular signatures, are more aggressive and exhibit a greater propensity for infiltrating cortical and subcortical networks. This aggressive biology accelerates cognitive decline and often limits the capacity for network reorganization and neuroplastic adaptation.

Mutations in TP53, commonly observed in astrocytic tumors, are associated with diffuse tumor infiltration and remote recurrence patterns, leading to poorer neurocognitive prognoses (Nakae et al., 2017). Conversely, 1p/19q co-deletions, frequently found in oligodendrogliomas, are linked to prolonged survival and milder cognitive impairment, often in the context of concurrent IDH mutations. This combination defines a distinct and relatively favorable molecular signature (Appay et al., 2018; Ichimura et al., 2009).

These genomic and molecular profiles do more than inform prognosis; they also influence the brain's adaptive potential. IDH-mutant gliomas, for example, may support early cortical remodeling even prior to surgical intervention, enabling greater functional compensation and resilience to cognitive decline (Lee & Jang, 2017). As multi-omics approaches and connectomic analyses continue to advance, integrating molecular, imaging, and neuropsychological data will be critical for the development of precision-guided cognitive rehabilitation strategies tailored to each patient's unique neurobiological profile.

6. Neurobiological Foundations of Cognitive Rehabilitatıon

The potential for cognitive recovery in glioma patients is underpinned by the brain's inherent capacity for neuroplasticity - a dynamic process that enables structural, functional, and molecular adaptations within neural circuits following injury. This plasticity provides the biological basis for compensatory mechanisms, allowing spared or reorganized brain regions to assume the functions of areas compromised by tumor growth or treatment (Berlucchi, 2011). Understanding these mechanisms is essential for the development of personalized, evidence-based rehabilitation protocols that optimize recovery potential.

Neuroplasticity: Cortical Reorganization and Synaptic Remodeling

Neuroplasticity involves the brain's ability to reorganize neural networks and remodel synaptic connections in response to both injury and rehabilitative stimulation. In glioma, this reorganization occurs in two primary ways:

perilesionally, where adjacent cortical regions adapt to assume lost functions, and contralesionally, where homologous areas in the opposite hemisphere are recruited to support recovery (Duffau, 2017).

Functional neuroimaging studies have demonstrated task-dependent network reorganization following tumor resection, particularly in language and executive domains. Increased activation of contralateral Broca's and Wernicke's areas has been observed in patients undergoing resections in language-dominant hemispheres, indicating interhemispheric compensatory shifts (Duffau, 2017; Ekert et al., 2024). Similarly, reorganization of the frontoparietal control networks has been linked to compensation for deficits in attention and executive functioning (Poologaindran et al., 2021).

At the cellular and molecular level, these adaptive changes are mediated by synaptic remodeling processes such as synaptogenesis, dendritic spine formation, and long-term potentiation (LTP). These mechanisms are driven by neurotrophic pathways, particularly the brain-derived neurotrophic factor (BDNF) and intracellular signaling cascades such as the MAPK/ERK and PI3K/Akt pathways, which are integral to synaptic strength, neural survival, and plasticity (Levin, 2006).

Emerging evidence indicates that interventional neurorehabilitation strategies, including non-invasive brain stimulation and AI-assisted cognitive training, can enhance these neuroplastic processes, accelerating recovery and maximizing functional outcomes (Ekert et al., 2024).

7. Neuroimaging Evidence of Adaptive Changes

Advances in neuroimaging technologies have been pivotal in elucidating the neural substrates of cognitive recovery and neuroplasticity in glioma patients. Modalities such as functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), and magnetoencephalography (MEG) provide complementary insights into both structural and functional reorganization during and after treatment.

Resting-state and task-based fMRI studies consistently demonstrate dynamic restoration of functional connectivity within key cognitive networks. Post-surgical reorganization within the default mode network (DMN) and frontoparietal control systems has been correlated with improved executive function and memory performance during rehabilitation (Rodríguez-García et al., 2023). Moreover, task-related fMRI frequently shows a shift in language processing from the dominant hemisphere to contralateral homologous regions, particularly in patients undergoing resections in eloquent cortical areas (Quiñones et al., 2021; Zimmermann et al., 2019).

DTI offers valuable insights into microstructural integrity and white matter remodeling during recovery. In patients demonstrating significant post-treatment cognitive improvement, longitudinal increases in fractional anisotropy and enhanced tract coherence have been observed in associative pathways, including the arcuate fasciculus and cingulum bundle, reflecting compensatory white matter reorganization (Zheng et al., 2023).

High temporal-resolution techniques such as MEG and EEG further enrich our understanding of neural adaptation. MEG studies reveal increased right-hemispheric activation and interhemispheric recruitment during language and executive tasks post-resection, indicating real-time network compensation and reorganization (Zimmermann et al., 2019; Quiñones et al., 2021). Complementary EEG findings demonstrate adaptive changes in cortical oscillatory dynamics, which correlate with improvements in attention and processing speed during structured rehabilitation programs (Laatsch et al., 2004).

Collectively, these multimodal neuroimaging approaches highlight the dynamic and individualized nature of neuroplasticity in glioma patients. Beyond providing mechanistic insights, such imaging biomarkers have the potential to predict responsiveness to rehabilitation, enabling the design of precision-guided cognitive interventions tailored to each patient's unique neural profile.

8. Exercise and Neurotrophic Factor Modulation

Physical exercise is increasingly recognized as a potent modulator of neuroplasticity in both healthy individuals and clinical populations, including patients with glioma. Aerobic and resistance training have been shown to stimulate the release of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF), all of which play pivotal roles in neuronal repair, synaptic plasticity, and cognitive performance (Hötting & Röder, 2013; Budde et al., 2016).

Among these, BDNF is particularly significant, as it enhances synaptic remodeling, supports dendritic spine growth, and facilitates long-term potentiation (LTP) within hippocampal and cortical circuits, key processes underlying learning and memory (Sleiman & Chao, 2015). IGF-1 and VEGF further promote angiogenesis and neurogenesis, fostering an enriched microenvironment conducive to neural repair and functional recovery (Ben-Zeev et al., 2022).

Beyond molecular modulation, exercise has been shown to exert anti-inflammatory effects, reducing systemic and central nervous system

inflammation, and promoting hippocampal neurogenesis. These effects are particularly important in mitigating treatment-related cognitive decline, which is often exacerbated by neuroinflammation and white matter damage. Evidence from preclinical and clinical studies demonstrates that structured physical activity can lead to increased hippocampal volume, improved white matter integrity, and measurable gains in executive function (Liang et al., 2021; Ezzdine et al., 2025).

Moreover, exercise appears to act synergistically with cognitive training, amplifying the effects of rehabilitation. Physical activity primes the brain for enhanced learning and adaptive plasticity by increasing neurotrophic availability and improving functional connectivity (Hötting & Röder, 2013). This synergy highlights the potential of integrated rehabilitation protocols, where tailored exercise regimens complement cognitive therapy to optimize recovery and quality-of-life outcomes in glioma survivors.

9. Translational and Clinical Evidence

A growing body of translational and clinical research underscores the biological plausibility and therapeutic potential of cognitive rehabilitation in glioma patients. Recovery of cognitive function is increasingly understood as a process driven by experience-dependent neuroplasticity, which can be harnessed through targeted behavioral, physical, and technological interventions.

Randomized controlled trials (RCTs) have provided encouraging evidence of efficacy. For example, Gehring et al. (2009) demonstrated that a structured cognitive rehabilitation program, combining computer-based attention retraining with compensatory strategies, led to improvements in verbal memory and attention at six-month follow-up, even when immediate post-treatment effects were modest. Similarly, Maschio et al. (2015) reported significant gains in short-term verbal and visuospatial memory among glioma patients experiencing tumor-related epilepsy following cognitive training.

Technology-assisted approaches are emerging as scalable, patient-friendly options. The ReMind tablet-based application, evaluated by van der Linden et al. (2021), demonstrated high adherence and patient satisfaction, although objective outcomes were not significantly superior to standard care. These findings suggest that digital tools may support accessibility and engagement, particularly when integrated into multimodal rehabilitation protocols.

Recent retrospective studies have expanded this evidence base. Wu et al. (2024) found that incorporating memory therapy into routine postoperative care significantly improved cognitive performance, reduced cancer-related fatigue,

and alleviated anxiety in glioma patients, with improvements persisting during 12-month follow-up periods.

Collectively, these findings highlight the importance of integrating neurobiologically informed rehabilitation strategies into standard care. However, heterogeneity in tumor biology, treatment regimens, and individual resilience continues to challenge the development of standardized protocols. Translational studies that integrate neuroimaging biomarkers, molecular profiling, and behavioral outcomes are increasingly critical for tailoring interventions to maximize clinical benefit and long-term survivorship.

10. Randomized Controlled Trials and Neuroimaging-Supported Studies

Although the evidence base for cognitive rehabilitation in glioma patients is less extensive than that for other neurological conditions, randomized controlled trials (RCTs) and neuroimaging studies provide growing support for its effectiveness. A landmark multicenter RCT by Gehring et al. (2009) demonstrated that a structured rehabilitation program-combining computer-based attention retraining with compensatory strategy training-yielded significant improvements in attention and verbal memory at six-month follow-up in patients with low-grade and anaplastic gliomas. Participants also reported reductions in mental fatigue, indicating both objective and subjective benefits.

Functional neuroimaging has provided crucial mechanistic insights into these behavioral outcomes. For instance, functional MRI (fMRI) studies in related neurological populations have revealed increased activation in task-relevant areas, including the dorsolateral prefrontal cortex and inferior temporal lobe, after structured cognitive interventions (Díez-Cirarda et al., 2016). In glioma populations specifically, resting-state fMRI has demonstrated partial restoration of connectivity within disrupted networks, such as the default mode network (DMN) and frontoparietal control systems, following cognitive or behavioral therapies (Wang et al., 2019). These findings highlight the capacity for network-level reorganization, reinforcing the biological plausibility of targeted rehabilitation protocols.

Pharmacological adjuncts, including methylphenidate and modafinil, have also been explored, though results remain mixed due to small sample sizes, heterogeneous treatment protocols, and variability in outcome measures. Recent work suggests that integrating neuroimaging biomarkers-such as diffusion tensor imaging (DTI) and functional connectivity mapping-can help predict which patients are most likely to respond to such interventions, facilitating a precision-medicine approach to cognitive rehabilitation (van der Linden et al., 2018).

11. Longitudinal Observations and Survivorship Data

Longitudinal studies have provided critical insights into the dynamic trajectory of cognitive function in glioma patients, illustrating how outcomes evolve across the disease continuum. Cognitive performance is shaped by a complex interplay of tumor biology, treatment intensity, baseline cognitive reserve, and neuroplastic potential.

Among molecular predictors, IDH mutation status consistently emerges as a key determinant of cognitive outcomes. Patients with IDH-mutant gliomas typically demonstrate preserved functional network integrity, slower cognitive decline, and greater potential for adaptive reorganization compared with those harboring IDH-wildtype tumors (Wang et al., 2019). This molecular advantage translates into better functional recovery and higher quality of life during survivorship.

The extent and location of surgical resection further modulate recovery trajectories. Maximal safe resections are associated with better local disease control but carry varying risks of functional disruption, particularly in eloquent cortical and subcortical regions. Adjuvant therapies-including radiotherapy and chemotherapy-also influence long-term neurocognitive outcomes, with cumulative exposure often correlating with more pronounced deficits over time.

Timing of rehabilitation plays a pivotal role. Data suggest that initiating cognitive rehabilitation within the first six months following treatment yields more robust and durable improvements in attention, memory, and executive functioning compared with delayed interventions (Gehring et al., 2009; Hansen et al., 2020). This window of heightened plasticity underscores the importance of early, structured, and individualized rehabilitation protocols to capitalize on the brain's adaptive capacity during the early recovery phase.

Collectively, these longitudinal observations reinforce the need for continuous cognitive monitoring throughout the treatment and survivorship trajectory. Such approaches not only guide tailored rehabilitation strategies but also inform prognostic counseling and quality-of-life planning for patients and caregivers.

12. Treatment-Specific Rehabilitation Strategıes in Glioma Care

The growing understanding of glioma heterogeneity has underscored the importance of individualized rehabilitation strategies that align with each patient's tumor biology, neural network profile, and cognitive needs.

Patients with IDH-mutant gliomas often exhibit preserved functional connectivity and greater neuroplastic potential, making them more responsive to network-preserving interventions and gradual, intensive cognitive retraining.

These targeted strategies aim to minimize disruption to critical neural pathways while optimizing the recruitment of compensatory networks, ultimately supporting better neurocognitive outcomes (Derks et al., 2019; Persico et al., 2022).

For patients with significant white matter disruption, particularly those with infiltration or treatment-induced injury, multimodal rehabilitation approaches are recommended. These protocols typically integrate structured cognitive training with non-invasive neuromodulation techniques, such as transcranial direct current stimulation (tDCS) or transcranial magnetic stimulation (TMS). Evidence suggests that combining neuromodulation with cognitive exercises enhances neuroplasticity, facilitates functional reorganization, and promotes measurable improvements in domains such as attention, memory, and executive function (Gehring et al., 2009).

Advances in artificial intelligence (AI) and connectomic mapping are transforming how rehabilitation strategies are designed and implemented. AI-guided platforms leverage patient-specific neuroimaging and molecular data to generate precision rehabilitation protocols that adapt in real time to patient progress and neural responses (Akbari et al., 2024; Davatzikos et al., 2020). By integrating connectomic analysis, these frameworks provide a nuanced understanding of functional network disruptions, allowing interventions to be tailored to each patient's unique neurobiological profile.

Furthermore, the concept of digital twins-virtual models of individual patients that simulate responses to various therapies-is emerging as a promising tool for predicting outcomes and optimizing rehabilitation intensity and modality (Innani et al., 2024; Califano, 2015). Although still experimental, such technologies highlight the potential for adaptive, closed-loop rehabilitation systems that continuously refine therapy delivery for maximal efficacy.

Collectively, these treatment-specific frameworks represent a significant shift from generic rehabilitation models toward personalized, mechanism-driven protocols. By aligning interventions with tumor biology, network integrity, and patient-specific factors, these approaches have the potential to improve both functional recovery and long-term quality of life for glioma survivors.

13. White Matter Disruption and Multimodal Rehabilitation

White matter tract injury, whether resulting from tumor infiltration, surgical resection, or adjuvant therapies such as radiotherapy and chemotherapy, presents a major barrier to effective cognitive recovery in glioma patients. Disruption of white matter integrity compromises the efficiency of neural

communication across cognitive networks, leading to persistent deficits in attention, executive function, and memory.

To address these challenges, multimodal rehabilitation approaches have gained traction. These strategies combine cognitive training programs with non-invasive neuromodulation techniques, particularly transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS). Evidence suggests that such combinations facilitate synaptic plasticity and functional network reorganization, offering measurable improvements in multiple cognitive domains (Gehring et al., 2009).

Mechanistically, neuromodulation enhances cortical excitability and functional connectivity, thereby creating a more receptive neural environment for learning during cognitive exercises. When integrated into structured rehabilitation protocols, tDCS and TMS have been shown to accelerate adaptive reorganization within disrupted cognitive networks, particularly in regions subserving attention and executive control. This synergistic effect underscores the importance of precision-targeted neuromodulation, tailored to the individual's neuroimaging profile and cognitive baseline.

Looking ahead, advanced neuroimaging and connectomic techniques are expected to refine these protocols further. By mapping the structural and functional integrity of white matter tracts, clinicians can customize neuromodulation parameters and cognitive task intensity to maximize rehabilitation gains, even in patients with extensive white matter compromise.

14. AI-Driven Precision Frameworks and Connectomics

The integration of artificial intelligence (AI) and connectomic science is reshaping the landscape of cognitive rehabilitation for glioma patients. Traditional one-size-fits-all rehabilitation models are increasingly being replaced by precision frameworks that leverage individualized neuroimaging, molecular, and clinical data to optimize therapy planning and delivery (Akbari et al., 2024; Davatzikos et al., 2020).

AI-driven platforms can analyze complex, multimodal datasets-ranging from functional and structural connectivity maps to genomic profiles-to generate patient-specific rehabilitation protocols. These systems adapt dynamically as new patient data emerge, enabling real-time personalization of therapy intensity, modality, and duration. This level of responsiveness allows clinicians to adjust interventions proactively, maximizing functional gains while minimizing cognitive fatigue and therapy-related burden.

Connectomic mapping, particularly using advanced techniques such as high-resolution diffusion tensor imaging (DTI) and resting-state functional MRI (rs-

fMRI), provides detailed insights into the structural and functional organization of brain networks. By identifying key nodes and pathways disrupted by the tumor or its treatment, connectomic analyses can highlight residual neural hubs with the greatest potential for adaptive reorganization. These findings can guide targeted stimulation, network-specific cognitive training, or multimodal interventions aimed at reinforcing functional recovery pathways.

The integration of AI with connectomics also opens new avenues for predictive modeling. By simulating potential outcomes based on a patient's unique neurobiological profile, clinicians can anticipate recovery trajectories and optimize treatment strategies before initiating therapy. Although still emerging, these precision frameworks represent a paradigm shift toward data-driven, individualized rehabilitation, offering the potential for improved neurocognitive outcomes and enhanced quality of life for glioma survivors.

15. Digital Platforms and Closed-Loop Systems

Digital technologies are rapidly transforming cognitive rehabilitation for glioma patients, offering scalable and adaptable solutions that complement traditional therapy. AI-enhanced platforms are particularly promising, as they allow for real-time monitoring and adaptive interventions that respond dynamically to patient performance and engagement levels (Innani et al., 2024; Califano, 2015).

One innovative concept gaining traction is the development of digital twins-virtual patient-specific models that integrate neuroimaging, molecular, and behavioral data to simulate responses to various rehabilitation protocols. By predicting how an individual's brain networks might adapt to specific interventions, digital twins have the potential to optimize therapy selection and dosing, ensuring that rehabilitation strategies are both efficient and personalized.

In addition to predictive modeling, closed-loop systems are being explored to create continuous feedback mechanisms during therapy. These systems collect real-time data on cognitive performance, neural activity, and physiological metrics, allowing for immediate adjustments to therapy parameters. For example, if a patient exhibits fatigue or plateauing in progress, the system can automatically reduce task difficulty or alter stimulation intensity to maintain engagement and therapeutic efficacy.

The integration of digital platforms into clinical practice also enhances accessibility, enabling patients to participate in rehabilitation remotely while maintaining continuous communication with clinical teams. This is particularly

valuable for glioma survivors facing mobility challenges or residing far from specialized centers.

While these technologies are still emerging, preliminary evidence suggests that digitally augmented and closed-loop rehabilitation models can enhance engagement, promote neuroplasticity, and support more consistent functional gains. Future clinical trials are needed to validate their long-term effectiveness, cost-efficiency, and feasibility for widespread clinical adoption.

16. Non-Invasive Stimulation and Pharmacological Synergy

The combination of non-invasive brain stimulation techniques with pharmacological agents represents a promising frontier in cognitive rehabilitation for glioma patients. Among these, transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) have shown the ability to enhance cortical excitability, promote synaptic plasticity, and facilitate network-level reorganization when integrated with structured cognitive training (Gehring et al., 2009).

tDCS delivers low-intensity electrical currents to targeted cortical regions, modulating neuronal membrane potentials and enhancing the efficiency of ongoing cognitive exercises. Similarly, TMS uses magnetic fields to induce localized neuronal firing, strengthening functional connectivity within disrupted networks. When combined with rehabilitation protocols, these approaches can accelerate adaptive plasticity, leading to measurable improvements in attention, memory, and executive function.

In parallel, pharmacological interventions are being investigated to augment neuroplasticity and cognitive recovery. Selective serotonin reuptake inhibitors (SSRIs), for example, have been associated with increased neurogenesis and synaptic remodeling, while dopamine modulators may enhance executive functioning by improving frontostriatal network efficiency (Anwer & Abdel-Rasol, 2025). When combined with tDCS or TMS, these agents may provide synergistic effects, creating a neurochemical environment that facilitates more robust cognitive gains.

Although early-phase studies are encouraging, the evidence base remains limited by small sample sizes, heterogeneous protocols, and a lack of standardized outcome measures. To fully realize the potential of these combined approaches, rigorous clinical trials with standardized dosing regimens, precise neuroimaging endpoints, and long-term follow-up are needed. These studies should also explore patient-specific predictors of response, such as molecular subtype, baseline network integrity, and genetic variability, to guide personalized stimulation and pharmacological strategies.

17. Lifestyle Interventions and Neurotrophic Support

Lifestyle-based interventions are increasingly recognized as critical adjuncts to formal cognitive rehabilitation in glioma care. Among these, aerobic exercise and targeted nutritional strategies have shown substantial potential to enhance neuroplasticity and support functional recovery by modulating neurotrophic and metabolic pathways (Persico et al., 2022).

Regular aerobic exercise has been shown to upregulate key neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1). These molecules are central to synaptic plasticity, neuronal survival, and angiogenesis, creating a neurobiological environment that facilitates learning, memory, and overall cognitive performance. Importantly, exercise-induced increases in BDNF are correlated with improved neuropsychological outcomes, including better executive function and processing speed in both healthy individuals and clinical populations.

Nutritional interventions may further augment these benefits. Diets rich in omega-3 fatty acids, antioxidants, and polyphenols have been associated with enhanced neurotrophic signaling, reduced neuroinflammation, and improved synaptic efficiency. Such dietary approaches may be particularly valuable in mitigating the pro-inflammatory and oxidative stress states common in glioma patients undergoing multimodal treatment.

Emerging evidence also highlights a synergistic interaction between lifestyle interventions and structured cognitive or physical rehabilitation. For instance, exercise not only primes neural circuits for adaptive plasticity but also enhances the efficacy of cognitive training, resulting in more durable and generalized improvements in functional domains. Similarly, optimal nutritional support may help sustain metabolic resilience and reduce cognitive fatigue, further improving patient adherence to rehabilitation protocols.

While the evidence base is still evolving, integrating exercise and nutrition into comprehensive rehabilitation frameworks holds considerable promise for improving quality of life and long-term cognitive outcomes in glioma survivors. Future research should focus on personalized lifestyle prescriptions, guided by individual neurobiological profiles, to maximize their neuroprotective and neurorestorative potential.

18. Future Directions

Future progress in cognitive rehabilitation for glioma patients will depend on multidisciplinary collaboration between neuro-oncologists, neuropsychologists, and computational neuroscientists. Advances in molecular profiling,

neuroimaging, and artificial intelligence hold potential for precision-based interventions, but large-scale validation is needed. Prospective, multi-center clinical trials incorporating biomarker-guided protocols and long-term follow-up will be essential to determine real-world effectiveness. Integrating cognitive monitoring into standard care pathways may further optimize quality of life and functional outcomes for glioma survivors. Despite encouraging findings, the current literature on cognitive rehabilitation in glioma patients faces several methodological limitations. Most available studies are limited by small sample sizes, heterogeneous tumor grades, and variability in cognitive assessment tools. Follow-up durations are often short, and the absence of standardized outcome measures complicates cross-study comparisons. Moreover, publication bias and selective reporting of positive outcomes may overestimate the true efficacy of interventions. There is also a lack of replication in large-scale, multi-center randomized controlled trials. Addressing these gaps is essential to establish the robustness and generalizability of current evidence.

19. Conclusion

Cognitive dysfunction remains a pervasive and underrecognized burden in glioma care, arising from complex interactions between tumor biology, treatment-induced neurotoxicity, and network disruption. Recent advances in neuroimaging, molecular profiling, and rehabilitation science have begun to reveal the mechanisms of neuroplastic recovery. Integrating these insights into clinical practice through personalized, biomarker-guided rehabilitation represents a critical next step. Moving forward, rigorous validation, early intervention, and multidisciplinary collaboration will be essential to transform cognitive rehabilitation from an adjunctive therapy into a standard component of comprehensive glioma management.

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Chapter 3

Mechanistic Pathways of Cognitıve Recovery

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Fundamental Audiological Perspectives on Inner Ear Malformations

Congenital inner ear malformations are among the most important causes of congenital hearing loss and represent a critical determinant in the audiological assessment and rehabilitation process. Structural abnormalities that arise during the early stages of embryological development can affect sensory structures such as the cochlea, vestibular system, and auditory nerve to varying degrees, resulting in a wide clinical spectrum. These malformations should be regarded not merely as anatomical variations but as a multidimensional problem area that directly shapes auditory processing, frequency resolution, temporal characteristics, vestibular function, and neuroplasticity-based auditory developmental processes. Congenital ear malformations are among the most important causes of congenital hearing loss and represent a critical determinant in the audiological assessment and rehabilitation process (Sennaroğlu & Saatçi, 2002). Structural abnormalities that arise during the early stages of embryological development can affect sensory structures such as the cochlea, vestibular system, and auditory nerve to varying degrees, resulting in a wide clinical spectrum. These malformations should be regarded not merely as anatomical variations but as a multidimensional problem area that directly shapes auditory processing, frequency resolution, temporal characteristics, vestibular function, and neuroplasticity-based auditory developmental processes. Although the widespread use of advanced imaging techniques has facilitated the identification of inner ear anomalies, relating these findings to audiological outcomes remains a complex process that requires interdisciplinary expertise. Even different malformation types classified under the same radiological category may present highly heterogeneous auditory profiles. This variability necessitates the comprehensive use of an audiological test battery, multidimensional evaluation of auditory performance, and long-term follow-up. One of the greatest challenges in the clinical management of inner ear malformations is the unpredictability of hearing aid performance and the

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anatomical risks associated with cochlear implant surgery. Responses to electrical stimulation, electrode placement, the status of residual hearing, and the integrity of the auditory nerve are key factors that determine the success of treatment. Therefore, specialists in audiology must approach inner ear malformations not merely as radiological findings, but as complex biological structures that shape auditory development from the outset. This section will provide an overarching framework for the embryological origins, classification systems, expected audiological findings, vestibular involvement profiles, and rehabilitation approaches associated with inner ear malformations. The aim is to equip clinicians with both theoretical knowledge and practical management strategies, thereby supporting the development of more effective, individualized, and evidence-based approaches for this complex patient population. Inner ear malformations arise as a result of arrested or abnormal development during specific stages of embryogenesis and represent important anatomical variations that directly influence audiological assessment and clinical management. According to current practice, the most widely used system is the classification proposed by Sennaroğlu and Saatci (2002). This framework is organized according to embryological weeks of development and serves as a highly informative guide for both clinicians and surgeons.

1. Complete Labyrinthine Aplasia (Michel Deformity)

The most severe and rare type within this classification is Complete Labyrinthine Aplasia (CLA), also known in the literature as Michel Deformity, and it is characterized by the arrest of embryonic membranous labyrinth development around the third week of gestation. CLA may present with different anatomical variants (Sennaroğlu & Yaralı., 2022). The first group is CLA accompanied by hypoplastic or aplastic petrous bone; in this condition, the petrous bone is either underdeveloped or absent, and the middle ear structures are typically adjacent to the posterior fossa.

This anatomical variation not only creates challenges in radiological and surgical evaluation but also results in complete sensorineural hearing loss, and cochlear implant placement is not possible due to the absence of anatomical structures; therefore, alternative approaches such as an auditory brainstem implant (ABI) are required for auditory rehabilitation (Ozgen et al., 2009).

The second group includes CLA without an otic capsule; despite normal development of the petrous bone, the otic capsule is hypoplastic or aplastic. In this case, the bony shell of the cochlea and vestibular labyrinth is insufficient, leading to complete loss of auditory function, and conventional hearing aids or cochlear implants are ineffective. Due to the absence of vestibular structures,

balance and motor development are severely affected.The third group consists of CLA with partially preserved otic capsule variants; although the cochlea and vestibular structures are absent or hypoplastic, the otic capsule may be partially present in some cases. Audiologically, hearing loss remains completely sensorineural, and rehabilitation options are limited. While partial presence of the otic capsule may facilitate radiological diagnosis, it provides only limited advantage for surgical or rehabilitative planning (Sennaroğlu & Bajin, 2022).

Audiologically, Complete Labyrinthine Aplasia is typically associated with complete sensorineural hearing loss, and this loss is generally bilateral. Hearing threshold measurements may be unobtainable or may exceed 120 dB. Stapedial and acoustic reflexes are typically absent, and otoacoustic emission (OAE) tests yield negative results because, even if the outer and middle ear are normal, the cochlear structure is absent. Cochlear implantation is not feasible in this condition; therefore, auditory rehabilitation strategies often rely on alternative methods such as auditory brainstem implants (ABI). Due to the absence of the vestibular system, balance and postural control are severely affected, and infants may exhibit delays in motor development. For this reason, a multidisciplinary approach, comprehensive audiological evaluation, and early rehabilitation planning are crucial in the management of patients with Michel Deformity (Sennaroğlu & Bajin, 2007; Ozgen et al., 2009).

2. Rudimentary Otocyst

A rudimentary otocyst is a rare malformation that results from an arrest in inner ear development at a very early embryologic stage. Embryologically, it is characterized by the failure of cochlear and vestibular structures to form around the third week of gestation. In this condition, the inner ear structures are not fully developed; instead, only a rudimentary (primitive) inner ear cavity or cystic structure is observed, and the cochlea, vestibular system, and otic capsule are typically hypoplastic or absent (Sennaroğlu & Saatçi, 2002; Brutto el al., 2023).

From an audiological perspective, patients with a rudimentary otocyst typically present with profound sensorineural hearing loss, with thresholds too elevated to be measured. Stapedial and acoustic reflexes are absent, and otoacoustic emission tests yield negative results. Cochlear implantation is generally not feasible due to the severe anatomical deficiencies; therefore, auditory brainstem implantation (ABI) is the preferred method for auditory rehabilitation. In addition, because the rudimentary structure provides inadequate vestibular function, balance disorders and delays in motor development may be observed. For this reason, early diagnosis and a

multidisciplinary approach are critical in the management of rudimentary otocyst cases (Sennaroğlu & Bajin, 2007).

3. Cochlear Aplasia

Cochlear aplasia is a malformation characterized by the complete absence of the cochlear portion of the inner ear. Embryologically, it results from an arrest in cochlear development around the fourth week of gestation. Cochlear aplasia may present with different radiologic variants. In some cases, the vestibular system and semicircular canals are normally developed, with only the cochlea being absent. In this subtype, preservation of the vestibular structures suggests that balance function may be only partially affected. In another variant, the vestibule appears dilated and enlarged; this presentation often accompanies cochlear absence and indicates a more severe compromise of vestibular function (Sennaroğlu & Bajin, 2007).

From an audiological perspective, patients with cochlear aplasia typically exhibit profound sensorineural hearing loss, with thresholds too elevated to be measured. Stapedial and acoustic reflexes are usually absent, and otoacoustic emission tests yield negative results. The condition of the vestibular structures plays an important role in postural control and motor development: while vestibular function may be partially preserved in cases with a normal labyrinth, significant balance disturbances and delays in motor development are common in those with a dilated vestibule. Cochlear implantation is not feasible due to the anatomical absence of the cochlea; therefore, alternative auditory rehabilitation options such as the auditory brainstem implant (ABI) are considered (Sennaroğlu & Bajin, 2007; Hota et al., 2023).

3. Common Cavity

Common cavity is a rare type of inner ear malformation in which the cochlea and vestibule fail to separate and instead form a single cystic space. Embryologically, this anomaly results from the incomplete separation of the cochlear and vestibular structures around the fourth week of gestation. Radiologically, no distinction is observed between the cochlea and the vestibule; the entire labyrinth appears as a single cystic cavity. The semicircular canals are typically hypoplastic or incompletely developed (Sennaroğlu & Bajin, 2007).

From an audiological perspective, patients with a common cavity usually present with severe to profound sensorineural hearing loss. Hearing thresholds often exceed 100–120 dB, and otoacoustic emission (OAE) tests are negative. Stapedial and acoustic reflexes are absent. Although cochlear implantation is

anatomically possible, electrode placement is challenging, and auditory outcomes tend to be more variable than in individuals with a normal cochlea.

Due to the inadequate vestibular structures, balance disorders are common, and motor development delays are frequently observed in infants. Therefore, the management of common cavity malformation requires special attention and a multidisciplinary approach in both audiological assessment and surgical planning (Mahboob et al., 2022).

5. Cochlear Hypoplasia

Cochlear hypoplasia is one of the rare inner ear malformations in which the cochlea is underdeveloped but not completely absent. Embryologically, it results from an arrest in cochlear development around the sixth week of gestation. Radiologically, the cochlea is reduced in size and its spiral morphology is altered.

Cochlear hypoplasia is classified into four subtypes: Type 1 (non-cystic hypoplasia), Type 2 (cystic hypoplasia), Type 3 (Mondini deformity / Incomplete Partition Type II), and Type 4 (small cochlea) (Sennaroğlu & Bajin, 2007)**.**

From an audiological perspective, in Type 1 the cochlea is small but non-cystic; hearing loss is typically severe sensorineural, with auditory thresholds frequently ranging between 90–110 dB. Otoacoustic emissions (OAEs) are absent, and stapedial reflexes are generally not elicited. In Type 2, the presence of cystic morphology and partial modiolus formation may allow minimal residual hearing at low frequencies, despite the presence of profound loss at most frequencies; OAEs are still typically absent. Type 3, or Mondini deformity, is characterized by a cochlea with approximately 1–1.5 turns and a cystic apical component. Residual low-frequency hearing may be present, and hearing loss ranges from severe to profound. Electrophysiological assessments (ABR) usually show elevated response thresholds and are important when considering candidacy for cochlear implantation. In Type 4, the cochlea is small but segmental structures are preserved; hearing loss is variable and may range from moderate to severe sensorineural, with some cases exhibiting residual low-frequency hearing (Brutto et al., 2021)**.** The condition of the vestibular system is also critical in audiological assessment. Patients with cochlear hypoplasia may exhibit semicircular canal hypoplasia or partial absence, which can lead to abnormalities on balance tests (posturography, rotational head movement tests, videonystagmography). Residual vestibular function may be partially preserved in Type 3, whereas Types 1 and 2 are often associated with significant vestibular dysfunction and delays in motor development. In all subtypes,

auditory rehabilitation planning involves evaluation for cochlear implantation or, when necessary, auditory brainstem implantation (ABI). Electrode placement considerations and surgical risks must be evaluated individually for each subtype (Brutto et al., 2021).

6. Incomplete Partitions

Incomplete Partition (IP) of the Cochlea is an inner ear malformation characterized by the incomplete separation of cochlear segments, typically resulting from arrested cochlear development between the fourth and sixth gestational weeks. This anomaly disrupts the structural integrity of the cochlea and is radiologically classified into several subtypes:

IP Type I is defined by a cystic cochlear configuration with complete absence of the modiolus and interscalar septa. Although the cochlea and vestibule appear undifferentiated, their relationship with the middle ear may sometimes be more clearly visualized. Audiologically, hearing loss is usually severe-to-profound sensorineural, with behavioral or electrophysiological thresholds often exceeding 100 dB. Stapedial and acoustic reflexes are absent, and otoacoustic emissions (OAEs) are typically negative. Cochlear implantation is feasible; however, the risks of CSF fistula and difficulties in electrode placement are substantial (Sennaroğlu & Saatci, 2002; Berrettini et al., 2013)

IP Type II (Mondini deformity) is the most common subtype and is characterized by a cochlea measuring approximately 1–1.5 turns with a cystic apex. Audiological findings vary from moderate-to-severe to profound sensorineural hearing loss. Residual low-frequency hearing may be preserved in some cases, making cochlear implantation a frequent choice in auditory rehabilitation. Electrophysiological assessments such as ABR often reveal elevated response thresholds. Vestibular function is partially preserved, although some patients may present with imbalance (Sennaroğlu & Saatci, 2002; Swords et al., 2023).

IP Type III features absence of the modiolus with partial preservation of interscalar septa and is associated with X-linked stapes gusher syndrome. Hearing loss is typically profound sensorineural. Cochlear implantation can be performed, but the risk of intraoperative CSF leakage and challenges in electrode insertion is high. Vestibular structures are generally severely compromised, and balance disorders are common Sennaroğlu & Saatci, 2002; Swords et al., 2023).

Overall, all IP subtypes tend to present with severe-to-profound sensorineural hearing loss, often accompanied by absent OAEs and absent stapedial reflexes. Vestibular involvement varies across subtypes: Type II may

exhibit relatively preserved vestibular function, whereas Types I and III often present with significant balance disturbances and delayed motor development. Decisions regarding cochlear implantation or auditory brainstem implantation (ABI) should be based on the specific anatomical characteristics of each subtype (Sennaroğlu & Saatci, 2002).

7. Enlarged Vestibular Aqueduct (EVA)

Enlarged Vestibular Aqueduct (EVA) is a common inner ear malformation characterized by an abnormally widened endolymphatic duct and sac, and it is one of the most frequent causes of progressive sensorineural hearing loss in children. Embryologically, it arises from incomplete development of the endolymphatic duct, and it may present unilaterally or bilaterally. EVA is typically diagnosed radiologically using computed tomography or magnetic resonance imaging; a vestibular aqueduct width of approximately 1.5 mm or greater is generally considered diagnostic (Valvassori & Clemis, 1978; Sennaroğlu & Saatci, 2002).

From an audiological perspective, EVA is commonly associated with low- and mid-frequency sensorineural hearing loss. Hearing loss is often mild to moderate at onset but tends to progress over time. Additionally, trauma, upper respiratory infections, or head injury may precipitate sudden deterioration in hearing. In individuals with EVA, otoacoustic emissions (OAEs) are often affected, though partial responses may be present in some cases. Stapedius reflexes and auditory brainstem response (ABR) findings vary depending on the severity of the hearing loss.

Although vestibular function is preserved in most patients, mild balance disturbances may occur in some cases (Jackler & De La Cruz, 1989). The clinical significance of EVA lies in the risk of progressive hearing deterioration, particularly when accompanied by low-frequency hearing loss, underscoring the need for early diagnosis and regular audiological monitoring. As hearing loss becomes more pronounced, hearing aids or, in appropriate cases, cochlear implantation may be considered for auditory rehabilitation. Patients should also be advised to avoid head trauma and rapid pressure changes, which may help prevent further auditory decline (Jackler & De La Cruz, 1989).

8. Cochlear Aperture Abnormalities

Cochlear Aperture Abnormalities are rare inner ear malformations characterized by an abnormally narrow or malformed opening between the cochlea and the internal auditory canal (IAC), where the modiolus and the cochlear nerve enter the cochlea. Embryologically, these abnormalities arise

from disruptions in the early development of the modiolus and the internal auditory canal. Radiologic imaging typically reveals a narrowed or malformed cochlear aperture, which may compromise the passage of cochlear nerve fibers and affect neural responsiveness (Sennaroğlu & Saatci, 2002; Yan et al., 2023).

From an audiological perspective, cochlear aperture abnormalities are commonly associated with severe to profound sensorineural hearing loss. In most cases, hearing thresholds range between 90–120 dB or higher. Otoacoustic emissions (OAEs) are absent, and stapedial reflexes are typically not measurable. Cochlear implantation may be technically challenging due to the narrowed aperture and limited electrode access; in some cases, an auditory brainstem implant (ABI) may be a more suitable option. Vestibular function is often partially preserved, though mild balance disturbances may occur in some patients (Sennaroğlu & Bajin, 2017; Tahir et al., 2017)

Early diagnosis and detailed radiologic evaluation are critical for determining appropriate auditory rehabilitation and for surgical planning. When considered alongside other severe inner ear malformations, cochlear aperture abnormalities require a multidisciplinary management approach, and auditory prognosis varies depending on anatomical characteristics (Yan et al., 2013; Sennaroğlu & Bajin, 2017).

9. Cochlear and Cochlear-Vestibular Nerve Abnormalities

Cochlear and cochlear-vestibular nerve variants play a critical role in both auditory and vestibular function and are closely associated with inner ear malformations. Magnetic resonance imaging (MRI) is the gold standard for assessing the presence, absence, or hypoplasia of these nerves, and each variant has distinct clinical and audiological implications.

In cases of hypoplastic cochlear nerve (Hypoplastic CN), the nerve is present but smaller than normal; auditory transmission is limited, and audiological findings typically reveal severe sensorineural hearing loss. Otoacoustic emissions are usually absent, and the stapedial reflex is not measurable. Cochlear implantation may be possible, but electrode placement and auditory outcomes are limited (Sennaroğlu & Saatci, 2002; Glastonbury et al., 2002)

In individuals with a normal cochlear nerve (Normal CN), auditory transmission is preserved or may show mild to moderate hearing loss; OAE and stapedial reflex responses are generally present, and the likelihood of successful cochlear implantation is high (Sennaroğlu & Saatci, 2002)

When the cochlear nerve is absent (Absent CN), auditory transmission is not possible, resulting in profound sensorineural hearing loss. Cochlear

implantation cannot be performed, and an auditory brainstem implant (ABI) is the preferred method of rehabilitation (Sennaroğlu & Saatci, 2002).

In cases of hypoplastic cochlear-vestibular nerve (Hypoplastic CVN), both auditory and vestibular transmission are limited; severe hearing loss occurs along with partial balance disturbances (Sennaroğlu & Saatci, 2002) With a normal cochlear-vestibular nerve (Normal CVN), auditory and vestibular functions are generally preserved, providing optimal conditions for surgical planning and rehabilitation. When the cochlear-vestibular nerve is absent (Absent CVN), both auditory and vestibular transmission are completely lost, resulting in profound sensorineural hearing loss accompanied by significant balance deficits and motor development delays (Sennaroğlu & Saatci, 2002). For these reasons, accurate radiological characterization and multidisciplinary evaluation of cochlear and cochlear-vestibular nerve variants are essential for planning auditory rehabilitation.

In summary, inner ear malformations constitute a highly heterogeneous group of conditions that extend far beyond structural abnormalities, exerting profound effects on auditory perception, vestibular function, and auditory development. As outlined in this chapter, variations in embryological arrest, anatomical configuration, and neural integrity give rise to diverse audiological profiles, necessitating individualized assessment and rehabilitation strategies.

A fundamental audiological perspective—grounded in an understanding of embryology, classification systems, and functional outcomes—is essential for accurate diagnosis, realistic prognostication, and effective intervention planning. By integrating radiological findings with comprehensive behavioral and electrophysiological evaluations, clinicians can better navigate the complexities of hearing rehabilitation, including cochlear implantation and auditory brainstem implantation. Ultimately, adopting a fundamental audiological framework enables evidence-based, patient-centered management and supports optimal auditory and developmental outcomes for individuals with inner ear malformations.

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Chapter 4

The Use of Bibliometric Analysis in Determining Current Research Trends in the Health Sciences

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Introduction

In the academic field, understanding the change in research focuses over time, identifying current and evolving methodologies, and recognizing influential authors and institutions related to a specific topic are important for the advancement of the field and the determination of future research directions (1). Questions such as which statistical methods are becoming prominent in health research, how international collaborations are shaping the development of biostatistical applications, or which journals serve as primary preferred publication outlets for methodological innovations are increasingly becoming important for biostatisticians and epidemiologists. While traditional literature review methods remain valuable, they struggle to capture the comprehensive landscape of a field when dealing with large volumes of publications. In this context, subjective selection bias, limited scope, and the inability to systematically measure trends are existing limitations.

Bibliometric analysis is considered a quantitative approach used to evaluate scientific literature, which has gained significant interest over the last decade (2, 3). The key feature of this methodology is its ability to transform large amounts of bibliometric data into meaningful patterns and trends using both statistical and visual techniques.

Bibliometric methods are used in numerous areas, such as mapping the evolution of epidemiological research over time, determining the focal points of health research, assessing the impact of funding on research productivity, and evaluating international collaboration networks in the health sector (4-7). Recently, the number of bibliometric studies in health-related academic journals has increased significantly, reflecting the growing recognition of these methods as essential tools for research evaluation and strategic planning.

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What is Bibliometric Analysis?

Bibliometric analysis is a set of quantitative methods used to examine information, patterns, and trends within the scientific literature (8). The analysis can focus on various desired units of measurement, including individual publications, authors, journals, institutions, or entire research fields. Although bibliometrics literally means 'book measurement,' its scope encompasses all forms of scholarly communication, including conference proceedings, journal articles, preprints, patents, and datasets. The methodology draws upon library science, information science, statistics, and network analysis to provide objective insights into research landscapes.

Accurate compilation and cleaning of bibliographic data are critical to the reliability of the analysis results. In a bibliometric study, researchers must first clearly define the scope of their analysis by specifying the time period, the databases to be searched, and the inclusion criteria. Data sources such as Scopus, Science Direct, Web of Science, PubMed, and Google Scholar have different characteristics that affect the comprehensiveness and accuracy of the results. Multiple databases are often queried to ensure sufficient coverage, but this brings with it challenges related to removing duplicate records and data standardization.

The most important feature that distinguishes bibliometric analysis from traditional literature reviews is its emphasis on quantitative measurement and visualization. In this context, rather than providing a narrative synthesis of research findings, bibliometric studies reveal the structural characteristics of scientific fields through metrics and maps (9). Citation analysis examines how publications reference each other, revealing influential works and the flow of ideas. Co-authorship analysis maps collaboration networks, showing how researchers and institutions work together. Co-citation analysis suggests thematic relationships by identifying publications that are frequently cited together. With bibliographic coupling, publications sharing common references are linked, allowing similar research foundations to be observed.

The citation counts of studies reveal which statistical methods or methodological articles are most influential in shaping research practices. Co-word analysis of keywords and abstracts identifies prominent topics. Author collaboration networks indicate whether the field of study is characterized by small, isolated research groups or larger international consortia. Journal mapping, on the other hand, shows which publication outlets are central to the methodological discourse and which serve specialized topics.

Temporal analysis is a fundamental component of bibliometric research, examining how the publication volume, citation patterns, and subject

prominence of studies change over time (10). Growth curves of studies can reveal whether a field is in a phase of exponential growth, linear expansion, or plateau maturity. The half-life of study citations indicates how quickly knowledge becomes obsolete. Temporal co-word networks can track the evolution of terminology, showing when new concepts emerge, rise in prominence, and when older terms decline in use.

It is also important to clarify what bibliometric analysis is *not*. This methodology does not provide a critical evaluation of individual studies; high citation counts do not necessarily indicate methodological rigor or validity. Therefore, bibliometrics should not be used in isolation to evaluate researcher productivity or research quality, as manipulation of metrics is possible, and disciplinary differences significantly affect citation behavior. Finally, bibliometric analysis is not a single technique but encompasses multiple approaches, each with specific applications, assumptions, and limitations.

Core Concepts and Mathematical Foundations

To understand bibliometric analysis, one must be familiar with several core concepts and metrics. Firstly, the H-index is one of the widely known bibliometric indicators, designed to measure both productivity and impact (11). Proposed by Jorge Hirsch in 2005, the h-index is defined as the largest number h such that an author has published h papers that have each been cited at least h times. For example, an h-index of 20 means the researcher has published 20 papers that have each been cited at least 20 times. According to this metric, neither publishing a large number of low-impact papers nor publishing a single highly cited paper alone will result in a high h-index. However, the h-index has limitations, such as its insensitivity to highly cited papers beyond the h-core, its discipline-dependent variability, and its dependence on career length.

The Impact Factor (IF) is a journal-level metric calculated annually by Clarivate Analytics for journals indexed in the Web of Science (12). The two-year impact factor is calculated by dividing the number of citations in a given year to articles published in that journal during the previous two years, by the total number of citable articles published in those two years. For instance, a journal with an IF of 4.5 indicates that articles published in 2022 and 2023 were, on average, cited 4.5 times in 2024. Despite its widespread use in academic evaluation, this metric has been criticized for several reasons: the distribution of citations is skewed, with a small percentage of articles receiving the majority of citations; the two-year period may not be appropriate for all fields; review articles can inflate the metric's value; and journal-level metrics may not reflect individual article quality.

Alternative Metrics (Altmetrics) have emerged to capture broader scholarly impacts beyond traditional citations (13). Altmetrics track interest shown in research through mentions on social media, coverage in news outlets, and inclusion in policy documents. The Altmetric Attention Score aggregates these various signals into a single number, but interpretation is challenging due to the heterogeneity of data sources and lack of standardization. Although altmetrics can reveal societal impact and public engagement, they are vulnerable to manipulation and may not correlate with scientific quality.

Co-citation strength measures the frequency with which two publications are cited together in subsequent works. If articles A and B are cited together by articles X, Y, and Z, they have a co-citation count of 3. High co-citation strength suggests intellectual kinship, as authors perceive the paired publications as addressing similar questions or representing complementary approaches (14). Co-citation analysis can be used to create maps of scientific fields; clusters of highly co-cited papers represent distinct research themes. Visualization techniques like multi-dimensional scaling or network maps make co-citation matrices interpretable.

Data Sources and Search Strategies

Selecting the appropriate databases is a critical decision for bibliometric research. Web of Science (WoS), produced by Clarivate Analytics, is generally considered the gold standard for bibliometric analysis due to its broad coverage, rigorous indexing standards, and inclusion of complete citation data (15). This database covers over 21,000 journals and has records extending back many decades. WoS provides the variety of structured data necessary for bibliometric analysis, including author affiliations, funding information, and cited references for studies.

Scopus, produced by Elsevier, offers broader journal coverage than Web of Science, indexing over 27,000 titles, and has a particularly strong representation in the biomedical and physical sciences (16). Compared to Web of Science, Scopus includes more non-English language journals and conference proceedings, making it more advantageous for detailed regional analyses.

PubMed, maintained by the National Library of Medicine, is freely accessible and covers the detailed content of the biomedical literature, including veterinary journals. Although PubMed does not inherently include citation data, citation information can be obtained by linking to other databases or by utilizing PubMed Central citation data for open-access articles. The Medical Subject Headings (MeSH) vocabulary in PubMed can enhance the accuracy of

bibliometric analyses in specific medical fields, as it allows for precise searching and subject classification.

Google Scholar has the broadest coverage, indexing not only peer-reviewed journals but also books, theses, conference papers, preprints, and technical reports. This comprehensiveness can come at the cost of quality, as Google Scholar includes duplicate entries and non-scholarly material. Citation counts in Google Scholar are typically higher than those in Web of Science or Scopus, due to both the wider source coverage and the inclusion of non-traditional citation sources. While Google Scholar is valuable for discovery searches and ensuring no important publications are missed, the lack of robust filtering and export functionalities limits its use for systematic bibliometric analysis.

Search strategies must be designed to strike a balance between sensitivity and specificity. Overly broad searches retrieve out-of-scope publications, creating a significant burden for data cleaning, while overly narrow searches can lead to missing important contributions (17). Keyword selection should include both controlled vocabulary terms (like MeSH in PubMed) when available, and natural language keywords appearing in titles and abstracts. Boolean operators (AND, OR, NOT) combine search terms logically, while truncation and wildcard symbols capture variations in word forms. For a bibliometric study on biostatistics, a search strategy might combine terms related to medicine (cancer, infection, organ, drug) with terms related to biostatistics (statistical analysis, survival analysis, regression, epidemiology). Pilot searches and iterative refinements performed upfront help to hone the search strategy before the final data collection.

Bibliometric Indicators and Metrics

The number of publications remains the most fundamental bibliometric indicator today, measuring research productivity at the level of publications, authors, institutions, or countries. While simple to calculate, publication counts must be interpreted carefully due to differences across disciplines and varying publication cultures (18). Adjusted publication counts, which weight contributions based on authorship position (first author, corresponding author, equal contribution), provide more nuanced assessments of productivity.

Citation count measures how many times a publication has been referenced by subsequent works. Citations are generally interpreted as indicators of scientific impact, influence, or quality. Highly cited articles may represent groundbreaking discoveries, methodological innovations, or comprehensive reviews that have become standard references. However, citations can also accumulate for negative reasons, such as articles cited as examples of flawed

methodology or refuted claims (19). The temporal dimension of citations is also important, as articles need time to accumulate citations, and citation rates vary significantly across disciplines. Field-normalized citation indicators adjust for these disciplinary differences, enabling cross-field comparisons.

The Journal Impact Factor has been previously introduced, but related journal metrics include the five-year impact factor, which uses a longer citation window; the Eigenfactor Score, which weights citations based on the prestige of the citing journal; and the Article Influence Score, which measures the average influence of a journal's articles. The SCImago Journal Rank (SJR) indicator applies the PageRank algorithm to journal citation networks, giving more weight to citations from prestigious journals (20). Source Normalized Impact per Paper (SNIP) adjusts for differences in citation practices across subject areas, acknowledging that fields with longer reference lists will naturally generate more citations.

Author-level metrics extend beyond the h-index to include the g-index, which gives more weight to highly cited papers; the i10-index, which counts publications with at least 10 citations; and the m-quotient, which normalizes the h-index by career length. Field-weighted metrics, such as the Field-Weighted Citation Impact, examine an author's citation performance against the world average for their field. The Relative Citation Ratio, developed by the National Institutes of Health, uses co-citation networks to define relevant field benchmarks.

Collaboration metrics measure teamwork in research. The collaboration index measures the average number of authors per publication, while the collaboration coefficient accounts for both single-authored and multi-authored papers. The international collaboration rate calculates the proportion of publications involving authors from multiple countries, a rate which has been associated with higher citation impact (21). Network centrality measures such as degree centrality, betweenness centrality, and closeness centrality characterize the structural position of authors or institutions within collaboration networks, identifying brokers who connect disparate research communities.

Visualization Techniques and Software Tools

Visualization transforms complex bibliometric data into interpretable graphical representations. Scientific mapping techniques create representations where proximity indicates similarity or linkage (22). Co-occurrence networks depict relationships between entities such as keywords, authors, or journals; nodes represent the entities, while edges represent co-occurrence relationships. Node size often indicates the frequency of occurrence, and edge thickness

indicates the strength of the co-occurrence. Community detection algorithms partition the networks into clusters that represent thematic areas or research communities.

VOSviewer, developed at Leiden University, is one of the most widely used tools for bibliometric network visualization (23). The software uses a layout algorithm that minimizes the distance between strongly related nodes while preventing overlap, producing readable maps even for large networks. It supports various bibliometric analyses, including co-authorship, co-citation, and co-word analysis. Its density visualization mode highlights areas of high activity using color intensity, enabling the identification of research hot spots. Through overlay visualizations, it can map temporal information onto the networks, demonstrating how research themes evolve over time.

CiteSpace, developed by Chaomei Chen, specializes in detecting and visualizing emerging trends and abrupt changes in research fields (24). The software introduced the concept of citation burst detection, which identifies papers or keywords that receive an unusual surge in citation or usage over specific time periods. Burst detection helps pinpoint paradigm shifts and the emergence of new research directions. CiteSpace's timeline visualization displays the temporal evolution of clusters, showing when research themes emerged, peaked, and declined. The software also calculates structural metrics such as betweenness centrality and sigma scores to identify pivotal papers that bridge different research areas.

Bibliometrix is an R package that provides comprehensive tools for scientific mapping and bibliometric analysis within the R statistical environment (25). The package imports data from one or multiple databases, performs extensive bibliometric calculations, and generates plots. Bibliometrix includes functions for analyzing collaboration networks, tracking intellectual structure through co-citation analysis, examining the thematic evolution of topics over time, and identifying themes through thematic mapping. Its integration with R's statistical analysis capabilities allows researchers to combine bibliometric indicators with statistical modeling and hypothesis testing.

Gephi is an open-source network analysis and visualization platform that offers advanced graphing options and layout algorithms. While not primarily designed for bibliometrics, Gephi's flexibility and visual quality make it popular for creating publication-ready network visualizations. The software supports interactive exploration of large networks, and its filtering, clustering, and ranking functions allow researchers to focus on relevant substructures (26).

Layout algorithms such as Force Atlas 2 and OpenOrd produce aesthetically and scientifically meaningful spatial arrangements.

Applications in the Field of Health

Bibliometric methods have been frequently applied in recent years to map the evolution of topics and statistical methodologies in health research. Time-series analysis of keyword frequencies can reveal when specific topics or statistical techniques enter the literature and how quickly they are adopted. For example, the term "mixed model" was rarely seen in health journals before 1990, but it increased steadily throughout the 1990s and 2000s, becoming a standard terminology today. Similarly, "machine learning" and "random forest" have shown exponential growth starting around 2015, reflecting the rapid adoption of these methods in the health sector (27).

Co-citation analysis of methodological papers reveals the intellectual foundations of the health field. Newer methodological papers on topics like causal inference or Bayesian statistics may initially appear peripheral but gradually integrate into the central knowledge structure as they gain acceptance. Tracking these structural shifts can provide insights into how the field's methodological paradigm is evolving.

Collaboration network analysis demonstrates that health research has become increasingly international and interdisciplinary in recent times (28). Early publications in the field often involved single authors or only local collaborators, whereas current research frequently involves author teams spanning multiple institutions and countries. Network analysis reveals that specific institutions serve as hubs, with high degree centrality indicating numerous collaborations, while others are in intermediary positions with high betweenness centrality, connecting otherwise disconnected research communities. The Small World property, characterized by short average path lengths and high clustering coefficients, is often observed in scientific collaboration networks and facilitates rapid information diffusion.

Journal analysis identifies the publishing outlets that are central to disseminating research. The observed citation flows between journals reveal interdisciplinary influences.

Topic modeling and text mining approaches extract thematic content from large volumes of abstracts and full texts. Latent Dirichlet Allocation (LDA) is a model that identifies hidden topics based on word co-occurrence patterns within documents (29). When applied to health literature, LDA can identify distinct topics such as "survival analysis in oncology," "mixed models for production data," "diagnostic test evaluation," and "Bayesian methods for disease

surveillance." Tracking the prevalence of these topics over time reveals shifting research priorities. For instance, topics related to antimicrobial resistance and One Health have grown significantly in recent years, reflecting broader societal concerns.

Methodological Considerations and Limitations

Database selection significantly affects bibliometric results, and multi-database searches are recommended to maximize coverage. For instance, for a given topic, it might be found that the Web of Science yields 3,000 publications, Scopus yields 3,800, and PubMed yields 4,200, but only 2,500 appear in all three databases. Database choice depends on the research question: WoS may be preferred for citation analysis due to its historical depth, Scopus for broader journal coverage, and PubMed for comprehensive biomedical literature retrieval (30).

Data cleaning is a laborious yet essential step in bibliometric analysis. Author name disambiguation addresses the problem that an author may appear under multiple name variants due to name abbreviations, name changes, or errors. Similarly, institution name disambiguation handles the multiplicity of organizational names, acronyms, and affiliations. Duplication detection removes redundant records that may arise from database overlap or indexing errors. Keyword standardization harmonizes synonyms and spelling variations to accurately generate co-word networks. Automated tools assist with these tasks but rarely achieve perfect accuracy, necessitating manual verification.

The time window for data collection influences both the volume of data and the observed citation metrics. A study examining publications from 2000–2024 in 2025 provides a 25-year perspective but must acknowledge that recent publications have had little time to accumulate citations, potentially underestimating their eventual impact. Citation windows of 3–5 years are often used to capture relatively recent work while allowing sufficient time for impact to emerge. Prospective designs, which track publications forward from a fixed point, allow for the assessment of citation accumulation over time.

Language bias is inherent in most bibliometric studies, as major databases predominantly index English-language journals. Research published in other languages may be invisible to bibliometric analyses, leading to an underrepresentation of contributions from non-English publications. While difficult to overcome entirely, researchers can mitigate this limitation by including regional databases such as SciELO for Latin American literature, CNKI for Chinese literature, or J-STAGE for Japanese literature.

The distinction between self-citations and external citations is relevant in some contexts. Self-citations occur when authors cite their own previous work; this is a normal scholarly practice for building one's research program but can inflate citation counts when excessive. Journal self-citation rates show the proportion of citations a journal receives from articles published within the same journal. A moderate journal self-citation rate is expected, especially in specialized fields, but very high rates may indicate coercive citation practices or limited external influence. Most bibliometric software can identify and optionally exclude self-citations from analyses.

The Matthew effect describes the tendency for highly cited papers to accumulate citations at an accelerating rate, while less visible works remain obscure, irrespective of quality (31). This preferential attachment process can lead to citation inequality, where a small fraction of articles receives the majority of citations. The Matthew effect is compounded by factors such as author prestige, institutional affiliation, and journal impact factor, which influence the attention of readers and citation decisions independently of intrinsic scientific merit. Bibliometric indicators thus reflect not only quality but also visibility and social processes within the scientific community.

Publication bias, just as it affects systematic reviews and meta-analyses, also affects bibliometric analyses. Studies with statistically significant or novel findings are more likely to be published and cited than studies with null results or replication studies. Time-lag bias results from the delay between study completion and publication, which varies by outcome and research area. Bibliometric studies based solely on the published literature present an incomplete and potentially skewed view of true research activity.

Emerging Trends in the Field of Health

Bibliometric analysis reveals trends that emerge over time. In recent years, with the development of artificial intelligence, the integration of machine learning (ML) and artificial intelligence (AI) methods has accelerated; publications on topics such as random forests, support vector machines, neural networks, and deep learning are exponentially increasing in the health literature (32). These methods are applied to predictive modeling of disease risk, diagnostic image analysis, and the analysis of high-dimensional omics data. The co-occurrence of keywords related to traditional statistical methods with machine learning suggests integration rather than replacement, indicating that ML is augmenting rather than supplanting classical biostatistical approaches.

Causal inference methods such as propensity scores, instrumental variables, difference-in-differences, and marginal structural models are gaining

importance in epidemiology. Publications explicitly addressing causal inference or using directed acyclic graphs (DAGs) to represent causal assumptions have increased substantially since 2015. This trend reflects a growing awareness that association does not imply causation and that careful study design and analytical methods are necessary to draw causal conclusions from observational data.

Bayesian statistics is another area showing growth, with increasing frequency of publications on Bayesian inference, Markov Chain Monte Carlo methods, and Bayesian hierarchical models. The flexibility of Bayesian approaches in incorporating prior knowledge, handling complex data structures, and quantifying uncertainty makes them attractive for health applications. Co-citation networks show strong links between health papers and foundational Bayesian texts, indicating methodological cross-pollination.

Missing data methods have seen increased attention, with a rise in publications related to multiple imputation, maximum likelihood methods, and inverse probability weighting. This trend is driven by the recognition that complete case analysis can produce biased results and that different missing data mechanisms require different analytical approaches. The proliferation of accessible software implementations, especially the MICE package in R, has made missing data handling more feasible for applied researchers.

Recommendations for Conducting Bibliometric Studies

Researchers planning to conduct a bibliometric study should start by defining clear research questions suitable for bibliometric methods. Questions about research trends, influential works, collaboration patterns, and emerging themes are appropriate for bibliometric analysis, whereas questions about the validity of specific findings or the quality of individual studies require different approaches. The defined research question guides decisions about data sources, time periods, units of analysis, and analytical methods.

Protocol development and pre-registration enhance the rigor and transparency of bibliometric research. A protocol should specify the research questions, databases to be searched, search strategies, inclusion and exclusion criteria, data extraction procedures, analytical methods, and visualization techniques. Although bibliometric studies are observational in nature, methodological rigor is essential in their execution.

Data management practices must ensure reproducibility and quality. All search strategies, including database names, search strings, retrieval dates, and filters applied, must be fully annotated and documented. Data files downloaded from the databases should be preserved and checked in their original format.

Data cleaning steps, including author disambiguation, institutional standardization, and duplicate removal, must be systematically recorded. It is advisable that the analysis code used is commented, organized, and ideally shared in a public repository.

The perspectives of multiple analysts further strengthen bibliometric research. Having two or more team members independently perform data extraction, classification, or interpretation reduces individual bias and improves study reliability. Reliability statistics such as Cohen's kappa can measure agreement. Interdisciplinary teams bring complementary perspectives that enhance the depth and validity of interpretations.

Sensitivity analyses test the robustness of findings against methodological decisions. For example, researchers can compare results obtained using different databases, different time windows, or different citation thresholds. If the results remain consistent across these variations, confidence in the findings increases. If the results are sensitive to specific choices, this should be acknowledged and discussed as a limitation.

Interpretation of bibliometric results requires caution and contextual knowledge. High citation counts indicate influence but do not necessarily indicate accuracy or quality. Publication counts reflect productivity but may also reflect salami-slicing or unnecessary publication. Journal impact factors are journal-level metrics that do not predict individual article quality. Network centrality indicates structural position but not individual merit. Bibliometric indicators should always be interpreted in conjunction with expert opinion and qualitative assessment.

Ethical considerations in bibliometric research must also be addressed, avoiding the misuse of metrics for evaluation and presenting results fairly. While bibliometric data is largely public, caution should be exercised when presenting potentially identifying information, especially about early-career researchers. Findings should be presented in a balanced manner, acknowledging both the strengths and limitations of the methods and data.

Limitations and Future Directions

Bibliometric analysis faces inherent limitations that must be acknowledged. The focus on published literature excludes unpublished work and practical knowledge that does not appear in academic journals. This publication bias limits the completeness of the picture that bibliometrics can provide. Retrospective studies only capture past trends and cannot definitively predict future developments. Emerging topics may not yet have sufficient publication volume for robust bibliometric analysis.

In recent times, artificial intelligence (AI) methods are increasingly being applied to enhance bibliometric analysis (33). Natural Language Processing (NLP) can extract structured information from unstructured text, automatically classify documents by topic, and identify sentiment or novelty in publications. Machine learning algorithms can predict which articles will be highly cited, identify potential collaborators, or suggest relevant literature upfront. While these tools offer efficiency gains, they also introduce new challenges related to interpretability, bias, and validation.

The integration of multiple data sources beyond traditional bibliographic databases enriches bibliometric analyses. Funding data from agencies like the National Institutes of Health or the European Commission allows tracking research investment and its relationship with publication output. Patent databases link basic research to technological applications. Clinical trial registries provide insights into research activity that precedes publication.

The movement towards open science is significant for bibliometric research. Open-access publications remove paywalls to full texts, enabling text mining and content analysis. Open data repositories make research materials available for re-analysis and integration. Open peer review platforms provide transparency into the evaluation process. These developments create opportunities for more comprehensive and transparent bibliometric analyses but also raise questions about privacy, commercial exploitation, and incentive structures.

Equity considerations in bibliometrics are also gaining attention. Citation practices and publishing opportunities vary systematically based on researchers' geography, institutional resources, and demographic characteristics. If bibliometric analyses do not account for structural inequalities, they can inadvertently reinforce existing hierarchies. Developing context-sensitive indicators, recognizing diverse research outputs beyond traditional publications, and critically examining whose contributions are visible and valued are essential for fair research evaluation.

Conclusion and Recommendations

Bibliometric analysis provides powerful tools for mapping the research landscape of the health field, identifying influential contributions, revealing collaboration patterns, and detecting emerging trends. The quantitative and visual nature of bibliometric methods offers objective, comprehensive, and reproducible assessments of large literature volumes, complementing traditional literature reviews. Ranging from simple publication counts to sophisticated network visualizations, bibliometric approaches span a range of complexity and

purpose, offering researchers the flexibility to address diverse questions about the structure and dynamics of their field.

The practical value of bibliometric analysis for health researchers is significant. Researchers gain the opportunity to identify knowledge gaps and promising directions for future research. Researchers can map the state of their field, identify potential collaborators, and observe influential works and authors. Funding agencies and policymakers can evaluate research investments, assess the impact of funding programs, and identify areas that are strong or neglected. Journal editors can benchmark their publications against competitors and identify emerging topics for special issues.

Recommendations for future research include the greater integration of bibliometric methods into systematic reviews and meta-analyses, where bibliometric approaches can guide literature searching, identify relevant studies, and detect publication bias. Longitudinal bibliometric tracking can monitor the diffusion of innovations, such as the adoption of new statistical methods or reporting guidelines, providing insights into the pace of methodological change. Comparative bibliometric analyses across disciplines can reveal how the health field relates to other quantitative domains.

Recommendations for health researchers include supporting data sharing and open science practices, which enable more comprehensive and transparent bibliometric analyses. Training in bibliometric literacy ensures that researchers can critically interpret bibliometric indicators and conduct their own bibliometric studies.

Finally, while bibliometric analysis offers valuable quantitative insights, it should never replace critical thinking, expert judgment, and deep engagement with the scientific content of publications. Bibliometrics can show us what is influential, where connections exist, and how fields evolve, but only careful reading and thoughtful interpretation can tell us which findings are valid and reliable, which methods are appropriate, and which directions are scientifically promising. The integration of bibliometric and traditional scholarly methods is the best pathway forward for understanding and advancing the current state of science in the health field.

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Chapter 5

Basic Principles of Meta-Analysis and Application Stages in Veterinary Clinical Research

Furkan Çağrı BEŞOLUK[1]

Introduction

In veterinary clinical research, the accumulation of evidence from the results of multiple studies is extremely valuable for making informed and correct decisions about diagnostic, therapeutic, and preventive strategies (1). Veterinary practitioners and researchers often encounter questions in daily life, such as which surgical technique yields more favorable outcomes in canine cranial cruciate ligament surgery, whether a specific antibiotic is more effective in treating feline urinary tract infections, or what the overall efficacy of a newly developed vaccine is in different populations. Disadvantages of individual studies often include limited sample sizes, conflicting results, or methodological differences that prevent drawing definitive conclusions. Reviews are useful for summarizing the literature but are subjective and lack the statistical rigor required to systematically synthesize quantitative evidence.

Meta-analysis is defined as a statistical methodology that quantitatively combines the results of multiple independent studies addressing the same research question, providing a more precise estimate of the effect or relationship (2,3). By pooling data from studies, meta-analysis increases statistical power, resolves uncertainties when studies conflict, and generates more robust evidence to guide clinical practice. Over the last three decades, meta-analysis has become a cornerstone of evidence-based medicine, and its adoption rate in veterinary medicine has significantly increased, particularly in companion animal research, livestock health, and disease control programs (4-6).

The fundamental principle of meta-analysis is that combining evidence from the results of multiple studies provides a more accurate estimate compared to a single study. This approach is particularly valuable in situations where individual studies lack sufficient power, where heterogeneity exists in study populations or methods, or where publication bias may distort the literature. Meta-analysis also allows researchers to explore sources of variation between

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studies through subgroup analyses and meta-regression, providing insights into factors that modify treatment effects or disease associations.

What is Meta-Analysis?

Meta-analysis is defined as the statistical synthesis of data from independent studies to produce a summary estimate of an effect size or relationship (7). Unlike traditional literature reviews that qualitatively describe study findings, meta-analysis uses statistical techniques to combine numerical results, weight studies according to their precision, and calculate confidence intervals around the pooled estimate. The process involves the systematic identification of relevant studies, data extraction on outcomes and study characteristics, assessment of study quality and risk of bias, statistical pooling of results, and evaluation of heterogeneity and publication bias.

The starting point for any meta-analysis is the determination of a clearly defined research question, typically formulated using the PICO framework: Population, Intervention, Comparison, and Outcome (8). For example, in a meta-analysis evaluating the efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) for postoperative pain control in dogs undergoing orthopedic surgery, the population would be dogs undergoing orthopedic procedures, the intervention would be NSAIDs, the comparison would be placebo or alternative analgesics, and the outcome would be pain scores or rescue analgesia rates. This structured approach ensures that the meta-analysis addresses a focused, answerable question and guides the systematic literature search.

Beyond simple statistical calculations, meta-analysis fundamentally demands meticulous attention to both clinical and methodological heterogeneity. Clinical heterogeneity encompasses variations in the study populations, interventions, chosen comparators, and outcome measurements. Conversely, methodological heterogeneity is defined by differences in study design, risk of bias assessment, and the specific measurement techniques employed. Understanding these sources of variation is crucial for determining whether it is appropriate to pool studies and for interpreting the results in a meaningful way.

The most important feature that distinguishes meta-analysis from other forms of evidence synthesis is the calculation of a pooled effect size with a confidence interval and the assessment of between-study variability. Effect sizes can be presented in different forms depending on the data type: risk ratios, odds ratios, or risk differences for binary outcomes; mean differences or standardized mean differences for continuous outcomes; and hazard ratios for time-to-event data. Each effect size has specific assumptions and interpretations.

Types of Meta-Analysis and Study Designs

Meta-analyses can be classified according to the type of data synthesized and the level at which the data is combined. The most common distinction is between aggregate data meta-analysis and individual participant data meta-analysis (9). Aggregate data meta-analysis uses summary statistics reported in published studies, such as mean differences, odds ratios, and their standard errors or confidence intervals. This is the most widely used approach as it does not require access to raw data from individual studies. Individual participant data meta-analysis, on the other hand, obtains the raw data from each study and performs a pooled analysis on the combined dataset. This approach offers greater flexibility for conducting subgroup analyses, adjusting for confounders, and standardizing outcome definitions, but it is more resource-intensive and requires collaboration with original study authors.

The type of outcome data dictates the specific meta-analytic approach. For binary outcomes such as mortality, treatment success, or disease occurrence, meta-analysis typically calculates pooled risk ratios, odds ratios, or risk differences (10). For continuous outcomes like body weight, pain scores, or biochemical parameters, meta-analysis calculates pooled mean differences or standardized mean differences. The standardized mean difference is used when studies measure the same outcome using different scales or units, allowing for comparison across studies. For time-to-event outcomes such as survival time or time to recurrence, meta-analysis pools hazard ratios from survival analyses. For diagnostic test accuracy studies, meta-analysis evaluates sensitivity and specificity together using bivariate or hierarchical models.

Network meta-analysis, also known as mixed-treatment comparison meta-analysis, is an extension that allows for the simultaneous comparison of multiple interventions, even when some comparisons have not been directly studied (11). For instance, if studies compare Treatment A to placebo, Treatment B to placebo, and Treatment C to B, network meta-analysis can estimate the relative efficacy of A versus C, even without a direct head-to-head study. This approach is increasingly used in veterinary medicine to rank treatment options and inform clinical guidelines.

Systematic Review and Literature Search

Every meta-analysis must be preceded by a systematic review, which is a transparent and rigorously conducted process to identify, select, and appraise all relevant studies (10). The systematic review protocol should be developed before the literature search begins and ideally registered in a public database, such as PROSPERO, to prevent selective reporting and protocol changes after

seeing the results. The protocol specifies the research question, inclusion and exclusion criteria, search strategy, data extraction procedures, risk of bias assessment, and planned statistical analyses.

The conducted literature search must be comprehensive and reproducible. Multiple databases should be searched, including databases such as PubMed, Web of Science, Scopus, Science Direct, CAB Abstracts, and CAB Direct. The strategy for selecting search terms should combine terms related to the population, intervention, and outcome using Boolean operators. To maximize sensitivity, both controlled vocabulary terms, such as Medical Subject Headings (MeSH), and free-text keywords should be used. The reference lists of included studies and relevant review articles should be manually screened to identify additional studies. Conference abstracts, theses, and grey literature should be considered to reduce publication bias, although the inclusion of these studies requires very careful quality assessment.

The inclusion and exclusion criteria must be clearly defined and consistently applied. Studies are typically screened in two stages: first by title and abstract, then by full-text review. At least two independent reviewers should perform the screening and data extraction, with disagreements resolved through discussion or the involvement of a third reviewer. Reasons for excluding studies at the full-text stage must be documented and reported in a PRISMA flow diagram (12). The PRISMA statement provides a checklist of items to be included in systematic reviews and meta-analyses, ensuring the transparency and completeness of reporting.

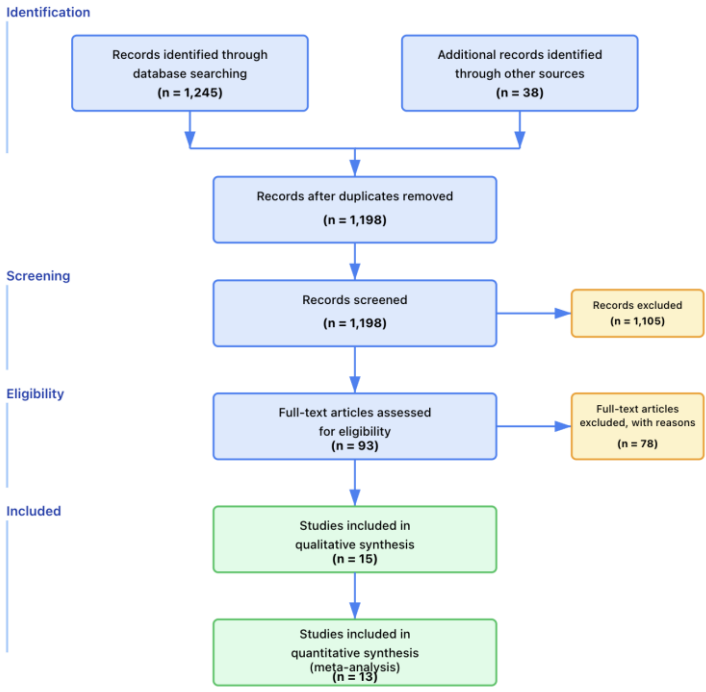

Figure 1. PRISMA Flow Diagram (n values are given as examples)

Data extraction from studies involves the systematic recording of information from each study, including study characteristics, participant demographics, intervention details, comparator specifics, outcome measures, sample sizes, effect sizes, measures of variability, and risk of bias assessments. Standardized data extraction forms should be used to ensure consistency. If study data is missing or ambiguous, the study authors should be contacted. If multiple publications report data from the same study population, care must be taken to avoid double-counting participants.

Risk of Bias Assessment

Assessing the risk of bias in included studies is a critical part of meta-analysis because biased studies can distort the pooled estimate (13). For randomized controlled trials, the Cochrane Risk of Bias tool is the most commonly used instrument. Bias evaluation utilizes this tool to investigate core criteria such as the method of random sequence generation, how allocation was concealed, blinding status of participants/personnel, blinding of outcome assessors, the completeness of outcome data, avoidance of selective reporting, and identification of any further bias concerns. For every study, the domains are

assigned a risk rating: low, high, or unclear, culminating in a single overall judgment regarding the risk of bias. When dealing with observational designs, dedicated instruments, including the Newcastle-Ottawa Scale or the ROBINS-I tool, are employed to evaluate biases pertaining to participant selection, confounding factors, measurement of exposure and outcome, and attrition.

Additional considerations may be necessary in veterinary research. Blinding is often challenging in surgical or management interventions, and the lack of blinding may have different impacts depending on the outcome. Objective outcomes, such as mortality or laboratory values, are less susceptible to detection bias than subjective outcomes, such as pain scores or quality-of-life assessments. The species, breed, age, and health status of the animals must be considered when assessing the applicability of the results.

Risk of bias assessments should be used to inform sensitivity analyses, where high-risk studies are excluded to evaluate whether they disproportionately influence the pooled estimate. If the results are robust against the exclusion of high-risk studies, confidence in the findings increases. If the results change significantly, the pooled estimate must be interpreted cautiously, and the impact of bias on the conclusions should be discussed.

Calculation of Effect Size and Data Preparation

The choice of effect size depends on the type of outcome data and the study design. For binary outcomes, the most common effect sizes are the risk ratio, odds ratio, and risk difference (10). The Risk Ratio (RR) compares the probability of the outcome in the intervention group to the probability in the control group. The RR is intuitive and easy to interpret but cannot be calculated if there are zero events in one group. The Odds Ratio (OR) compares the odds of the outcome in the intervention group to the odds in the control group. It is symmetrical and can be calculated even when events are rare, but it is less intuitive than the RR. The Risk Difference (RD) is the absolute difference in the outcome rates between the groups and provides information on the public health impact of an intervention.

For continuous outcomes, the Mean Difference (MD) is used if all studies measure the outcome on the same scale, while the Standardized Mean Difference (SMD) is used if different scales are used across studies (14). The SMD is calculated by dividing the mean difference by the pooled standard deviation, yielding a unitless measure that can be compared across studies. Interpretation of SMDs often follows general guidelines: values of 0.2 are considered small, 0.5 moderate, and 0.8 large effects. Nevertheless, the clinical

context must always be considered when interpreting the magnitude of an effect.

For time-to-event outcomes, the Hazard Ratio (HR) derived from Cox proportional hazards regression is the preferred effect size. The HR represents the relative instantaneous risk of the event in the intervention group compared to the control group. For diagnostic test accuracy studies, sensitivity and specificity are the primary effect sizes, and a bivariate meta-analysis is often used to account for the correlation between these measures.

Data transformation may be necessary when studies report results in different formats. If a study reports only medians and interquartile ranges instead of means and standard deviations, transformation formulas or estimation methods can be used, although they introduce additional uncertainties. If a study only reports p-values or confidence intervals without effect estimates, effect sizes can sometimes be calculated using standard formulas. If the data cannot be obtained in a usable format, despite contacting the authors, the study may need to be excluded from the quantitative synthesis but should still be discussed qualitatively.

Statistical Models for Meta-Analysis

There are two fundamental statistical models used in meta-analysis: the fixed-effect model and the random-effects model (15). The choice between these models depends on the assumptions about the underlying distribution of the true effects across studies. The fixed-effect model operates on the premise that every included study attempts to measure an identical underlying true effect, attributing all observed variation in results exclusively to sampling error. In this approach, the synthesized estimate is calculated as a weighted average of the individual findings. Weighting is determined by the inverse of the variance within each study, meaning that studies characterized by larger sample sizes and greater precision are assigned a higher influence. The fixed-effect model is preferred when studies are methodologically and clinically homogeneous and when the aim is to estimate the average effect in the specific set of studies included.

The random-effects model assumes that the true effects vary across studies due to differences in populations, interventions, or settings (16). In contrast to the fixed-effect approach, the random-effects model accounts for two components of variance: sampling error (within-study variance) and genuine differences in true effects (between-studies variance, or heterogeneity). The calculation of the pooled estimate involves a weighted average, with weights determined inversely by the total of the within-study and between-studies

variances. This weighting mechanism results in a comparatively greater influence of smaller studies when compared to the fixed-effect alternative. Generally favored in meta-analysis, the random-effects model is more resilient to heterogeneity and yields a more cautious estimate, reflected in wider confidence intervals. Crucially, it facilitates the generalization of findings to a wider universe of studies, extending beyond those actually synthesized.

Methods such as the DerSimonian-Laird method, restricted maximum likelihood, or the Paule-Mandel method are commonly used in estimating the between-studies variance. The choice of estimation method can influence the results, especially when heterogeneity is high or the number of studies is small. The robustness of the findings can be assessed through sensitivity analyses using different methods.

Assessment of Heterogeneity

Heterogeneity refers to the variability in study results being greater than expected by chance alone from sampling error (17). The assessment and understanding of heterogeneity are crucial because significant heterogeneity may indicate that pooling studies is inappropriate or that the pooled estimate obscures important differences between studies. Heterogeneity can be assessed through visual, statistical, and inconsistency measurement methods.

Visual assessment is performed using forest plots which display the effect size and confidence interval of each study along with the pooled estimate. If the confidence intervals substantially overlap and the point estimates cluster around the pooled estimate, heterogeneity is likely low. If the confidence intervals are widely dispersed or the point estimates vary considerably, heterogeneity may be present. Nevertheless, visual assessments are subjective and must be supported by statistical tests.

The Cochran Q test is a statistical test for heterogeneity that assesses whether the variation in observed effect sizes is greater than what is expected by chance (18). The test yields a p-value, and when $p < 0.05$, it suggests significant heterogeneity. However, the Q test has low power when the number of studies is small and excessive power when the number of studies is large, indicating that it should not be used as the sole measure of heterogeneity.

The I^2 statistic measures the proportion of the total variation in effect sizes that is due to heterogeneity rather than chance (19). It is calculated as $I^2 = 100 \times (Q-df)/Q$, where Q is the Cochran Q statistic, and df is the degrees of freedom (number of studies minus one). I^2 ranges from 0% to 100%, and values of 25% are conventionally considered low, 50% moderate, and 75% high heterogeneity. Unlike the Q test, I^2 is not affected by the number of studies, making it a more

interpretable measure. However, I^2 must be interpreted in the context of the magnitude and direction of the effects, as statistical heterogeneity does not always imply clinical or practical heterogeneity.

Tau-squared (τ^2) is the estimated between-studies variance in the random-effects model, providing a measure of the absolute amount of heterogeneity. Unlike I^2, which is a relative measure, τ^2 is expressed on the scale of the effect size and can be used to estimate the distribution of true effects across studies. Prediction intervals, which incorporate τ^2, indicate the range within which the true effect in a new study is expected to fall, offering a more complete picture of heterogeneity than confidence intervals.

When significant heterogeneity is detected, researchers should explore its sources rather than merely reporting the pooled estimate. Subgroup analyses and meta-regression are the primary tools for exploring heterogeneity in this case. Subgroup analysis separates studies into groups based on a categorical characteristic, such as species, disease stage, or study quality, and performs a comparison of the pooled estimates between the groups. Meta-regression uses a regression model to examine the relationship between study-level covariates and effect sizes. For example, meta-regression can assess whether the effectiveness of a treatment changes depending on the administered dose or the length of follow-up. These exploratory analyses should be interpreted cautiously because they are observational, have limited power, and are susceptible to confounding at the study level.

Publication Bias and Small Study Effects

Publication bias typically occurs when the likelihood of a study being published depends on its results, usually favoring statistically significant or positive findings (20). This bias can distort the pooled estimate in a meta-analysis, leading to an overestimation of treatment effects or associations. Small studies with statistically non-significant or unexpected results may remain unpublished or be published in less accessible journals, while larger studies are more likely to be published regardless of their findings. The presence and impact of publication bias must be assessed in every meta-analysis.

Funnel plots are the most common graphical tool used to detect publication bias. The funnel plot is a graphical tool where each study's effect size is mapped on the horizontal axis and its corresponding precision (often standard error or sample size) on the vertical axis. Ideally, in the absence of publication bias, the points form a symmetrical inverted funnel shape. This symmetry reflects that less precise, smaller studies exhibit wider variation, while larger studies congregate closely around the overall pooled effect. Asymmetry in the funnel

plot, especially when smaller studies show larger effect sizes, suggests potential publication bias or small-study effects. However, asymmetry can also be caused by heterogeneity, differences in study quality, or genuine differences in effect sizes between large and small studies, so the interpretation of a funnel plot requires caution.

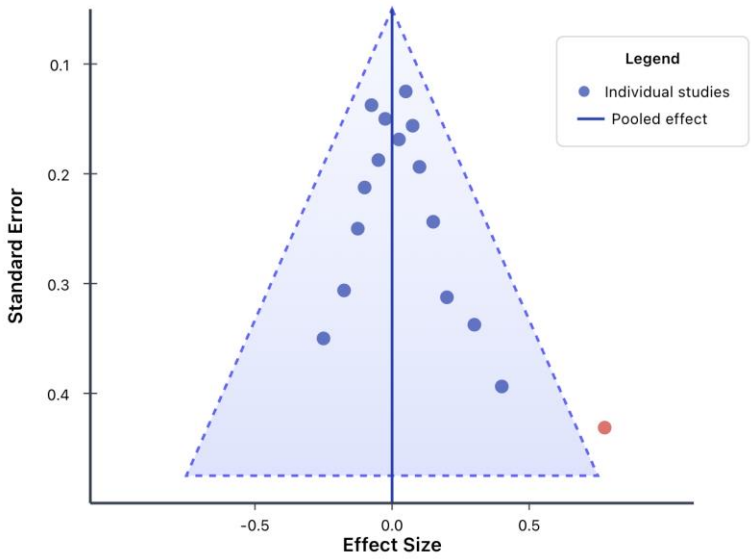

Figure 2. Funnel Plot for Publication Bias Assessment

Statistical tests for funnel plot asymmetry include Egger's regression test and Begg's rank correlation test (21). Egger's test performs a linear regression analysis of the standardized effect size on its precision and tests whether the intercept is significantly different from zero. A significant intercept suggests asymmetry. Begg's test assesses the rank correlation between the effect sizes and their variances. These tests have limited power when the number of studies is small, typically requiring at least ten studies for reliable results. Furthermore, a significant test does not definitively establish publication bias, as other factors can also cause asymmetry.

The trim and fill method is a statistical approach that adjusts for funnel plot asymmetry by imputing missing studies and recalculating the pooled estimate (22). The method identifies the most extreme small studies causing the asymmetry and removes them, estimates the number and effect sizes of the

missing studies, imputes them symmetrically on the opposite side of the funnel plot, and then recalculates the pooled estimate. The adjusted estimate provides an indication of how publication bias might have affected the results. However, the method assumes that the asymmetry is due to publication bias and that the bias follows a specific model, which may not always be true.

Other strategies to address publication bias include searching for unpublished studies, such as conference abstracts, trial registries, and regulatory agency reports. Contacting study authors and pharmaceutical companies may uncover unpublished data. Comparing the pooled estimate derived from published studies with a subset estimate including unpublished studies can be useful in gauging the impact of publication bias. Sensitivity analyses that exclude small studies or stratify by publication status can also provide insights into the robustness of the findings.

Subgroup Analysis and Meta-Regression

When heterogeneity exists, understanding the sources of heterogeneity is crucial for interpreting the results of a meta-analysis. Subgroup analysis and meta-regression are the primary methods for exploring heterogeneity and identifying factors that modify the effect of an intervention or the strength of an association (23). These analyses are observational and should be prespecified in the systematic review protocol to prevent data dredging and spurious findings.

Subgroup analysis divides studies into groups based on a categorical variable, such as species, age group, disease severity, intervention dose, or study quality, and calculates separate pooled estimates for each subgroup. The subgroups are then compared to determine if the effect is significantly different between them. For instance, a meta-analysis of NSAID effectiveness for postoperative pain might perform subgroup analyses by species to assess whether the effect is consistent across dogs, cats, and horses. A formal test of subgroup differences, often based on the Q statistic, assesses whether the between-subgroup variability is greater than expected by chance.

Subgroup analyses have several limitations. They are underpowered because they rely on between-study comparisons rather than within-study comparisons. They are susceptible to confounding because subgroups may differ in multiple characteristics simultaneously. Multiple subgroup analyses increase the risk of false-positive findings due to multiple testing. Therefore, the number of subgroup analyses should be restricted to a few prespecified variables, and the results should be interpreted as hypothesis-generating rather than definitive.

Meta-regression is a more flexible approach that models the relationship between study-level covariates and effect sizes using regression analysis (24).

Meta-regression can accommodate both categorical and continuous covariates, such as dose, duration, or mean age, and can include multiple covariates simultaneously. The regression coefficient represents the change in the effect size associated with a one-unit change in the covariate. For example, meta-regression might reveal that the efficacy of a vaccine linearly increases with the antigen dose or that treatment effects diminish with longer follow-up periods.

Meta-regression requires a sufficient number of studies, typically at least ten studies per covariate, to avoid overfitting and yield reliable estimates. The analysis is performed at the study level, not the individual participant level. Observed associations between study-level variables and effect sizes may not actually hold true at the individual level. Meta-regression is also susceptible to confounding because studies differing in one characteristic may differ in others. Sensitivity analyses and cautious interpretation are necessary to avoid over-interpreting meta-regression results.

Software and Practical Application

Conducting a meta-analysis requires specialized statistical software capable of performing the meta-analytic models, heterogeneity assessment, publication bias evaluation, and sensitivity analyses. Several software packages are commonly used in meta-analysis research, each with strengths and weaknesses compared to one another.

R is a free, open-source statistical programming environment with extensive meta-analysis capabilities through packages such as "meta," "metafor," and "metaphor" (25). These packages support a wide range of meta-analytic models, including fixed-effect and random-effects models, subgroup analyses, meta-regression, and diagnostic test accuracy meta-analysis. R provides flexibility and reproducibility because analyses can be scripted and shared via code. However, R has a steep learning curve and requires coding and programming knowledge.

Stata is a commercial statistical software with built-in meta-analysis commands like "meta," "metan," and "metareg." Stata is user-friendly and provides comprehensive output, including forest plots, funnel plots, and heterogeneity statistics. The software is widely favored in epidemiology and clinical research. However, Stata requires a paid license, and its meta-analysis functionality is less comprehensive compared to specialized R packages.

RevMan (Review Manager) is free software developed by the Cochrane Collaboration specifically for conducting Cochrane systematic reviews and meta-analyses. It offers a user-friendly interface for data entry, risk of bias assessment, and meta-analysis. RevMan is designed for users with limited

statistical expertise and seamlessly integrates with Cochrane review standards. However, its functionality is more limited for advanced analyses such as meta-regression and network meta-analysis.

Comprehensive Meta-Analysis (CMA) is commercial software with a point-and-click interface designed specifically for meta-analysis. It supports a wide range of effect sizes and models, provides comprehensive graphical output, and includes tools for publication bias assessment and subgroup analysis. The software is accessible to users without coding and programming skills but requires a paid license and is less flexible than R or Stata.

A reproducible workflow is essential when conducting a meta-analysis. Data extraction should be performed in a structured format, such as a spreadsheet, with clear documentation of variable coding and missing data. The statistical analysis code should be saved and annotated to ensure reproducibility. Results should be systematically organized, with separate files for forest plots, funnel plots, subgroup analyses, and sensitivity analyses. Following reporting guidelines such as PRISMA ensures transparency and completeness.

Reporting Standards and Best Practices

Transparent and complete reporting of meta-analyses is essential for readers to assess the validity of the findings and for other researchers to replicate the analysis. The PRISMA statement provides a comprehensive checklist of items that should be included in the reports of systematic reviews and meta-analyses (12). The checklist covers all stages of the review process, from protocol development to dissemination, and includes items related to the search strategy, study selection, data extraction, risk of bias assessment, statistical methods, and interpretation of results.

The PRISMA flow diagram serves as an essential reporting element, offering a visual and transparent summary of the entire study selection procedure. This graphic must detail key numerical stages: the quantity of records found via database searches and other channels, the count of screened records, the number of full-text articles evaluated for eligibility, and the final totals for both qualitative and quantitative synthesis inclusion. It is imperative to meticulously document and list the specific reasons for excluding articles during the full-text review phase.

The forest plot constitutes the principal graphical tool for presenting meta-analysis findings and must be incorporated into all primary analyses. This visual representation illustrates the effect size and corresponding confidence interval for every individual study, alongside the summarized pooled estimate, its calculated weight, and confidence limits. The plot should include a vertical line

representing the null effect, typically zero for mean differences or one for risk ratios, to facilitate interpretation. Studies should be logically ordered, perhaps by publication year, sample size, or risk of bias. Statistics for heterogeneity, including I^2, τ^2 and the Q test p-value, should be displayed on the plot.

Risk of bias assessments should be presented in a risk of bias graph or summary table, showing the judgment for each domain for every study. This presentation allows readers to assess the overall quality of the evidence and identify potential sources of bias. The impact of the risk of bias on the pooled estimate should be explored through sensitivity analyses, with results reported separately for low-risk and high-risk studies.

Assessments of publication bias, including funnel plots and statistical tests, should be reported. Where publication bias is suspected, adjusted estimates from the trim and fill method or other sensitivity analyses should be presented. The implications of publication bias for the interpretation of the findings must be discussed.

Results of subgroup analyses and meta-regression should be reported with appropriate caution, noting that these analyses are exploratory and observational. The number of studies in each subgroup, the pooled estimate and confidence interval for each subgroup, and the p-value for the test of subgroup differences should be provided. Meta-regression results should include the regression coefficient, its standard error and confidence interval, and the p-value.

Researchers frequently employ the GRADE framework to evaluate the certainty of evidence synthesized from multiple studies (26). This system assesses evidence quality according to five key factors: risk of bias, inconsistency, indirectness, imprecision, and publication bias, resulting in a certainty grade (high, moderate, low, or very low). Ultimately, this grading assists readers in establishing confidence in the meta-analysis results and helps guide clinical recommendations.

Limitations and Challenges in Veterinary Meta-Analysis

Meta-analysis in veterinary medicine faces several challenges that can affect the validity and interpretation of the results. For many research questions, the limited number of available studies is a major constraint. Unlike human medicine, where hundreds of studies may be available for a single intervention, veterinary research often has only a handful of studies, which limits statistical power and the ability to explore heterogeneity or publication bias. Small meta-analyses are more susceptible to the influence of outlier studies and may yield imprecise estimates with wide confidence intervals.

Heterogeneity in study populations is often greater in veterinary research than in human research. Animals of the same species can vary widely in breed, size, age, and genetic background, all of which can modify treatment effects. Studies may include mixed populations or focus on specific breeds, making it unclear whether the results are generalizable. For example, a treatment effective in large-breed dogs may not be effective in small breeds due to differences in pharmacokinetics or disease pathophysiology. Decisions on whether to pool studies with diverse populations require careful clinical judgment.

Outcome measures in veterinary studies are often heterogeneous, with different studies using different scales, definitions, or time points. Pain is often assessed using various scoring systems that are not directly comparable, making it difficult to pool results. Biochemistry results in animals may be measured using different laboratory methods or reported in different units. Survival may be defined differently, such as all-cause mortality in some studies versus disease-specific mortality in others. Standardizing outcomes or using standardized mean differences can address some of these issues, but it may also obscure clinically meaningful differences.

Study quality and reporting standards in veterinary research are often lower compared to human medicine. Many veterinary studies fail to report sufficient details on randomization, blinding, or allocation concealment, making risk of bias assessment difficult. Studies may be underpowered due to the lack of sample size calculations. Attrition and missing data are common but poorly reported. These quality issues can introduce bias into the meta-analysis and reduce confidence in the findings.

Ethical and regulatory differences also impact veterinary research. Unlike human clinical trials, veterinary studies are not always registered in public databases, making it challenging to identify unpublished studies and assess publication bias. Conflicts of interest related to drug or device manufacturers may not be disclosed. Ethical review processes vary widely across institutions and countries, and some studies may not have undergone formal ethical review. These factors can compromise the transparency and integrity of the evidence base.

Advanced Methods and Extensions

As meta-analysis methods evolve, several techniques have been developed to address specific challenges, such as those encountered in veterinary research. Network meta-analysis (NMA) allows for the simultaneous comparison of multiple interventions by combining direct and indirect evidence (11). This approach is particularly useful when multiple treatment options exist, but head-

to-head comparisons are absent or sparse. Bayesian NMA further allows for the incorporation of prior information and the estimation of treatment rankings, helping clinicians identify the most effective intervention.

Meta-analysis of diagnostic test accuracy requires specialized methods because sensitivity and specificity are correlated, and threshold effects can introduce variability. Bivariate and hierarchical summary receiver operating characteristic (HSROC) models account for these issues by jointly modeling sensitivity and specificity and allowing for between-study variability in thresholds (27). These models provide summary estimates of diagnostic accuracy and can compare the accuracy of different tests.

Individual participant data (IPD) meta-analysis offers significant advantages over aggregate data meta-analysis, allowing for more sophisticated analyses (9). With access to individual-level data, researchers can standardize outcome definitions, adjust for confounders, conduct time-to-event analyses, and perform subgroup analyses based on individual characteristics rather than just study-level features. However, obtaining IPD is challenging, requiring collaboration with study authors, data-sharing agreements, and substantial resources for data harmonization and analysis.

Prospective meta-analysis is an emerging approach where studies are explicitly designed and conducted with the intention of being combined in a meta-analysis (28). This method allows for the standardization of protocols, interventions, and outcomes across studies, reducing heterogeneity and increasing the precision of the pooled estimate. Prospective meta-analysis is particularly useful for rare diseases or outcomes where no single study can achieve adequate power. However, it requires extensive coordination and funding.

Living systematic reviews and meta-analyses are continually updated as new evidence emerges, providing an up-to-date synthesis of the evidence (29). This approach is facilitated by automation tools for literature screening and online platforms for dissemination. Living meta-analyses are valuable, especially in rapidly evolving fields.

Clinical Application and Evidence-Based Practice

The ultimate goal of meta-analysis in clinical practice is to guide clinical decision-making and improve animal health outcomes. Veterinarians can use the findings of meta-analyses to inform treatment choices, counsel clients, and develop clinical guidelines. For example, a meta-analysis demonstrating that a specific surgical technique reduces complication rates can influence surgical

practice, while a meta-analysis showing that a vaccine provides long-term immunity can inform vaccination protocols.

Understanding the limitations of the meta-analysis is crucial for application. The results represent the average effect across studies and may not apply to individual patients with specific characteristics. Confidence intervals indicate the precision of the estimate, and wide intervals suggest uncertainty. Heterogeneity suggests that the effect may vary across populations, settings, or interventions, and subgroup analyses or meta-regression can help identify which patients are most likely to benefit. The quality of evidence, as assessed by GRADE, provides guidance on the confidence that can be placed in the findings.

Meta-analysis findings should be integrated with clinical expertise and patient values in a shared decision-making process. Evidence from meta-analysis is one component of evidence-based practice, along with clinical experience and an understanding of pathophysiology. When high-quality evidence from meta-analysis is available, it should be given substantial weight, but it does not replace clinical judgment.

Future Directions and Innovations

The future of meta-analysis in veterinary medicine is likely to be shaped by several trends and innovations. The increasing availability of electronic health records and large databases will allow researchers to collect more comprehensive data and facilitate individual participant data (IPD) meta-analysis. Machine learning and natural language processing techniques can automate data extraction and risk of bias assessment, potentially reducing the time and effort required to conduct systematic reviews and meta-analyses.

Standardization of outcome measures through core outcome sets will improve the consistency and comparability of studies, facilitating meta-analysis. Core outcome sets define a minimum set of outcomes that should be measured and reported in all studies on a particular condition, ensuring that studies can be combined in future meta-analyses. International collaborations and consortia can drive the development and adoption of core outcome sets in veterinary research.

Bayesian methods are increasingly being used in meta-analysis to incorporate prior knowledge, address small sample sizes, and provide probabilistic interpretations of results (30). Bayesian meta-analysis allows researchers to update their beliefs based on new evidence and make probability statements about treatment effects, such as the probability that one treatment is

superior to another. These methods are particularly useful when data is sparse or when decision-making requires probabilistic reasoning.

The integration of multi-omics data—including genomics, transcriptomics, proteomics, and metabolomics—into meta-analyses will enable personalized medicine approaches in veterinary practice. Meta-analyses incorporating biomarker data can identify subgroups of animals most likely to respond to specific treatments, guiding precision medicine strategies.

Conclusion and Recommendations

Meta-analysis is a powerful tool for synthesizing evidence and informing clinical practice in veterinary medicine. By systematically combining results from multiple studies, meta-analysis provides more precise estimates of treatment effects and associations, resolves uncertainties when studies disagree, and identifies gaps in the evidence base. The appropriate conduct and reporting of meta-analyses require adherence to rigorous methodological standards, including comprehensive literature searches, risk of bias assessment, appropriate statistical methods, and transparent reporting.

Several recommendations are offered for researchers planning to conduct a meta-analysis. First, a detailed protocol outlining the research question, inclusion criteria, search strategy, and analysis plan should be developed before starting the review. The protocol should be registered in a public database to enhance transparency. Second, a comprehensive and reproducible literature search that includes both published and unpublished studies should be conducted. Third, the risk of bias in included studies should be assessed using validated tools and the results reported transparently. Fourth, appropriate statistical methods should be selected based on the type of outcome data and the presence of heterogeneity. The random-effects model should be preferred by default unless there is a strong justification for the fixed-effect model. Fifth, heterogeneity should be assessed through visual inspection, statistical tests, subgroup analyses, and meta-regression. Sixth, publication bias should be assessed using funnel plots and statistical tests, and sensitivity analyses should be conducted to evaluate its impact. Seventh, results should be reported following PRISMA guidelines, including all necessary details to allow for replication and critical appraisal. Eighth and last, findings should be interpreted with caution, acknowledging limitations and avoiding over-interpretation of subgroup or meta-regression analyses.

Several recommendations apply to clinicians interpreting meta-analysis findings. First, the quality of the meta-analysis should be assessed by checking if it followed systematic review standards, assessed risk of bias, and reported

results transparently. Second, the forest plot should be examined to understand the consistency of results across studies and the precision of the pooled estimate. Third, the applicability of the study populations, interventions, and outcomes to your clinical setting and patient population should be evaluated. Fourth, the certainty of evidence should be assessed using frameworks like GRADE to determine the confidence that can be placed in the findings. Fifth, meta-analysis findings should be integrated with clinical expertise and patient values in shared decision-making.

The application of meta-analysis in veterinary medicine has grown significantly over the last two decades, and this trend is expected to continue. As the volume of veterinary research increases, the need for rigorous evidence synthesis becomes even greater. Meta-analysis provides a systematic and transparent approach to combining evidence, identifying knowledge gaps, and guiding future research priorities. The adoption of standardized methods, reporting guidelines, and quality appraisal tools will enhance the reliability and impact of meta-analyses in veterinary clinical research.

Veterinary researchers, clinicians, educators, and policymakers all have important roles in promoting the use of meta-analysis and evidence-based practice. Researchers should design high-quality studies with standardized outcomes and transparent reporting to facilitate future meta-analyses. Clinicians should seek out and critically appraise meta-analyses to inform their practice and contribute to systematic reviews by sharing unpublished data and clinical insights. Educators should incorporate training in systematic review and meta-analysis methods into veterinary curricula to build capacity for evidence synthesis. Policymakers should support funding for systematic reviews and meta-analyses as essential research activities that maximize the value of primary research.

Finally, ethical considerations must be at the forefront of meta-analytic research. The conduct of systematic reviews and meta-analyses should adhere to the principles of scientific integrity, including transparency, reproducibility, and honest reporting of results. Conflicts of interest should be disclosed, and publication bias should be minimized by efforts to identify and include unpublished studies. The welfare of animals in primary studies included in meta-analyses is paramount, and systematic reviews can play a role in reducing unnecessary animal use by identifying situations where sufficient evidence already exists or where further research is unlikely to change the conclusions. The 3Rs principles—replacement, reduction, and refinement—should be considered when interpreting meta-analysis findings and planning future studies.

Meta-analysis is a critically important tool in advancing evidence-based veterinary medicine. By synthesizing evidence across multiple studies, meta-analysis provides robust estimates of treatment effects, identifies sources of variability, and guides clinical decision-making. The rigorous application of meta-analytic methods, adherence to reporting standards, and careful interpretation of findings will continue to strengthen the evidence base in veterinary medicine and ultimately improve the health and welfare of animals under veterinary care.

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Footnote

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Chapter 6

Protective Effects Of 4′-(3,4 Dihydroxybenzoyloxymethyl) Phenyl-*O*- B -D-Glucopyranoside Supplementation On Acute Exercise-Induced Oxidative Stress In Rats

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ABSTRACT

Objective: *This study focused on the protective effects of 4'-(3,4-dihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside (PG) isolated from the aerial parts of Origanum micranthum on acute exercise-induced oxidative stress in rat skeletal muscle tissues.*

Material and Method: *A total of 38 male rats were used in this study. The rats were divided into four groups: Control (C), acute exercise (AE), PG-supplemented (PS), acute exercise and PG-supplemented (AE/PS) groups. The rats in the AE and AE/PS groups were run on a treadmill. PG (20 mg/kg) was administrated intraperitoneally (i.p.) 30 min before running. The skeletal muscle tissues were carefully removed and divided into two parts for histopathological and biochemical analyses.*

Result and Discussion: *PG supplementation has decreased the levels of the tissue malondialdehyde MDA and myeloperoxidase (MPO). PG supplementation has increased the levels of the tissue superoxide dismutase (SOD) and glutathione (GSH) activity. In conclusion, PG supplementation may prevent exercise-induced oxidative stress by preventing lipid peroxidation and increasing antioxidant enzyme activity.*

Keywords: *Acute exercise, oxidative stress, 4'-(3,4-dihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside*

ÖZ

Amaç: *Bu çalışma, Origanum micranthum'un toprak üstü kısımlarından izole edilen 4'-(3,4-dihidroksibenzoiloksimetil)fenil-O-β-D-glukopiranosidin (PG) sıçan iskelet kas*

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dokularında akut egzersiz kaynaklı oksidatif stres üzerindeki koruyucu etkilerine odaklanmıştır.

Gereç ve Yöntem: *Bu çalışmada toplam 38 erkek sıçan kullanıldı. Sıçanlar dört gruba ayrıldı: Kontrol (C), akut egzersiz (AE), PG takviyeli (PS), akut egzersiz ve PG takviyeli (AE/PS) grupları. AE ve AE/PS gruplarındaki sıçanlar koşu bandında koşturuldu. PG (20 mg/kg), koşudan 30 dakika önce intraperitoneal (i.p.) olarak uygulandı. İskelet kas dokuları dikkatlice çıkarıldı ve histopatolojik ve biyokimyasal analizler için iki parçaya ayrıldı.*

Sonuç ve Tartışma: *PG takviyesi, doku malondialdehit MDA ve miyeloperoksidaz (MPO) seviyelerini düşürmüştür. PG takviyesi, doku süperoksit dismutaz (SOD) ve glutatyon (GSH) aktivitesi seviyelerini artırmıştır. Sonuç olarak, PG takviyesi, lipit peroksidasyonunu önleyerek ve antioksidan enzim aktivitesini artırarak egzersiz kaynaklı oksidatif stresi önleyebilir.*

Anahtar Kelimeler: *Akut egzersiz, oksidatif stress, 4'-(3,4-ihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside*

INTRODUCTION

Physical exercise induces many adaptive metabolic and compositional changes in skeletal muscle [1]. Recently, there has been a great deal of interest in the role of oxidative stress in exercise-induced tissue damage and fatigue [2]. During running, excessive reactive oxygen species (ROS) production occurs. This increased production has been attributed to high oxygen consumption (up to 100-200 times higher than normal) in skeletal muscle, resulting in a substantially increased mitochondrial electron flux [2,3]. Elevation of electron leakage from the mitochondrial transport system disturbs the intracellular pro-oxidant and antioxidant homeostasis [4]. It has been shown that xanthine oxidase (XO) and myeloperoxidase (MPO) are the two main sources of extracellular free radicals during strenuous exercise, and that they are responsible for the tissue damage caused by exhaustive exercise [2]. Since oxidative stress contributes to fatigue, tissue damage, and impaired recovery from exhaustive exercise, much research has focused on supplementation with nutraceutical agents to reduce the these effects [5]. Some *Origanum* species (especially, *O. vulgare*, *O. majorana*) (Lamiaceae) have been used for common cold, respiratory system diseases, gastrointestinal disorders, urinary system disorders in the world [6]. *Origanum* species are widely used all over the world as a very popular spice and used traditionally in many other ways as their essential oils have antimicrobial, cytotoxic, and antioxidant activity [7]. *Origanum vulgare* is known to consist of many effective antioxidants, such as rosmarinic acid, caffeic acid, and various flavonoids [8]. *O. vulgare*, *O. majorana* and *O. onites* have been used for cold, respiratory system disease,

gastrointestinal disorders, urinary system disorders and as spice in Türkiye [9,10]. Historically, 4′-(3,4-dihydroxybenzoyloxymethyl)phenyl-*O*-*β*-D-glucopyranoside (PG, Figure 1) was first isolated from *O. vulgare* by Nakatani and Kikuzaki, and investigated its antioxidant activity with ferric thiocyanate [11]. In a previous study, it has been showed that PG exhibits free radical scavenging activity, antioxidant and cytoprotective effects on liver and skin cells [11,12].

Twenty seven taxa of *Origanum* genus grow in Türkiye [13]. The aerial parts of *O. micranthum*, which is an endemic species for Türkiye, have been used as a herbal tea in Kozan District (Adana Province, Türkiye). PG was isolated from the aerial part of *O. micranthum* growing in Kozan [14].

The aim of the this study was to determine if PG isolated from the aerial parts of *O. micranthum* has an antioxidant effect against acute exercise-induced oxidative stress in rats.

MATERIAL AND METHODS

Animals and Exercise Protocol

Six-month-old male Sprague-Dawley rats (200-250 g each) were used. The rats were fed standard rat chow and water ad libitum. The animals were kept on a 12 h light/12 h dark regime and maintained at 23 °C. The animals were divided randomly into the following groups: Group 1 (n= 10; 220±17 g), control group (C; no exercise); Group 2 (n= 10; 227±20 g), acute exercise group (AE; the rats were exercised on a treadmill once for 60 min at 20 m/min and 0% grade); Group 3 (n= 9; 226±17 g), PG-supplemented (PS); Group 4 (n= 9; 230±16 g), acute exercise and PG-supplemented group (AE/PS).

The rats were exercised for 60 min on a treadmill at 20 m/min and 0% grade. PG was dissolved in normal saline (0.9% NaCl). PG (20 mg/kg) was administrated i.p. 30 min before running [15]. Anesthetic drug (ketamine/xylazine 90/10 mg/kg) was administered to rats. The right hind limb gastrocnemius skeletal muscle tissues were removed carefully under anesthesia from the rats and skeletal tisuues were divided into two parts. The first part was rinsed in ice-cold normal saline, blotted dry, and stored at -80 °C for further analysis. The second part was stored in 10% formaldehyde for histopathological examination.

After removing the skeletal muscle tissues, rats were sacrified by cervical dislocation.

Isolation of PG

The aerial parts of *O. micranthum* were collected from Kozan District (Adana Province, Türkiye). A voucher specimen is deposited at the Ankara

University, Herbarium of the Faculty of Pharmacy (AEF 25873). The aerial parts of the plant were dried and powdered. The powdered plant (410 g) was extracted by refluxing with methanol (3 L x 3). Methanolic extract was concentrated and dried under reduced pressure to give a residue (98 g). Methanolic extract was partitioned with chloroform and ethyl acetate (EtOAc), respectively. Chloroform subextract was 26.7 g and EtOAc subextract 12.7 g. Several chromatographic studies (column and thin layer chromatography) were used to isolate PG from the EtOAc subextract. The EtOAc subextract (12 g) was subjected to silica gel column eluting by $CHCl_3$:MeOH:H_2O (80:20:2, 70:30:3, 50:50:5) solvent system. Fractions 27-28 were subjected to Sefadex LH-20 column chromatography using by MeOH. Fractions 7-9 gave PG. The structure of PG was elucidated using by spectroscopic methods such as ^{1}H NMR, ^{13}C NMR, and ESI-MS [14].

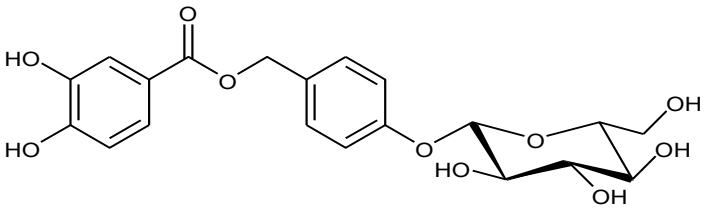

Figure 1. 4′-(3,4-dihydroxybenzoyloxymethyl)phenyl-*O*-*β*-D-glucopyranoside (PG)

Analysis of Oxidative Stress-Associated Parameters

The right hind limb gastrocnemius muscle tissues (approximately 100 mg) were homogenized in ice-cold 50 mM phosphate-buffered saline (PBS; pH 6) containing 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical Corp., St. Louis, MO) mixed 1:10 (w/v) with IKA Ultra Turrax T 25 Basic (IKA Labotechnik, Staufen, Germany) at 12000 rpm for 10 min. The homogenates were then measured for MPO activity. Aliquots (0.3 mL) were added to 2-3 mL of a reaction mixture containing 50 mM PBS (pH 6), o-dianisidine, and 20 mM hydrogen peroxide (H_2O_2). H_2O_2 was used as a substrate for MPO. Oxidized o-dianisidine forms a stable chromophore with maximal absorption at a wavelength of 460 nm [16]. One unit of enzyme activity was defined as the amount of MPO required to produce a change in absorbance at 460 nm for 3 min. One unit of MPO activity was defined as the amount required to degrade 1 μmol of H_2O_2 per minute at 25 °C. MPO activity was recorded as the activity per gram of tissue (U/g protein).

The right hind limb gastrocnemius muscle tissues (~200 mg) were homogenized in ice-cold 50 mM PBS (pH 7.4) mixed 1:10 (w/v) with IKA Ultra Turrax T 25 Basic (IKA Labotechnik) at 16000 rpm for 5 min. The homogenates were analyzed for the amount of GSH, amount of MDA, and SOD activity. All procedures were performed at 4 °C. The MDA concentration in the homogenates was determined spectropohotometrically (Benchmark Plus, Bio-Rad, Hercules, CA) by measuring for thiobarbituric acid-reactive substances [17]. The results are expressed as nanomoles per gram of tissue. The GSH concentration was measured spectrophotometrically using Elman's reaction [18]. The results are expressed as μmol per gram of tissue. SOD enzyme activity was measured based on the production of H_2O_2 from xanthine by xanthine oxidase and the reduction of nitroblue tetrazolium, as described previously [19]. The results are expressed as the units per gram of tissue.

Histopathologic Examination

The right hind limb gastrocnemius muscle tissues were fixed in 10% formaldehyde. After processing, the tissues were embedded in paraffin and cut into 4-μm thick sections. The muscles were evaluated for inflammation and bleeding using hematoxylin and eosin staining. Mast cells in muscle tissue were evaluated with toluidine blue staining.

Statistical Analysis

Statistical analysis was performed using a commercial software package (SPSS version 17.0 for Windows, SPSS Inc., Illinois, USA). The data was reported as mean±SD. The means of the four groups were compared by the Kruskal-Wallis test. If the result was statistically significant, then the Mann Whitney U test was applied to compare the difference between pairs. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Table 1. Effects of PG on lipid peroxidation (MDA), myeloperoxidase (MPO), glutathione (GSH) and superoxide dismutase (SOD) values in muscle of exercised rats (Mean±SD).

	Control (C) $\overline{X}$ **± SD**	**Acute Exercise (AE)** $\overline{X}$ **± SD**	**PG-Supplemented (PS)** $\overline{X}$ **± SD**	**Acute Exercise + PG-Supplemented (AE/PS)** $\overline{X}$ **± SD**
MDA (nmol/g protein)	10.47 ± 1.1	13.26 ± 1.8[a,b]	9.70 ± 1.1	11.00 ± 1.4[c]

MPO (U/g protein)	0.81 ± 0.1	1.39 ± 0.4[d,e]	0.70 ± 0.3	0.90 ± 0.3[f]
SOD (U/g protein)	7.02 ± 0.9	4.57 ± 1.0[g,h]	7.51 ± 0.8	6.78 ± 1.2[i]
GSH (µmol/g protein)	74.55 ± 15.7	52.36 ± 7.8[j,k]	78.83 ± 12.2	64.68 ± 9.4[l]

The MDA, MPO, SOD and GSH values for the different groups have been shown in Table 1. The levels of MDA ($p < 0.01$) and MPO ($p < 0.01$) were significantly increased in the skeletal muscle tissue of the acute exercise group compared to the control group. However, decreases in the lipid peroxidation product MDA and MPO concentrations were found in the AE/PG group ($p < 0.05$). Acute exercise caused significant decrease in gastrocnemius muscle tissue GSH ($p < 0.01$) and SOD levels ($p < 0.001$) when compared to the control group. After PG administration, there were increases in the activities of SOD ($p < 0.001$) and GSH ($p < 0.05$) in the AE/PG group, as compared to acute exercise group.

In our study, the gastrocnemius muscle tissue sections were evaluated by light microscopy following hematoxylin and eosin staining (Figure 2). Nucleus of muscle and connective tissue cells were increased in PG supplemented group (Figure 3a, 3b). We found an increased number of nuclei and a few centered nuclei (Figure 3c) in the muscle cells of the rats in the exercise group (Figure 3d), as compared to those in the PG-supplemented group. Using toluidine blue staining, we detected increased numbers of degranulated mast cells in the exercise group (Figure 3d). In addition, fewer neutrophils were present in the PG-supplemented group, as compared to the exercise group (Figure 3e). Capillary dilation was also seen in the PG-supplemented group (Figure 3f-g). The results for the control group were similar to those for the PG-supplemented group.

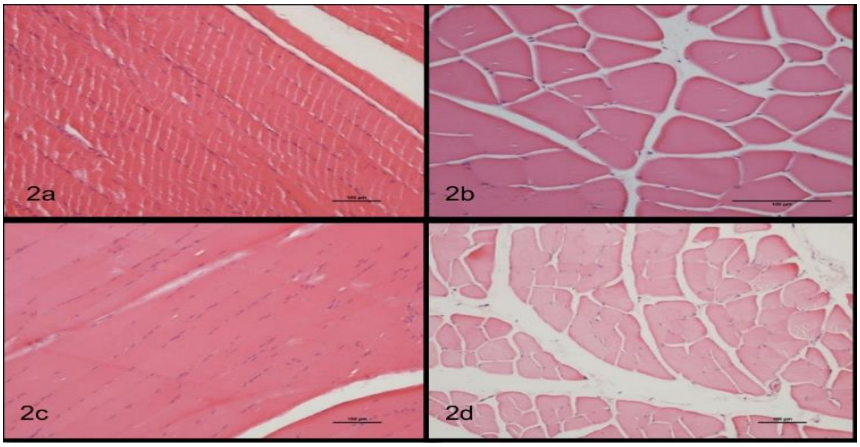

Figure 2. Skeletal muscle in group 1: sagittal section (2a), transvers section (2b). Sagital (2c)
and transvers (2d) sections of group 2. Group 2 is similar as group 1. Bar 100 μm.

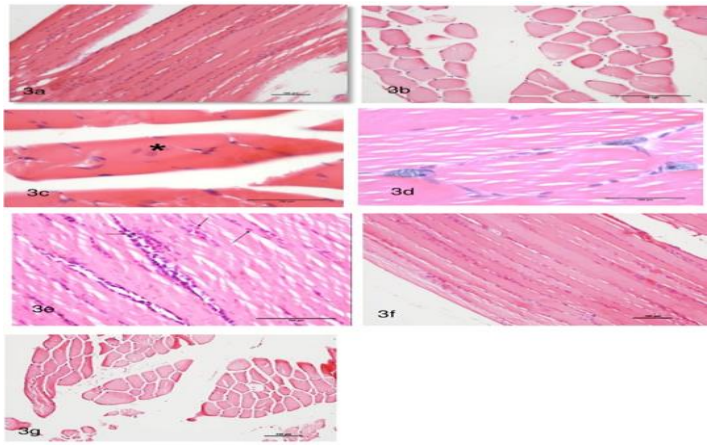

Figure 3. Nuclei of muscle and connective tissue cells increased in group 3 showing in sagital (3a) and transvers (3b) sections. A few centered nuclei (*) (3c). Many number of mast cells (→) around muscle cells (3d). Many number of neutrophils (→) between muscle fibers in group (3e). Decreased number of nuclei in muscle tissue and dilated capillaries in group 4. Sagital section (3f), transvers section (3g). Bar 100 μm.

CONCLUSION AND DISCUSSION

Skeletal muscle cells continuously generate ROS, which play a critical role in the modulation of muscle contractility: low and physiological levels of ROS are required for normal force production, but high levels of ROS promote contractile dysfunction, resulting in muscle weakness and fatigue, likely to be due to the oxidative damage of several molecular targets [20]. Exercise increases the utilization of oxygen in the body and, therefore, enhances the production of ROS and impairs both enzymatic and non-enzymatic antioxidant defense systems in target tissues and the blood [21,22]. Oxidative stress during exercise can be caused by an increase in oxidant compounds and/or a decrease in antioxidant defense systems [23,24]. Exhaustive exercise causes the release of MPO from neutrophils, which then induces severe oxidative damage [25]. MPO is regarded not only as an index of inflammation but also as an index of oxidative damage [26]. MDA levels increase after acute exhaustive exercise in plasma and skeletal muscle [27]. ROS are scavenged by a sophisticated

antioxidant defense system, which includes enzymes, SOD, catalase, GSH, and GSH peroxidase [28].

MPO is one of the main sources of extracellular free radicals during strenuous exercise, and it is responsible for the tissue damage caused by exhaustive exercise [2]. Our results show that there was a significant decrease in MPO levels in the PG-supplemented group. Oh-Ishi *et al*. measured MPO (found in abundance in neutrophils) in the diaphragm, and the ability of neutrophils to generate MPO in response to exercise as an index of extracellular oxidative stress, and found a significant increase in MPO levels in untrained rats after acute exhaustive exercise, consistent with the present results [29].

The data presented here show that the acute exhaustive exercise protocol caused increased MDA levels in plasma (Table 1). Furthermore, PG supplementation reduced the level of MDA and played a protective role against increased oxidative damage in muscle tissue. Aydin et al. found decreased MDA levels in liver, heart and kidney tissue in exhaustive swimming exercise [4]. Huang *et al*. found decreased MDA levels in skeletal muscle, liver, and kidney tissue homogenates from L-arginine-supplemented rats after exhaustive exercise [2]. Ana *et al*. [26] investigated the effect of stanozolol treatment on oxidative stress induced by acute exercise in rat skeletal muscle and found decreased levels of MDA-lysine in mitochondria. Our results are concordant with these studies.

GSH is a major non-enzymatic endogenous antioxidant that plays an important role in protecting skeletal and heart muscle from exercise-induced oxidative stress [30,31]. Wadley and McConell investigated the effects of vitamin C supplementation and found an increase in the ratio of oxidized to total GSH in the gastrocnemius muscle [32]. Aydin *et al*. found decreased GSH levels in liver, heart and kidney tissue in exhaustive swimming exercise [4]. Veera *et al*. reported that GSH levels, which increased in exercised rats, declined after selenium supplementation [33]. In our study, the GSH levels decreased after AE, but PG supplementation reduced oxidative damage and significantly increased the level of GSH.

In the present study, SOD was decreased after AE. The observed decrease in antioxidant enzyme activity may reflect the allosteric down-regulation of enzymes in addition to enzyme inactivation attributable to overwhelming oxidative stress. Belviranli et al. demonstrated that an acute exhaustive exercise protocol might induce a decrease in SOD and GSH peroxidase activity because of the increased production of ROS in animal tissues, especially skeletal muscle [34]. Shu-Ping et al. showed that animals receiving TRF-50 appeared to have higher levels of SOD in their liver and muscles, with significant decreases in

lipid peroxidation after acute swimming exercise [35]. Confirming these results, we found increased SOD levels following PG supplementation.

Bigard *et al*. found centrally located nuclei in the muscle cells of exercised animals [36]. We observed a few centrally located nuclei in the animals in the exercise group; after PG treatment, the sections were similar to those in the control group.

PG is a natural phenolic compound. The antioxidant activity of phenolic compounds is well known. There have been a lot of in vivo and in vitro studies on the antioxidant activity of phenolic compounds [37]. PG attenuates oxidative stress after acute exercise in rat skeletal muscle tissue by increasing tissue SOD and GSH activities and decreasing MDA and MPO levels. PG also improves morphological alterations which occur after periods of acute exercise. Briefly, this study provides evidence that PG, which is a molecule with antioxidant activity, may be beneficial as a supplement during heavy exercise.

AUTHOR CONTRIBUTIONS

Concept: H.Ç., T.F., U.Ö., E.S.K.; Design: H.Ç., T.F., U.Ö., E.S.K.; Control: H.Ç., T.F., U.Ö.; Literature: H.Ç., T.F., U.Ö.; Materials: H.Ç., T.F., U.Ö., E.S.K.; Collection and/or Processing: H.Ç., T.F., U.Ö.; Analysis and/or interpretation: H.Ç., T.F., U.Ö.; Writing the article: H.Ç., T.F., U.Ö.; Critical review: H.Ç., T.F., U.Ö.; Others:-

CONFLICT OF INTEREST DECLARATION

The authors declare that there is no actual, potential or perceived conflict of interest for this article.

ETHICAL CONSIDERATIONS

This study was approved by the Animal Care and Use Local Ethics Committee of Bolu Abant İzzet Baysal University (Bolu, Türkiye) (No: 9/4/2010- 2010/10). All experiments were performed according to the Guidelines for Animal Care and Experimentation.

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Chapter 7

Natural Language Processing in Healthcare: Applications in Veterinary and Human Medicine

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Introduction

The amount of data generated in the healthcare sector has increased exponentially over the past twenty years. Electronic health records, clinical notes, laboratory reports, radiology reports, and scientific literature constitute an accumulation of unstructured text data reaching millions of pages daily. The vast majority of this data is in free text format and is quite challenging to process using traditional data analysis methods. (1,2).

Natural language processing is a subfield of artificial intelligence that develops computers' ability to understand, interpret, and generate human language. Natural language processing technologies play a critical role in the execution of clinical decision support systems, disease surveillance, epidemiological studies, and clinical research by converting unstructured health data into meaningful, structured information. In recent years, with the development of deep learning algorithms and the emergence of large language models, natural language processing has become a transformative technology in the healthcare field (3,4).

Natural language processing applications in veterinary medicine and human medicine share fundamental principles but present different challenges and opportunities specific to each field. Veterinary clinical records are generally less standardised, contain multi-species information, and exhibit greater variability in terminology usage. In human medicine, although standardised terminologies are widely used, abbreviations in clinical notes, jargon usage, and context-dependent expressions remain significant challenges (5,6).

This study comprehensively addresses the fundamental concepts, methods, and current applications of natural language processing in healthcare. Key application areas such as information extraction from electronic health records, clinical entity recognition, disease classification, and automatic coding are

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examined in detail. Furthermore, natural language processing applications in veterinary and human medicine are evaluated from a comparative perspective, highlighting the commonalities, unique challenges, and development potential of these two fields. Finally, current limitations, ethical and methodological issues, and future research and application perspectives are discussed.

Fundamental Concepts of Natural Language Processing

Natural language processing is an interdisciplinary field that processes textual data by combining linguistic rules and statistical models. Natural language processing applications in healthcare require domain-specific terminology, abbreviations, and context understanding, unlike general-purpose natural language processing methods. In a clinical note, the abbreviation "CHF" stands for "congestive heart failure," while "CKD" stands for "chronic kidney disease," and the correct recognition of these abbreviations is critical (7).

The first step in the natural language processing process is called text pre-processing and includes sub-processes such as tokenisation, sentence segmentation, stop word removal, and normalisation. Tokenisation is the process of dividing text into words or meaningful units. Normalisation involves converting different forms of words into a standard form (8,9).

Natural language processing methods used in healthcare can be divided into three main categories: rule-based approaches, machine learning methods, and deep learning models (10-12).

Rule-based systems extract information from text using linguistic rules and regular expressions defined by domain experts. The advantage of this approach is high accuracy and interpretability, while the disadvantage is its inability to accommodate linguistic diversity and the constant need for new rules (13).

Machine learning-based approaches learn from labelled datasets to perform tasks such as text classification, entity recognition, and relation extraction. Traditional machine learning algorithms have yielded successful results when used with feature engineering. However, the performance of these methods depends on the quality of manually designed features (14,15).

Deep learning models, particularly large language models based on the transformer architecture, have revolutionised natural language processing in recent years. BERT, GPT, and their healthcare-specific versions demonstrate high performance with minimal feature engineering. These models are pre-trained on corpora consisting of billions of words and then fine-tuned for specific tasks (16,17).

Natural Language Processing in Electronic Health Records

Electronic health records form the backbone of modern healthcare systems and contain critical information for patient care, billing, quality improvement, and research. A typical electronic health record contains both structured fields (age, gender, laboratory values) and unstructured free-text fields (medical history, physical examination findings, radiology reports, discharge summaries). Unstructured text constitutes more than 80% of the total information in electronic health records and contains the most valuable information for clinical decision-making (18,19).

The use of electronic health records in veterinary medicine has increased significantly in recent years. Large-scale veterinary databases such as VetCompass, VetCompass Australia, and SAVSNET have become valuable resources for epidemiological studies and disease surveillance. These systems contain the clinical records of millions of animals and provide important insights into disease prevalence, risk factors, and treatment outcomes. However, analysing the free-text notes in these records is practically impossible without automated natural language processing tools (20,21).

Studies conducted on the VetCompass database have demonstrated the challenges of automatically extracting disease diagnoses from free-text clinical notes. Kennedy et al. (2019), developed a natural language processing algorithm to identify false positive disease references in veterinary clinical notes. The study emphasised the need to distinguish general statements such as "pancreatitis is seen in cats" from actual patient diagnoses. The algorithm achieved an 87% accuracy rate using contextual clues without requiring manual annotation (22).

Systems developed for information extraction from electronic health records in human medicine are more mature. Tools such as cTAKES, developed by the Mayo Clinic, MetaMap, developed by the National Library of Medicine, and Comprehend Medical, offered by Amazon, have strong capabilities for recognising entities in clinical text, matching them to standard medical terminologies, and extracting relationships (23-25).

These systems can recognise various types of clinical entities, such as diseases, symptoms, medications, dosages, anatomical regions, and procedures.

One of the most important applications of natural language processing in electronic health records is clinical entity recognition and normalisation. Entity recognition involves identifying diseases, drugs, symptoms, and other medical concepts mentioned in the text, while normalisation involves mapping these entities to standard terminologies such as SNOMED-CT, ICD-10, and RxNorm. For example, if a veterinary note contains the phrase "heart failure in a dog,"

this phrase should be identified and mapped to the SNOMED-CT code . Similarly, in human medicine, the phrase "type 2 diabetes mellitus" should be mapped to the ICD-10 code E11 (26,27).

Natural Language Processing Models in Veterinary Medicine

Although the development of natural language processing models specific to veterinary medicine is a relatively new field, significant progress has been made in the last five years. DeepTag, developed by Nie et al. (2018), is a deep learning-based system for automatic diagnosis extraction from veterinary clinical notes. DeepTag was trained on a corpus of 239,000 clinical notes obtained from Stanford University Veterinary Hospital and achieved an accuracy rate of over 85%. The model can extract disease diagnoses from free-text notes and map them to standard disease codes (28).

VetTag, developed by Zhang et al. (2019), is an extension of the DeepTag model and supports a broader disease taxonomy. VetTag can perform disease coding not only in dogs and cats but also in horses, cattle, and small mammals. Using a transfer learning approach, the model has demonstrated high performance even in species with limited data. VetTag's most significant contribution is its multi-species support and cross-species knowledge transfer (29).

PetBERT, developed by Farrell et al. (2023), is an adaptation of the BERT architecture for veterinary clinical texts. PetBERT was pre-trained on a corpus of 5 million clinical notes collected from primary care veterinary clinics in the UK. The model was used for ICD-11 syndromic disease coding and achieved an 89% F1 score in outbreak detection applications. PetBERT's success demonstrates the importance of domain-specific pre-training (30).

Jiang et al. (2024), developed VetLLM to investigate the use of large language models for veterinary diagnosis prediction. The study used general-purpose large language models such as GPT-3 and GPT-4 to predict diagnoses from veterinary notes and evaluated the models' performance. The results showed that the ability of large language models to learn from very few examples provided an advantage in rare diseases with limited labelled data. However, the models' risk of producing hallucinations and lack of explainability were identified as significant limitations (31).

One of the greatest challenges of natural language processing in veterinary medicine is the lack of standardisation in clinical records. Hur et al. (2019), applied natural language processing to understand antimicrobial usage patterns using VetCompass Australia data. The study found that the same drug was recorded differently by different clinics; for example, it could be written using

various abbreviations such as "amoxicillin", "amoxy", or "amox". This necessitated the development of special algorithms for normalising drug names (32).

Natural Language Processing Models in Human Medicine

Natural language processing models in human medicine are more mature and diverse than those in veterinary medicine. Models such as ClinicalBERT, BioBERT, BlueBERT, and Med-PaLM have been customised for different clinical tasks and subjected to extensive evaluations (33-35).

ClinicalBERT, developed by Huang et al. (2019), was pre-trained on a corpus of 2 million clinical notes obtained from the MIMIC-III database. The model demonstrated state-of-the-art performance in hospital readmission prediction, mortality prediction, and clinical outcome classification tasks (36).

GatorTron is a massive language model developed by the University of Florida and trained on a clinical text corpus of 90 billion words (37).

GatorTron outperforms previous models in relation inference, entity binding, and medical question answering tasks. The model's size and training data volume have significantly enhanced its ability to understand complex clinical scenarios. However, the model's computational cost and environmental impact have sparked significant debate (38,39).

Med-PaLM and Med-PaLM 2 are large language models developed by Google and optimised specifically for medical question answering. Med-PaLM 2 has demonstrated expert-level performance on US medical licensing exam questions, achieving an 86.5% accuracy rate. While these models possess a rich medical knowledge base, their integration with real clinical records is limited, and their use in clinical decision support systems requires careful evaluation (40,41).

One important application of natural language processing in human medicine is the automatic summarisation and structuring of radiology reports. Veen et al. (2023), developed a system called RadAdapt to automatically summarise radiology reports and highlight important findings (42). The system converted long and complex reports into short, understandable summaries, accelerating clinicians' decision-making process. Similar approaches are being developed for pathology reports and discharge summaries (43).

Bürgisser et al. (2024), examined the use of large language models for disease detection in electronic health records, using crystal arthropathies as an example. The study demonstrated that the GPT-4 model could accurately detect gout and pseudogout diagnoses from clinical notes. The model was able to resolve complex diagnostic scenarios by using not only explicit diagnostic

statements but also contextual clues. Such studies demonstrate the potential of large language models in clinical decision support (44).

Clinical Entity Recognition and Relationship Extraction

Clinical entity recognition is one of the most fundamental tasks in natural language processing and involves identifying medical concepts mentioned in the text (45).

Entity recognition systems are generally formulated as sequence labelling problems and utilise conditional random fields, hidden Markov models, or deep learning-based approaches. In recent years, BiLSTM-CRF and transformer-based models have demonstrated the highest performance. These models recognise entities by combining word embeddings, character-level features, and contextual information (46).

Relationship inference involves determining the semantic relationships between recognised entities. For example, in the sentence "Antibiotic prophylaxis was administered to the patient after surgical intervention," there is a causal relationship between "surgical intervention" and "antibiotic prophylaxis." Relationship inference is critical for clinical reasoning and decision support systems because it structures complex clinical information such as treatment-outcome relationships, disease-symptom relationships, and drug-drug interactions (47).

Sammani et al. (2021), used deep neural networks for the automatic detection of ICD-10 codes from Dutch cardiology discharge letters. Formulated as a multi-label classification problem, the model achieved a 91% F1 score. The system made independent predictions for each ICD-10 code, taking into account that multiple diagnoses could be present in a discharge letter. This approach has the potential to significantly reduce the manual coding workload (48).

Disease Classification and Syndromic Surveillance

One important application of natural language processing is disease classification and syndromic surveillance from free-text clinical notes. Syndromic surveillance involves monitoring specific symptoms or syndromes at the population level and provides early warning for public health interventions (49).

Bollig et al. (2020), used machine learning for syndromic surveillance from veterinary necropsy reports. In the study, free-text necropsy reports were automatically classified into categories such as respiratory syndrome, digestive syndrome, and neurological syndrome. The model detected syndromes with 88

per cent accuracy, and a system was developed that could be used for the early detection of disease outbreaks (50).

Davies et al. (2024), conducted a comprehensive two-part study on text mining in veterinary clinical data. In the first part, the linguistic characteristics of veterinary clinical records were examined, and word search strategies were developed. The second part addressed methods for training computers to recognise features in clinical texts. The study analysed in detail the unique structure of veterinary clinical language and how this structure affects natural language processing algorithms (51,52).

Kennedy et al. (2023), used a gradient boosting model to identify cases from free-text veterinary records. The study developed machine learning algorithms to automatically identify specific disease cases from clinical notes. The model achieved 92 per cent sensitivity and 89 per cent specificity compared to manual review and significantly accelerated case identification for epidemiological studies (53).

In human medicine, Comito et al. (2023), developed an AI-powered clinical decision support system that improves disease diagnosis by using patient similarity. The system analyses free-text notes in electronic health records to identify similar patient cases and provides diagnosis suggestions based on this similarity. Patient similarity is calculated based on multiple factors such as symptoms, laboratory results, and treatment history (54).

Automatic Coding and Terminology Matching

The conversion of diagnoses, procedures, and conditions in clinical records into standard codes is necessary for billing, quality measurement, epidemiological research, and public health reporting. Medical coding systems such as ICD-10, SNOMED-CT, CPT, and HCPCS enable the standardised representation of health information. However, manual coding is a time-consuming, error-prone, and costly process. Natural language processing can automate this process, thereby increasing efficiency and reducing error rates (55).

Boguslav et al. (2024), fine-tuned foundational models for diagnosis coding from veterinary health records. In the study, pre-trained language models were fine-tuned on veterinary clinical notes to develop automated diagnosis coding systems. The results revealed that fine-tuned models performed significantly better than general-purpose models (56).

Venkataraman et al. (2019, 2020), developed a system called FasTag to perform automatic text classification of unstructured medical narratives. FasTag has a structure that can be applied to both veterinary and human medical records

and automatically maps free-text notes to disease codes. The system achieved high performance with limited labelled data by employing active learning strategies (57,58).

Huang et al. (2024), conducted a critical evaluation of using ChatGPT to extract structured data from clinical notes. The study systematically analysed the potential and limitations of large language models for clinical knowledge extraction. The results revealed that the models were successful in simple information extraction but showed inconsistencies in tasks requiring complex clinical reasoning (59).

Limitations, Challenges, and Ethical Issues

Natural language processing applications in healthcare face numerous challenges and limitations. The first and most significant challenge is the lack of training data. Deep learning models require large amounts of labelled data to perform well (60).

However, labelling medical data is an expensive and time-consuming process that requires domain expertise. This problem is even more pronounced in veterinary medicine because there is less research funding and smaller databases compared to human medicine (61).

Data privacy and security are critical ethical issues in the processing of health data. Clinical notes contain patient and animal owner information, clinician notes, and sensitive health information. Anonymising and securely storing this data are legal and ethical requirements. Data protection regulations such as GDPR and HIPAA set strict rules for the use of health data. Compliance with these regulations is mandatory when developing natural language processing systems (62).

Model explainability and reliability are critical in clinical applications. Deep learning models are often referred to as "black boxes" because their decision-making processes are not transparent. Understanding why a model recommends a particular diagnosis is necessary for clinicians to trust the model and assume clinical responsibility. Explainability techniques such as SHAP and LIME help interpret model decisions, but their adequacy in a clinical context is debatable (63).

A systematic review by Du et al. (2024), identified common issues with the use of generative large language models in electronic health records. Low accuracy in rare cases, erroneous treatment recommendations, hallucination generation, limited explainability, and heterogeneity in evaluation metrics are among the most significant problems. The study emphasised the need to develop standardised methodological criteria and evaluation frameworks (64).

Specific challenges in veterinary medicine include multi-species support, terminological variation, and lack of data standardisation. When a natural language processing model is trained for dogs, it may not perform equally well for cats, horses, or farm animals because disease terms, symptom definitions, and treatment approaches differ between species. Additionally, the software and record formats used in veterinary clinics are highly heterogeneous (30,56).

Future Perspectives and Innovations

The future of natural language processing in healthcare will be shaped by several key developments. Multimodal learning will provide a more comprehensive clinical understanding by integrating text data with imaging, laboratory results, and genetic data. For example, processing images alongside a radiology report could improve diagnostic accuracy. Similarly, integrating clinical notes with histopathology reports could yield more precise results in oncological diagnosis (65,66).

Transfer learning and few-shot learning techniques will help overcome the limited data problem. Large general-purpose language models can be adapted to small veterinary or specific medical fields to achieve high performance. Meta-learning approaches will enable models to adapt quickly to new tasks. These techniques are particularly valuable for rare diseases and underrepresented species (67,68).

The development of real-time clinical decision support systems will accelerate the integration of natural language processing technologies into clinical workflows. While a clinician is writing a patient note, the system can provide real-time suggestions for potential diagnoses, treatment recommendations, drug interactions, and guideline recommendations. These systems can reduce clinical errors and support evidence-based practice (69).

Natural language processing for multilingual models and low-resource languages will increase the global accessibility of health technologies. Currently, the vast majority of natural language processing research focuses on English, and resources for other languages are limited. Developing clinical natural language processing systems in languages such as Turkish, Arabic, and Hindi will support the digital transformation of healthcare systems in these regions (70).

In the future, the role of natural language processing in healthcare will expand further. Multimodal models will provide a more comprehensive clinical understanding by integrating text, image, and numerical data. Real-time systems will provide clinicians with instant decision support. Personalised medicine approaches will develop treatment recommendations optimised for individual patient characteristics. Global health equity will be supported by systems adapted

for multilingual and low-resource settings. These developments have the potential to revolutionise the delivery of healthcare services and patient outcomes.

Conclusions and Recommendations

Natural language processing in healthcare is a powerful tool for extracting valuable information from unstructured text in electronic health records, supporting clinical decision-making, disease surveillance, and research. In both veterinary and human medicine, natural language processing technologies have the potential to increase the efficiency of clinical applications, reduce error rates, and support evidence-based practice. In recent years, the development of deep learning and, in particular, transformer-based large language models has significantly improved natural language processing performance. However, challenges such as limited and labelled datasets, privacy concerns, and model explainability hinder the widespread adaptation of these technologies in the clinical setting (71).

Natural language processing in veterinary medicine, while less mature than in human medicine, is developing rapidly. Models such as VetTag, PetBERT, DeepTag, and VetLLM have been developed to address the unique challenges of veterinary clinical texts. The success of these models demonstrates the importance of domain-specific pre-training and transfer learning. However, multi-type support, terminological variation, and data standardisation remain significant challenges.

In human medicine, clinical decision support systems provide significant benefits to healthcare professionals by optimising diagnosis and treatment processes using information extracted from medical records (72). Models such as ClinicalBERT, BioBERT, GatorTron, and Med-PaLM have demonstrated high performance in various clinical tasks and achieved results at or above human level in some cases. These models are used in applications such as information extraction from electronic health records, automatic coding, clinical decision support, and medical question answering. However, limitations such as the risk of hallucination, lack of explainability, and low performance in rare cases persist. Although transformer-based large language models have high potential in clinical applications, the lack of comprehensive evaluation and application guidance is slowing down their integration into healthcare services (73). Furthermore, although large language models can generate consistent and contextually appropriate responses , they can occasionally provide incorrect or misleading information (74). As this poses a significant risk, particularly in clinical decision support systems, it is essential that models are carefully validated and continuously monitored in clinical settings (75).

Several conditions must be met for natural language processing systems to be successful in clinical applications. First, the creation and sharing of high-quality,

labelled datasets should be encouraged. The active participation of domain experts in the data labelling process is critical for model accuracy. Secondly, standardised metrics and test sets should be developed to evaluate model performance. Thirdly, methods should be developed for model explainability and reliability, and the necessary transparency should be provided for clinicians to trust the models.

Data privacy and security should be a priority in the design and implementation of natural language processing systems. Anonymisation techniques, access controls, and encryption methods should be standard practice for protecting patient data. Compliance with GDPR, HIPAA, and other data protection regulations is a legal and ethical imperative. Privacy-preserving technologies such as federated learning can facilitate inter-institutional collaboration.

Multidisciplinary collaboration is essential for the success of natural language processing systems. Clinicians, data scientists, software engineers, and ethics experts should work together throughout all stages of the system development process. Clinicians define real-world needs and the clinical context, data scientists develop appropriate algorithms, and software engineers integrate the systems into clinical workflows. This collaboration ensures the development of user-friendly, clinically useful, and ethically responsible systems.

Education and capacity building are essential for the adoption of natural language processing technologies. Data science, artificial intelligence, and natural language processing topics should be included in the curricula of veterinary and medical schools. Continuing professional development programmes should enable existing clinicians to understand these technologies and evaluate them critically. Training opportunities in natural language processing methods for researchers should be increased, and interdisciplinary research should be encouraged.

Policy makers and regulatory bodies should develop frameworks to ensure the safe and effective use of natural language processing technologies. Medical device regulations should be updated to cover AI-supported decision support systems. Clinical validation requirements, performance standards and post-market surveillance mechanisms should be established. At the same time, balanced regulations that prioritise patient safety without hindering innovation should be established. In this context, transparent communication and interaction among stakeholders in the healthcare field play a critical role in the development and implementation of artificial intelligence systems (76). These interactions can help ethical committees and legal regulators establish policies that encourage responsible use (77). Furthermore, focusing on the transparency and explainability of artificial intelligence models will accelerate their integration into clinical decision-making processes by increasing healthcare professionals' trust in these systems (78).

In conclusion, natural language processing (NLP) in healthcare stands out as a transformative technology that maximises the value of unstructured health data, supporting clinical decision-making processes, improving care quality, and enhancing the overall efficiency of healthcare systems. The effective analysis of large volumes of data, such as electronic health records, clinical notes, and free-text reports, contributes significantly to diagnosis, treatment, monitoring, and health management processes in both human and veterinary medicine.

The responsible, ethical, and evidence-based development and implementation of NLP applications in veterinary and human medicine will contribute to the creation of more reliable clinical decision support systems in the future and, consequently, to better patient and animal health outcomes. In this process, not only technological advances but also ethical principles, data security, and clinical context awareness play a central role.

Realising the full potential of natural language processing in healthcare requires multidisciplinary and coordinated collaboration between researchers, clinicians, educators, policymakers, and technology developers. It is particularly important to systematically evaluate the performance of AI-based models integrated into clinical applications under real-world conditions through empirical studies. In this context, developing open, standardised, and ethically compliant datasets representing different clinical scenarios will both increase model comparability and accelerate scientific progress in the field.

Additionally, to support the safe and effective use of large language models in healthcare, controllability and steerability mechanisms that facilitate interaction between healthcare professionals and researchers with these models must be developed. Approaches such as prompt engineering enhance the context-appropriate nature of model outputs while also offering significant advantages in terms of transparency and reliability.

Finally, the adoption of federated learning and similar privacy-focused approaches is critical to protect sensitive health data and increase the generalisability of models across different institutions and populations. Such methods enable the development of AI models with high clinical value in a sustainable manner without compromising data privacy.

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Chapter 8

Needle EMG and Basic MUAP Analysis

Hasan YAŞAR[1]

Electromyography (EMG) consists of nerve conduction studies and needle EMG evaluation. It examines pathologies from motor units to anterior horn cells. Needle EMG is particularly important in the diagnosis of radiculopathy, myopathy, plexopathy, and motor neuron disease(1).

Needle EMG

The muscle is examined by moving the needle in short steps (0.5-1 mm) along a straight line into the muscle(2). The needle's registration area is a 1 mm diameter area(3).Insertional activity is assessed. Subsequently, the presence of spontaneous activity while the muscle is at rest is examined. Motor unit action potential (MUAP) are evaluated during low, medium, and maximum contraction. During the insertional activity, short-term electrical activity is normally observed; if it lasts longer than 300 ms, it is pathological and may be seen in cases of nerve damage, myopathy, or inflammation (1).

Spontaneous Activities and Denervation

When a needle is inserted into the muscle, silence is normally expected at rest; however, findings such as fibrillation, positive sharp waves, and complex repetitive discharges (CRD) indicate pathology. Healthy resting muscles are normally electrically silent; the only exception is activity occurring at the neuromuscular junction (NMJ)(1, 4). If there are signs of denervation such as fibrillation potentials and positive sharp waves, a neurogenic process should be considered(5). The sound produced by this potential is similar to the sound of rain hitting a tin roof. CRD is type of abnormal spontaneous potential that can arise from the activation of adjacent muscle fiber groups. Motor unit-derived spontaneous potentials include fasciculations, myokymia, cramps, and neuromyotonia(6). Myotonic discharges are another type of spontaneous activity originating from muscle fibers. These fluctuations are similar to the highly distinctive electrical sound of a dive bomber. Myotonic discharges occur not only in myopathies accompanied by myotonia (myotonic dystrophy and congenital paramyotonia), but also in other diseases such as hyperkalemic

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periodic paralysis, polymyositis, toxic myopathy, and several axonal disorders. Fasciculation often produces a sound similar to popping corn. It is more common in pathological conditions such as chronic neurogenic diseases (motor neuron disease, peripheral axonal neuropathies, and radiculopathies) and also in metabolic conditions such as hyperthyroidism(1).

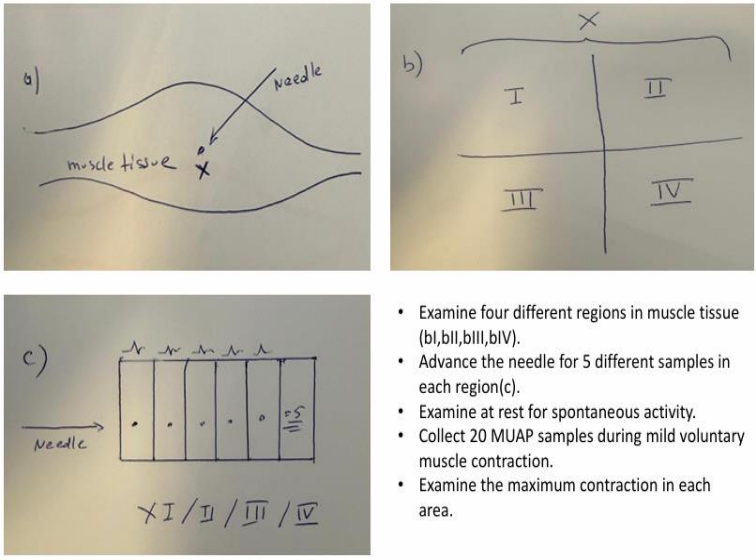

Figure 1: In needle EMG examination, a needle is inserted into the muscle tissue(a). The tissue is divided into 4 quadrants(b). In one quadrant, 5 different sections are examined for denervation and MUAP by advancing the needle. The needle is withdrawn under the skin. The other quadrant is entered(c). Five points are examined in each quadrant. At least 20 areas are scanned for spontaneous denervations. 20 MUAPs are examined during voluntary contraction and during maximum contraction(2, 7)..

MUAP Analysis

Voluntary MUAP represents the temporal and spatial aggregate of single muscle fiber action potentials in the electrode recording area, generated by the firing of a frontal horn cell(3).

Screen settings during MUAP analysis

For sweep time settings, 10 ms/div is generally preferred. For amplitude adjustment, 200 µV/div or 500 µV/div are commonly used(2). For MUAPs, having pointed tops is generally considered an indicator of good quality(8). During MUAP analysis, slight muscle contraction should be ensured. A small number of MUAPs are seen on the screen and analyzed. Excessive contraction

causes numerous MUAPs to overlap, making analysis difficult. Samples are taken from different regions. Various MUAPs are recorded and examined by passing the needle through different areas of the muscle. At least 20 MUAP samples should be collected for each muscle. The average duration, amplitude, and number of phases should be calculated from these samples. A typical MUAP should have a duration of 5–15 ms, an amplitude of 300–3000 μV, and a phase number of 2–4. Its shape should be pointed, symmetrical, and stable. In neurogenic changes, the duration is prolonged (>15 ms), the amplitude increases (>3000 μV), the number of phases increases (>4), and the MUAP shape becomes polyphasic and irregular. In myopathic changes, the duration is shortened (<5 ms), the amplitude is decreased (<300 μV), the phase number is generally normal, and the shape is small, pointed, and short. When calculating the MUAP duration, the number of segments between the start and end of the MUAP is counted. The duration of a segment is displayed on the screen as ms/div. When calculating the MUAP amplitude, the vertical divisions between the lowest and highest parts of the waveform are counted. The value of each division is indicated in μV in the display settings. An average can be taken by examining several consecutive discharges from the same motor unit. Only MUAPs with a short rise time should be considered(2).

Specifically, motor unit loss is visually assessed according to the fullness of the interference pattern at maximum effort(9). Amplitude is determined only in 2-12 muscle fibers very close to the needle tip(1).

Conclusion

Spontaneous potentials can be observed in both neuropathic and myopathic diseases. MUAP parameters alone are not sufficient; they should be evaluated together with clinical findings. A typical MUAP should be thin and high-amplitude if close to the needle tip, and thick but low-amplitude if further away. Large amplitude and thick MUAP, whether near or far from the needle tip, are neurogenic(10). As the power is increased, different motor units start engaging at different times depending on their proximity to the needle tip. Therefore, the MUAPs that engage at different times are displayed on the screen. In neuropathic disease, motor units are reduced and collaterals develop, so there will be a limited number of motor units in the needle's recording area. There will not be a rich variety of motor units when increasing muscle contraction. A healthy, complete interference pattern exhibits rapid movement and increasing amplitude of MUAPs, culminating in a thick line with an amplitude of 2-4 mV when maximum contraction is reached. In neuropathic disorders, a fence pattern forms at maximum muscle contraction. Depending on the characteristics of

myopathies, spontaneous activity may be observed; positive sharp wave and fibrillation potentials may be seen in inflammatory myopathies, while myotonic discharges may be seen in some myopathies. Therefore, the key feature of needle EMG in differentiating chronic myopathies from chronic neuropathies is to evaluate the interference pattern(1).

The most sensitive parameter widely accepted as specific to myopathy is the shortening of the MUAP duration. However, these changes in MUAP are not always associated with myopathy. When interpreting EMG findings, we must consider the absence of any abnormalities specific to myogenic disorders(11).

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Chapter 9

Needle EMG and Basic MUAP Analysis

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Comparison of 3D Printed Models with Conventional Plaster Models

Digital dentistry has significantly changed clinical and laboratory procedures in recent years. Digital impressions, model fabrication, and design steps are now much faster, which helps reduce clinical working time (1). At the same time, many new dental materials—such as glass ceramics, zirconia, various resins, and PEEK—have become widely used by clinicians and technicians (2). Production methods have also evolved, and one of the most important innovations is three-dimensional (3D) printing, an additive manufacturing technique (3).

3D printing is applied in several dental procedures, including model production, temporary crowns, and surgical guides. The process begins with creating a digital model. For this, an intraoral scanner captures data, which is then saved in formats such as STL, PLY, or OBJ (4). STL contains only the surface shape, while OBJ also includes color and texture, making it useful for smile design (5). During the slicing step, the operator chooses the layer thickness and printing direction. Layer thickness affects both the number of layers and the total printing time. A thinner layer generally increases accuracy but reduces mechanical strength (6). After slicing, the printer starts producing the model, and this step has a major effect on accuracy and strength.

The International Organization for Standardization (ISO-TC 261/ISO 17296-2:2015) has classified the technologies used in additive manufacturing into seven categories: Vat-polymerization (VP), material jetting (MJ), material extrusion (ME), powder bed fusion (PBF), binder jetting (BJ), sheet lamination (SL), and direct energy deposition (DEP)(7,8). Among the most widely used technologies is *Vat-Polymerization* (VP), which cures liquid photopolymer resins layer by layer using ultraviolet (UV) light (9). VP systems are commonly used to fabricate diagnostic models, surgical guides, provisional restorations,

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and various appliances (7). The two main VP technologies are stereolithography (SLA) and digital light processing (DLP) (10). In SLA, a laser cures each point individually. This point-by-point polymerization provides more controlled shrinkage and stress distribution, resulting in higher precision. Because the laser beam can scan a large area, SLA is especially suitable for printing larger models, such as those used in orthodontics (11,12). DLP, on the other hand, uses a digital micromirror device (DMD) to project and polymerize an entire layer at once (13). This simultaneous curing significantly reduces printing time, making DLP advantageous for fast or high-volume production (14). Although DLP generally produces models more quickly, several studies report that its accuracy may be slightly lower than that of SLA for certain applications, such as small restorations (12,15). However, implant-related studies have shown that DLP can outperform SLA when printing models containing multiple implant analogs (16,17). Because DLP polymerizes each layer uniformly, it distributes energy more homogeneously and reduces shrinkage variability across the model. Park et al. (18) compared the surface characteristics of SLA- and DLP-printed models using SEM and found that DLP models exhibited more pronounced layer lines, whereas SLA models had smoother and more homogeneous surfaces.

PolyJet technology operates by jetting low-viscosity photopolymer droplets through multiple nozzles, leveling the material with a roller, and curing it with ultraviolet (UV) light. The presence of multiple nozzles allows simultaneous printing of different materials, colors, and mechanical properties within a single build (19). However, its high operational cost and the requirement for specialized low-viscosity resins are considered major disadvantages (20).

Material Extrusion (ME), commonly known as Fused Deposition Modeling (FDM), is another widely used technique in dentistry. In this method, a thermoplastic filament is heated and extruded through a nozzle, depositing material layer by layer to form the object (21,22). FDM is generally less expensive than other 3D printing systems (23), and many of its printed materials are autoclavable, which is beneficial for sterilization. This technique enables the fabrication of high-performance thermoplastics such as PEEK and PEKK—biocompatible, lightweight, and durable polymers often considered alternatives to titanium (24). These materials are used in applications such as implant-supported bar frameworks, removable denture bases, and provisional restorations (25).

Many studies have compared these printing technologies in terms of resolution, speed, and clinical applications. One of the key parameters in evaluating them is accuracy (5), which consists of trueness and precision. Trueness reflects the degree of deviation between the printed model and the

actual reference model, while precision refers to the reproducibility of repeated prints.

Dental models serve as three-dimensional replicas of the patient's oral structures, and their accuracy is essential for maintaining precision throughout all stages of treatment. Even micron-level deviations in a model can affect the fit of the final restoration; for example, passive fit may be compromised in implant-supported prostheses (26,27). For this reason, model accuracy has become a major focus in the literature. Although conventional plaster models have long been regarded as the gold standard (28), advances in digital dentistry and the introduction of new materials have led to extensive comparisons between plaster and 3D-printed models. These studies have examined a variety of parameters and generally report that 3D-printed models can serve as reliable alternatives to traditional plaster models, while continuing to improve as printing technologies evolve (17,29–31).

Marta Czajkowska et al. (2020) evaluated the accuracy of 3D-printed and plaster models using three different surface-based measurements. All deviation values were below 1 mm, which was considered clinically acceptable, indicating that 3D printing can serve as a viable alternative to plaster models (29). A comparable investigation by Ellakany et al. (2022) converted digital scans obtained from two intraoral scanners (TRIOS 3Shape and Dental Wings) into SLA-printed models and compared them with conventional plaster casts. The printed models demonstrated similar accuracy to plaster models; however, noticeable distortion occurred during interarch measurements (30). This distortion remained below the 5% clinical threshold reported by Czarnota et al. (32). Studies examining DLP technology—including those by Alshawaf (2018), Banjar (2021), and Gagnon-Audet (2023)—also found that DLP-printed models produced clinically acceptable results, with deviations below 200 μm (33–35). Higher error rates observed along the vertical (Z-axis) direction were attributed to resin polymerization shrinkage (36). Additional comparisons between SLA and plaster models by Al-Imam et al. and Alshawaf et al. concluded that plaster models were more accurate overall (33,37). Similarly, Sim et al. reported that plaster outperformed DLP, particularly in full-arch models (38,39).

Implant-related studies further expanded the literature. These investigations varied implant number, angulation, and positioning to evaluate their effects on model accuracy. Buda et al. (16), for example, compared conventional, PolyJet, and SLA models for a single implant. Deviations were assessed vertically, horizontally, and rotationally. PolyJet and plaster models demonstrated high accuracy, whereas SLA exhibited greater deviation. Revilla-León et al. (40) also reported superior accuracy for PolyJet models when compared with plaster, using various scan bodies and implant analog designs. Both studies placed

implant analogs vertically. In contrast, Tan et al. (2024) examined two implant analogs positioned at buccolingual angles of 0°, 10°, and 20°. Models produced by both conventional and DLP methods were assessed. Although DLP maintained high accuracy for parallel implants, deviations increased with greater angulation, while still remaining within clinically acceptable limits. This reduction in accuracy was attributed to increased polymerization shrinkage in angled implant situations (41). For this reason, epoxy-based or hybrid resins may be more suitable than methacrylate-based materials in such cases (12,42,43). Derakhshi et al. (2025) further investigated whether polymerization shrinkage in resins and hardening expansion in plasters affected all regions of the model uniformly. Using DLP, LCD, and plaster casts produced from the same master model, they simulated both implant-supported and tooth-supported restorations. Plaster demonstrated the least distortion in interdental areas. However, all methods showed greater deformation interdentally than inter-implant. The complex anatomy of teeth reduced the accuracy of 3D-printed models, whereas DLP yielded higher accuracy in inter-implant regions (17).

In addition to comparing plaster and printed models, many studies have also evaluated differences among various 3D-printing technologies. These comparisons typically focus on factors such as production cost, layer thickness, printing speed, resolution, and overall accuracy. Camardella et al. reported that PolyJet printing produced more accurate results than SLA, which they attributed to shrinkage behavior associated with SLA systems (44). Similarly, Lee et al. found PolyJet to be more accurate than FDM when fabricating 3D-printed teeth (45). In contrast, Rebong et al. suggested that FDM may serve as a better alternative to plaster models due to the greater shrinkage observed in both SLA and PolyJet prints (46). Abdeen et al. (2022) compared conventional plaster models with 3D-printed models produced using several technologies—Straumann® P30+ (DLP, 50 µm), BEGO Varseo S (DLP, 100 µm), Formlabs Form 3b (SLA, 100 µm), and M2 Carbon (CLIP, 100 µm). Their results showed that CLIP and SLA yielded the highest trueness, while deviations from the other printers remained within clinically acceptable limits. When comparing two layer-thickness settings within the same technology, the 50-µm prints demonstrated higher accuracy than those printed at 100 µm (47). Brown et al. conducted another study involving orthodontic patients in the retention phase, comparing DLP, PolyJet, and plaster models. PolyJet demonstrated superior accuracy in the Z-axis, which the authors attributed to its finer layer thickness (16 µm) compared to DLP (50 µm) (36). Additionally, DLP models require post-curing, which contributes to polymerization shrinkage and explains the greater vertical deviation observed (48). Overall, these studies highlight that

layer thickness has a direct influence on production speed, surface quality, mechanical strength, and dimensional accuracy (6,31).

Studies evaluating layer thickness commonly compare 50-µm and 100-µm settings. Ahn and Choi (2025) assessed several parameters in maxillary models and found that although layer thickness did not significantly influence overall accuracy, the 50-µm setting produced slightly more precise prints (49). In contrast, Jin et al. reported that thicker layers reduced polymerization shrinkage in implant-analog models; despite the higher resolution of 50-µm prints, the 100-µm group demonstrated superior positional accuracy (50). Sherman et al. similarly found no meaningful accuracy difference between 50-µm and 100-µm layers, suggesting that 100-µm may be preferable when faster printing is required (51). Sim et al. also noted that thinner layers increase printing time but have the potential to improve accuracy (38).

Layer thickness additionally influences mechanical behavior. Alshamrani et al. examined DLP-printed samples at 25, 50, and 100 µm and observed the highest flexural strength in the 100-µm group (52). Tahayeri et al. reported that specimens printed at 25 µm and 100 µm demonstrated greater fracture strength than those printed at 50 µm, although the overall effect of layer thickness on mechanical strength was limited (53). Alharbi et al. maintained a 50-µm layer thickness and investigated the effect of build orientation on compressive strength, finding that vertical orientation minimized crack formation and delamination (54). Shim et al. assessed flexural strength at various printing orientations and reported the highest values at 0°, corresponding to horizontal printing (55). Although these findings may appear inconsistent, they evaluate different mechanical properties: compressive strength is clinically relevant for temporary restorations subjected to occlusal loading, whereas flexural strength is critical for components such as denture bases.

Mechanical strength in printed models is influenced by several factors, including layer thickness and post-processing procedures such as washing time, curing time, curing temperature, and the incorporation of nanoparticles. After printing, residual unpolymerized monomers remain on the model surface, and washing is performed to remove them. Hwangbo et al. evaluated a wide range of washing durations (3–90 minutes) and found that prolonged washing—particularly 90 minutes—resulted in the lowest mechanical strength. They also reported that insufficient washing (<15 minutes) may increase cytotoxicity (56). Jang et al. observed that both fracture strength and degree of conversion improved as washing time increased, recommending a 10-minute IPA wash (57). Xu et al., who investigated orthodontic splints, similarly used IPA for post-processing and found that mechanical strength decreased after 1 hour of washing, while surface cracking occurred following 12 hours of exposure (58).

The choice of washing solution also has a significant effect on mechanical performance. Scherer et al. examined several solvents—IPA-91, IPA-99, bio-ethanol (100%), tripropylene glycol monomethyl ether (100%), and a water-miscible formulation (ResinAway)—at rinsing times of 5–8 minutes. The highest flexural strength values were achieved with IPA-91 and IPA-99, particularly at 7 and 8 minutes (59). Bardelcik et al. further demonstrated that PMMA samples washed with IPA exhibited reduced strength and increased brittleness, whereas the combined use of hydrogen peroxide and detergent produced more flexible and resilient materials (60).

Post-processing procedures extend beyond washing and include post-curing, which completes the polymerization of residual unpolymerized monomers. Both curing temperature and curing duration are known to influence the mechanical behavior of printed resins (61). In their study, Bayarsaikhan et al. investigated curing temperatures of 40°C, 60°C, and 80°C combined with curing times of 15, 30, 60, 90, and 120 minutes. The specimens cured at 40°C demonstrated the lowest strength, while those cured at 60°C and 80°C showed comparable performance. The highest strength and hardness were achieved at 80°C for 120 minutes (62). Aati et al. (2022), using a light-curing protocol, also reported increases in flexural strength, surface hardness, degree of conversion, and reductions in water absorption as curing time increased (63). Generally, higher curing temperatures enhance hardness but reduce flexibility, which may lead to increased brittleness once a certain thermal threshold is exceeded (64,65).

Hague et al. evaluated three curing regimens: standard UV curing for 90 minutes; UV curing followed by thermal curing at 80°C for 2 hours; and an accelerated aging protocol involving thermal curing at 80°C for 24 hours. The extended high-temperature treatment resulted in over-curing and pronounced brittleness (66). Similarly, Jindal et al. observed that increasing curing temperature and time improved mechanical strength up to an optimal point, beyond which brittleness and crack formation occurred (67). Their findings also indicated that short-duration, high-temperature curing (e.g., 10 minutes at 80°C) produced better flexural strength than longer curing at lower temperatures (e.g., 20 minutes at 60°C) (43).

Overall, the literature consistently indicates that extending curing time decreases the amount of residual monomers and enhances mechanical performance. In contrast, inadequate polymerization weakens resistance to functional forces and compromises long-term stability (42,68).

Beyond curing, the incorporation of nanoparticles provides an additional means of improving the mechanical behavior of 3D-printed resins. Nanoparticles increase strength, limit crack propagation, reduce water absorption by filling microvoids, and—depending on their composition—may impart antibacterial properties (69–72). Different nanoparticles contribute unique benefits: zirconia (ZrO_2) and alumina

(Al_2O_3) improve overall mechanical strength (73,74); silica (SiO_2) decreases water absorption and enhances dimensional stability (75); titanium dioxide (TiO_2) increases light transmission and thus reduces residual monomer content (76); and silver (Ag) particles provide antimicrobial activity.

Alshaikh et al. examined heat-cured acrylic and 3D-printed resins reinforced with varying concentrations of ZrO_2 (0%, 0.5%, 1%, 3%, and 5% by weight). Flexural strength and hardness increased up to 3%, after which a decline was observed due to particle agglomeration (77). These findings align with those of Aati et al. Similarly, Protopapa et al. reported improved impact resistance in all nanoparticle-reinforced groups, although high particle loads reduced homogeneity and promoted agglomeration (79). Shirkavand et al., who used TiO_2, also observed reduced tensile strength when excessive nanoparticle content caused clustering (69). Gad et al. evaluated SiO_2-modified 3D-printed resins and found increased flexural strength and reduced surface roughness at low concentrations. However, as with previous studies, higher concentrations resulted in agglomeration, increased surface roughness, and elevated water absorption (80).

Water absorption plays a key role in determining the dimensional stability and mechanical performance of printed models (81). Penetration of water into voids within the polymer matrix reduces hardness and fracture toughness and promotes crack propagation (63,82). Incorporating nanoparticles helps limit this effect, as the particles occupy microvoids and restrict fluid penetration (77).

Aati et al. (2022) demonstrated that longer curing times decrease water absorption. Their specimens were stored in artificial saliva for 48 hours and 6 months, and increased cross-linking at higher curing durations produced a chemically more stable surface with reduced fluid uptake (63). Consistent with this, Greil et al. reported that curing time influences water absorption; however, excessive curing may induce microcracks, ultimately increasing water sorption (82).

Layer thickness also contributes to water absorption behavior. Bayarsaikhan et al. found that thinner layers showed reduced water uptake due to stronger cross-link formation (62). Nevertheless, extremely thin layers—as reported by Quan et al. in the 10–25 μm range—may delaminate, and weak interlayer bonding can lead to increased water absorption (12).

In summary, this review highlights that the performance of 3D-printed dental models is the result of multiple interacting variables, including printing parameters (layer thickness, build angle), post-processing conditions (washing and curing protocols), and material characteristics (polymer network structure, water absorption tendency). When these factors are appropriately optimized, 3D-printed models can serve as viable alternatives to conventional plaster models.

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Chapter 10

Evaporation Prediction Using Machine Learning Models Enhanced with cVAE–cWGAN-GP Based Synthetic Data Generation

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1. Introduction

Neurorehabilitation is a specialized and evolving field of rehabilitation sciences that aims to optimize functional recovery, independence, and quality of life in individuals with neurological disorders by promoting neuroplasticity through structured, repetitive, and task-specific training. Neurological conditions such as stroke, spinal cord injury (SCI), Parkinson's disease (PD), and multiple sclerosis (MS) are among the leading causes of long-term disability worldwide, placing a substantial burden on individuals, caregivers, and healthcare systems.

Traditional neurorehabilitation models rely predominantly on episodic, clinic-based assessments and therapist-guided interventions. Clinical decision-making is commonly informed by standardized outcome measures, observational assessments, and patient self-reports. Although these approaches remain foundational, they present important limitations. Assessments conducted in controlled clinical environments may not accurately reflect performance in real-world contexts, where environmental variability, cognitive demands, and fatigue significantly influence motor behavior (Dobkin & Dorsch, 2011).

Furthermore, many commonly used clinical scales are ordinal in nature and may lack sensitivity to subtle but clinically meaningful changes in motor performance. Inter-rater variability and ceiling effects further limit their utility, particularly in individuals with mild impairments or during the chronic stages of recovery. As a result, clinicians may underestimate residual disability or fail to detect gradual functional decline or improvement over time (Patel et al., 2012).

Wearable technologies have emerged as promising tools to address these challenges by enabling continuous, objective, and ecologically valid monitoring of movement and physiological responses during daily life. Advances in sensor miniaturization, wireless communication, and data analytics have facilitated the development of wearable systems that can be seamlessly integrated into routine

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activities. These technologies allow for the collection of high-resolution data on motor performance, physical activity, and physiological load outside the clinic, offering a more comprehensive understanding of functional ability and recovery trajectories (Alt Murphy et al., 2024).

In recent years, the integration of wearable technologies into neurorehabilitation has expanded beyond assessment to include intervention delivery, biofeedback, and long-term monitoring. Wearable systems support personalized rehabilitation by enabling clinicians to tailor interventions based on individual performance patterns and contextual factors. Moreover, they play a central role in home-based rehabilitation and telerehabilitation models, which are increasingly recognized as essential components of sustainable rehabilitation care, particularly in the chronic phase of neurological conditions (Dobkin et al., 2015).

This book chapter aims to provide a comprehensive and critical overview of wearable technologies in neurorehabilitation. The chapter discusses the types of wearable sensors commonly used, their clinical applications, and their role across major neurological conditions. In addition, implementation challenges, ethical considerations, and future directions are examined to inform both clinical practice and research.

2. Overview of Wearable Technologies in Neurorehabilitation

Wearable technologies refer to electronic devices designed to be worn on the body, capable of continuously collecting biomechanical, physiological, and contextual data during movement or daily activities. In the context of neurorehabilitation, wearable systems have gained increasing attention due to their potential to provide objective, real-time, and ecologically valid measurements of motor performance beyond traditional clinical settings (Patel et al., 2012; Alt Murphy et al., 2024).

Conventional neurorehabilitation assessments are typically conducted in clinical environments and rely on clinician observation, standardized rating scales, and time-based performance tests. While these tools are widely used and clinically meaningful, they often provide only a snapshot of performance and may not capture variability in motor behavior across different contexts or over time. Moreover, the artificial nature of clinical testing environments may influence patient performance, leading to results that do not fully represent functional abilities in everyday life (Dobkin & Dorsch, 2011).

Wearable technologies address these limitations by enabling continuous monitoring of movement and physiological responses in natural environments. This capability enhances the ecological validity of assessments and allows

clinicians to examine how individuals perform functional tasks in real-world settings. From a clinical perspective, wearable technologies serve three primary functions in neurorehabilitation: (i) objective assessment of motor and physiological function, (ii) support of therapeutic interventions through real-time or delayed feedback, and (iii) long-term monitoring of activity, adherence, and participation (Lobo et al., 2024).

The rapid evolution of wearable technologies has been driven by advances in sensor accuracy, battery life, wireless data transmission, and data processing algorithms. These developments have facilitated the integration of wearable systems with digital health platforms, electronic health records, and telehealth infrastructures. As a result, wearable technologies are increasingly positioned as key components of data-driven and personalized neurorehabilitation models.

2.1 Inertial Measurement Units

Inertial measurement units (IMUs) are among the most widely used wearable sensors in neurorehabilitation research and clinical practice. IMUs typically consist of tri-axial accelerometers, gyroscopes, and, in some configurations, magnetometers. These sensors enable the measurement of linear acceleration, angular velocity, and spatial orientation of body segments during movement (Patel et al., 2012).

IMUs have been extensively applied to the assessment of gait, balance, postural control, and upper limb movements in individuals with neurological disorders. In gait analysis, IMU-derived metrics include walking speed, step and stride length, cadence, stance and swing phase durations, gait symmetry, and variability. These parameters are particularly informative in neurological populations, where motor impairments often manifest as asymmetrical and highly variable movement patterns (Dobkin et al., 2015).

One of the major advantages of IMUs is their portability and suitability for use outside laboratory environments. Unlike optical motion capture systems, which require specialized equipment and controlled settings, IMUs can be used in clinics, homes, and community environments. This allows clinicians to assess gait and movement under conditions that more closely reflect everyday activities, thereby enhancing ecological validity and clinical relevance (Alt Murphy et al., 2024).

In upper limb neurorehabilitation, IMUs are used to quantify reaching movements, joint coordination, movement smoothness, and arm use during functional tasks. These objective measures provide valuable insights into motor control strategies and compensatory behaviors that may not be detected by conventional clinical assessments. Importantly, IMU-derived metrics have been

shown to be sensitive to changes over time, making them useful for tracking recovery and evaluating intervention effectiveness (Lobo et al., 2024).

Despite their advantages, challenges remain regarding standardization of sensor placement, data processing, and interpretation of IMU-derived outcomes. Ongoing research efforts aim to establish standardized protocols and clinically meaningful thresholds to facilitate broader clinical adoption.

2.2 Physiological Wearables

Physiological wearable sensors play a critical role in capturing internal bodily responses associated with movement and exercise during neurorehabilitation. These devices include surface electromyography (EMG) sensors, heart rate and heart rate variability monitors, electrodermal activity sensors, and wearable electroencephalography (EEG) systems.

Surface EMG sensors are widely used to assess muscle activation patterns, timing, and coordination during functional tasks. In neurological populations, EMG wearables provide objective information about abnormal muscle co-contraction, altered recruitment strategies, and spasticity-related muscle activity. This information supports both assessment and intervention planning by guiding exercise selection, progression, and neuromuscular re-education strategies (Patel et al., 2012).

Cardiovascular wearables, such as heart rate and heart rate variability monitors, offer insights into physiological load, aerobic capacity, fatigue, and autonomic regulation during rehabilitation sessions. These measures are particularly relevant in conditions such as stroke and MS, where reduced cardiovascular fitness and fatigue are common and significantly influence functional performance and participation (Lobo et al., 2024).

Wearable EEG systems, although less commonly used in routine clinical practice, have demonstrated potential for assessing cortical activity during movement and rehabilitation tasks. These systems contribute to understanding the neural mechanisms underlying motor recovery and support the development of neurofeedback and brain–computer interface-based interventions (Chang et al., 2022).

By complementing motion-based sensors, physiological wearables provide a multidimensional perspective on motor performance, linking observable movement outcomes with underlying neuromuscular and physiological processes.

2.3 Smart Textiles and Garments

Smart textiles and wearable garments represent an emerging category of wearable technologies in neurorehabilitation. These systems integrate sensors directly into clothing, enabling unobtrusive and continuous monitoring of movement and physiological signals during daily activities.

Smart garments may incorporate textile-based sensors capable of measuring joint angles, muscle activity, respiratory patterns, and pressure distribution. By embedding sensors into garments, these systems reduce the need for external attachments and frequent repositioning, thereby improving comfort and usability—key factors for long-term monitoring in neurological populations, including older adults and individuals with significant mobility impairments (Patel et al., 2012).

One of the primary advantages of smart textiles is their suitability for extended wear in home and community environments. Improved comfort and ease of use enhance adherence and data quality, making smart garments particularly valuable for monitoring functional activity, posture, and movement patterns over prolonged periods (Alt Murphy et al., 2024).

Despite their potential, challenges related to sensor durability, data accuracy, washability, and cost currently limit widespread clinical adoption. However, ongoing advances in textile engineering, flexible electronics, and data analytics are expected to further enhance the feasibility and clinical utility of smart garments in neurorehabilitation (Lobo et al., 2024).

3. Clinical Applications of Wearable Technologies in Neurorehabilitation

Wearable technologies have transitioned from experimental tools to clinically meaningful instruments in neurorehabilitation, enabling objective assessment, personalized intervention, and long-term monitoring across diverse neurological populations. By continuously capturing movement, physiological, and contextual data in real-world environments, wearable systems address key limitations of traditional clinic-based assessments, which are often episodic, subjective, and constrained by time and resources (Dobkin & Dorsch, 2011; Patel et al., 2012). This section explores the primary clinical applications of wearable technologies in neurorehabilitation, focusing on assessment, therapeutic intervention and feedback, and long-term monitoring of functional recovery.

3.1 Wearable Technologies for Assessment in Neurorehabilitation

3.1.1 Objective Measurement of Motor Performance

One of the most significant contributions of wearable technologies to neurorehabilitation is the ability to provide objective, quantitative measurements of motor performance. Inertial measurement units (IMUs), consisting of

accelerometers, gyroscopes, and magnetometers, enable detailed analysis of joint kinematics, postural control, and gait parameters in both clinical and ecological settings (Muro-de-la-Herran et al., 2014; Picerno, 2017).

In contrast to traditional clinical outcome measures such as the Fugl-Meyer Assessment, Berg Balance Scale, or Unified Parkinson's Disease Rating Scale (UPDRS), wearable-derived metrics offer high temporal resolution and sensitivity to subtle changes in motor behavior (Patel et al., 2012). Parameters such as step length variability, gait symmetry, trunk sway, movement smoothness, and joint angular velocity can be continuously monitored, providing a more comprehensive representation of functional ability (Horak et al., 2015).

Recent evidence highlights the validity and reliability of wearable sensors for assessing gait and balance impairments in neurological populations. Wearable-derived mobility metrics show strong associations with established clinical mobility scales and are capable of detecting impairments that may not be captured during brief clinical assessments (Fino et al., 2024). In addition, IMU-based gait analysis has demonstrated good to excellent agreement with laboratory-based motion capture systems, supporting its validity for clinical and research applications (Picerno, 2017).

3.1.2 Assessment in Real-World and Ecological Contexts

A key advantage of wearable technologies lies in their capacity to assess motor function in real-world environments rather than controlled laboratory or clinic settings. This ecological validity is particularly important in neurorehabilitation, where performance during daily activities often differs from observed performance during structured assessments (Dobkin & Dorsch, 2011).

Wearable sensors allow continuous monitoring of mobility, upper limb use, and physical activity levels during activities of daily living (ADLs). For example, accelerometer-based activity monitors can quantify arm use asymmetry in individuals with stroke, providing insights into learned non-use that may not be evident during clinic-based evaluations (Lang et al., 2013). Similarly, wearable devices have been used to assess fall risk by identifying patterns of instability and near-fall events in individuals with balance disorders (Weiss et al., 2013).

The Frontiers in Neurorobotics review emphasizes that ecological assessment using wearables enables clinicians to capture fluctuations in symptoms and performance across different contexts and times of day, which is particularly relevant for conditions characterized by variability, such as Parkinson's disease and multiple sclerosis (Del Din et al., 2022) .

3.1.3 Digital Biomarkers and Data-Driven Assessment

Wearable technologies have also facilitated the emergence of digital biomarkers—objective, quantifiable indicators of health and function derived from sensor data. In neurorehabilitation, digital biomarkers can reflect disease severity, functional capacity, and recovery trajectories (Patel et al., 2012).

For example, gait speed, stride-to-stride variability, and turning metrics derived from IMUs have been proposed as digital biomarkers of mobility impairment in neurological disorders (Del Din et al., 2016). Physiological wearables measuring heart rate variability (HRV), skin conductance, and muscle activation (via surface electromyography) further contribute to multidimensional assessment of physical and autonomic function (Baig et al., 2021).

The integration of machine learning techniques with wearable data has enhanced the ability to classify movement patterns, predict clinical outcomes, and detect early signs of deterioration (Shull et al., 2014). The MDPI Sensors article underscores the growing role of artificial intelligence in transforming raw wearable data into clinically interpretable metrics that support decision-making in neurorehabilitation .

3.2 Wearable Technologies for Intervention and Therapeutic Feedback

3.2.1 Biofeedback and Augmented Feedback

Beyond assessment, wearable technologies play an increasingly important role in delivering therapeutic interventions through real-time feedback. Biofeedback systems provide users with information about their movement or physiological state, enabling motor learning through augmented sensory input (Sigrist et al., 2013).

Wearable-based biofeedback can be delivered via visual, auditory, or haptic modalities. For instance, vibrotactile feedback has been used to cue gait timing and improve step symmetry in individuals with stroke or Parkinson's disease (Afzal et al., 2015). Visual feedback through smartphone applications or head-mounted displays can support postural control and upper limb coordination during task-oriented training.

Evidence suggests that wearable biofeedback enhances motor performance by promoting error-based learning and increasing patient engagement (Sigrist et al., 2013). The Physical Therapy Journal article highlights that real-time feedback from wearable sensors can support task-specific practice and improve movement quality during rehabilitation exercises.

3.2.2 Wearable Technologies in Motor Learning and Neuroplasticity

Motor recovery following neurological injury relies on principles of neuroplasticity, including repetition, task specificity, and feedback. Wearable

technologies align closely with these principles by enabling high-dose, individualized practice supported by continuous performance feedback (Kleim & Jones, 2008).

By monitoring movement parameters during therapy sessions, wearables allow clinicians to adjust task difficulty and progression based on objective performance metrics. This adaptive approach supports optimal challenge points, which are critical for effective motor learning (Guadagnoli & Lee, 2004).

Furthermore, wearable devices can promote self-management and active participation by empowering individuals to monitor their own performance. This aligns with contemporary rehabilitation models that emphasize patient-centered care and self-efficacy (Dobkin, 2005).

3.2.3 Integration with Robotics and Virtual Reality

Wearable technologies are increasingly integrated with robotic devices and virtual reality (VR) systems to enhance therapeutic interventions. IMUs and physiological sensors can be used to control virtual avatars, track movement accuracy, and adapt task difficulty in real time (Laver et al., 2017).

The integration of wearable technologies with virtual reality and robotic systems enables immersive, data-driven rehabilitation environments that support patient motivation and adherence to therapy (Del Din et al., 2022). Wearable sensors also enable objective evaluation of training intensity and movement quality during technology-assisted rehabilitation, addressing a common limitation of traditional therapy documentation.

3.3 Long-Term Monitoring and Outcome Tracking

3.3.1 Monitoring Recovery Trajectories

Long-term monitoring is essential for understanding recovery trajectories in neurorehabilitation, particularly in chronic and progressive neurological conditions. Wearable technologies enable continuous or intermittent monitoring over weeks or months, providing insights into functional changes beyond the clinic (Patel et al., 2012).

Wearable-derived metrics can be used to track improvements in mobility, upper limb use, and physical activity levels, supporting data-driven evaluation of rehabilitation outcomes. This longitudinal perspective is particularly valuable for identifying plateaus, regressions, or delayed improvements that may warrant modifications in intervention strategies.

3.3.2 Supporting Personalized and Adaptive Rehabilitation

The ability to monitor individuals in their natural environments supports personalized rehabilitation approaches tailored to individual needs, goals, and

contexts. Data from wearable devices can inform individualized goal setting, progression of exercises, and adjustment of therapy intensity (Shull et al., 2014).

Wearable-based monitoring supports precision rehabilitation by enabling adaptive interventions based on real-time performance data, an approach that aligns with broader trends toward personalized medicine and value-based healthcare (Patel et al., 2012; Stoppa & Chiolerio, 2014).

3.3.3 Clinical Decision-Making and Health System Integration

From a clinical perspective, wearable technologies offer opportunities to enhance decision-making by providing objective, longitudinal data that complement clinical judgment. Integration of wearable data into electronic health records and clinical workflows remains a challenge but holds significant potential for improving continuity of care (Patel et al., 2012).

Moreover, wearable technologies support outcome measurement for research and quality improvement initiatives, facilitating benchmarking and evaluation of rehabilitation programs.

4. Wearable Technologies Across Neurological Conditions

Wearable technologies have demonstrated substantial potential across a wide range of neurological conditions by enabling condition-specific assessment, intervention, and monitoring strategies. While the core technological components may be similar, their clinical applications vary considerably depending on the underlying pathophysiology, symptom profile, and rehabilitation goals of each condition. This section provides a detailed overview of the use of wearable technologies in stroke, spinal cord injury, Parkinson's disease and movement disorders, multiple sclerosis, and other neurological conditions, with particular emphasis on functional recovery, activity monitoring, and clinical decision-making.

4.1 Stroke Rehabilitation

Stroke remains one of the leading causes of long-term disability worldwide, frequently resulting in impairments in motor control, balance, gait, and upper limb function. Wearable technologies have emerged as valuable tools for addressing the heterogeneity of post-stroke impairments and supporting personalized rehabilitation strategies (Langhorne et al., 2011).

4.1.1 Assessment of Motor Impairments After Stroke

Wearable sensors, particularly IMUs and accelerometers, are widely used to quantify gait and upper limb movement in individuals with stroke. Parameters

such as gait speed, step length asymmetry, trunk instability, and movement smoothness provide objective indicators of motor impairment severity (Muro-de-la-Herran et al., 2014; Picerno, 2017).

Upper limb accelerometry has been extensively applied to assess real-world arm use, revealing discrepancies between clinical motor capacity and actual arm activity during daily life (Lang et al., 2013). This phenomenon, often referred to as "learned non-use," is a critical barrier to functional recovery and may not be detected through conventional clinical assessments alone.

Wearable-derived mobility metrics have been shown to be sensitive to functional changes in neurological populations and capable of capturing clinically meaningful improvements that may not be reflected in conventional ordinal clinical scales (Fino et al., 2024).

4.1.2 Wearable-Supported Interventions in Stroke

Wearable technologies support stroke rehabilitation through real-time feedback, task-specific training, and activity monitoring. Biofeedback systems using vibrotactile or auditory cues have been shown to improve gait symmetry, step timing, and postural control (Afzal et al., 2015).

In upper limb rehabilitation, wearable sensors can provide feedback on movement amplitude, speed, and repetition, supporting high-intensity, goal-oriented practice. When combined with virtual reality or gamified platforms, wearables enhance motivation and adherence to therapy, particularly in the chronic phase of stroke recovery (Laver et al., 2017).

4.1.3 Long-Term Monitoring and Community Reintegration

Following discharge from formal rehabilitation services, wearable technologies enable long-term monitoring of physical activity and participation in community-based activities. Step count, walking bouts, and arm use metrics provide valuable insights into real-world recovery and participation (Patel et al., 2012).

This long-term perspective supports clinicians in identifying individuals at risk of functional decline and tailoring follow-up interventions accordingly.

4.2 Spinal Cord Injury

Spinal cord injury (SCI) results in complex and heterogeneous impairments affecting motor, sensory, and autonomic systems. The severity and level of injury significantly influence functional outcomes, making individualized assessment and rehabilitation essential. Wearable technologies offer unique advantages in addressing the multifaceted rehabilitation needs of individuals with SCI.

4.2.1 Assessment of Mobility and Functional Performance in SCI

Wearable sensors enable detailed assessment of residual motor function, gait characteristics (in ambulatory individuals), and wheelchair mobility in individuals with SCI. IMUs placed on the trunk and lower limbs allow quantification of postural control, balance strategies, and compensatory movements during standing and walking (Nooijen et al., 2015).

For wheelchair users, wearable accelerometers and gyroscopes have been used to evaluate propulsion patterns, upper limb loading, and activity levels during daily life. These measures are particularly relevant given the high prevalence of upper limb overuse injuries in this population (Cowan et al., 2009).

Wearable technologies enable ecologically valid assessment of motor performance by capturing movement patterns across diverse real-world contexts and time periods, which is particularly relevant for neurological conditions characterized by symptom variability (Del Din et al., 2022).

4.2.2 Wearable Technologies in Locomotor and Upper Limb Training

Wearable devices support locomotor training in individuals with incomplete SCI by providing feedback on gait parameters such as step length, cadence, and weight shifting. Real-time feedback facilitates motor learning and encourages active participation during body-weight-supported treadmill training or overground walking (Harkema et al., 2012).

In upper limb rehabilitation, wearable sensors can monitor movement quality during reaching, grasping, and functional tasks. This is particularly relevant for individuals with tetraplegia, where subtle improvements in hand and arm function can have a profound impact on independence.

4.2.3 Monitoring Physical Activity and Health-Related Outcomes

Physical inactivity is a major concern in SCI and is associated with secondary complications such as cardiovascular disease, obesity, and reduced quality of life. Wearable activity monitors enable continuous tracking of physical activity levels, sedentary behavior, and energy expenditure (van den Berg-Emons et al., 2010).

Wearable technologies support a holistic approach to neurorehabilitation by integrating kinematic, physiological, and activity-related data, thereby facilitating personalized intervention planning and long-term health management (Patel et al., 2012; Stoppa & Chiolerio, 2014).

4.3 Parkinson's Disease and Movement Disorders

Parkinson's disease (PD) and related movement disorders are characterized by bradykinesia, rigidity, tremor, postural instability, and fluctuations in motor

performance. Wearable technologies are particularly well suited to these conditions due to their ability to capture symptom variability and movement patterns in real-world settings.

4.3.1 Objective Quantification of Motor Symptoms

Wearable sensors have been extensively used to quantify cardinal motor symptoms of PD, including tremor amplitude, gait freezing, step variability, and turning performance (Del Din et al., 2016). These objective measures complement clinical rating scales such as the UPDRS, which are subject to inter-rater variability and limited temporal resolution.

IMU-based gait analysis enables detection of freezing of gait episodes and subtle changes in mobility that may precede clinical deterioration. The ability to capture these events in daily life is a major advantage over clinic-based assessments.

4.3.2 Monitoring Motor Fluctuations and Treatment Response

Motor fluctuations related to medication cycles are a hallmark of PD. Wearable technologies enable continuous monitoring of motor performance across the day, providing insights into on–off fluctuations and dyskinesias (Patel et al., 2009).

Continuous wearable-based monitoring enables the capture of motor symptom variability across daily contexts, thereby supporting individualized medication management and optimization of rehabilitation timing in Parkinson's disease (Del Din et al., 2022).

4.3.3 Wearable-Based Interventions in PD

Wearable cueing systems delivering auditory or vibrotactile stimuli have been shown to improve gait initiation, cadence, and step length in individuals with PD (Nieuwboer et al., 2007). Such systems support external cueing strategies that bypass impaired internal timing mechanisms.

When integrated into home-based training programs, wearable cueing devices promote autonomy and long-term adherence to rehabilitation.

4.4 Multiple Sclerosis and Other Neurological Conditions

Multiple sclerosis (MS) is a chronic, immune-mediated neurological condition characterized by fluctuating symptoms, including fatigue, balance impairment, spasticity, and cognitive dysfunction. Wearable technologies are particularly valuable in MS due to their capacity to capture symptom variability over time.

4.4.1 Assessment and Monitoring in MS

Wearable sensors enable objective assessment of gait, balance, and physical activity in individuals with MS. Gait variability, walking endurance, and postural sway metrics derived from IMUs have been shown to correlate with disease severity and functional status (Spain et al., 2012).

Continuous monitoring allows detection of subtle functional changes that may indicate disease progression or response to rehabilitation.

4.4.2 Fatigue and Activity Regulation

Fatigue is one of the most disabling symptoms in MS and is often poorly captured by self-report measures. Wearable activity monitors provide objective insights into activity patterns, rest–activity cycles, and energy expenditure, supporting fatigue management strategies (Learmonth et al., 2013).

4.4.3 Other Neurological Conditions

Beyond stroke, SCI, PD, and MS, wearable technologies have been applied to a range of neurological conditions, including traumatic brain injury, cerebellar disorders, and peripheral neuropathies. In these populations, wearables support objective assessment of balance, coordination, and functional mobility, contributing to individualized rehabilitation planning (Patel et al., 2012).

5. Wearable Technologies in Home-Based Rehabilitation and Telerehabilitation

The increasing demand for accessible, cost-effective, and continuous rehabilitation services has accelerated the adoption of home-based rehabilitation and telerehabilitation models. Wearable technologies play a central role in these models by enabling remote assessment, monitoring, and intervention, thereby extending neurorehabilitation beyond traditional clinical settings (Dobkin & Dorsch, 2011).

5.1 Rationale for Home-Based Wearable Rehabilitation

Conventional neurorehabilitation is often constrained by limited therapy time, geographic barriers, and healthcare resource shortages. These limitations are particularly evident in chronic neurological conditions, where long-term rehabilitation is required but often inadequately supported (Langhorne et al., 2011).

Wearable technologies address these challenges by allowing continuous monitoring of motor performance and physical activity in home and community environments. This approach enhances ecological validity and provides clinicians

with insights into functional performance during daily life rather than isolated clinic visits (Patel et al., 2012).

5.2 Remote Monitoring and Teleassessment

Wearable sensors facilitate teleassessment by transmitting movement and physiological data to clinicians in real time or asynchronously. IMU-derived gait metrics, activity counts, and posture data can be remotely reviewed to assess functional status, adherence, and progression (Del Din et al., 2016).

Wearable-based mobility measures have demonstrated sensitivity to functional changes and suitability for remote outcome tracking, thereby supporting their integration into telerehabilitation services (Fino et al., 2024).

5.3 Enhancing Adherence and Self-Management

Adherence to home-based rehabilitation programs is a persistent challenge. Wearable technologies promote engagement through feedback, goal setting, and progress tracking. Visual dashboards and smartphone applications allow individuals to monitor their performance, fostering self-efficacy and motivation (Dobkin, 2005).

Gamification and feedback-driven exercise programs supported by wearables have been shown to improve adherence and sustain participation in long-term rehabilitation (Laver et al., 2017).

6. Implementation Challenges, Ethical Considerations, and Data Security

Despite their potential, the widespread adoption of wearable technologies in neurorehabilitation faces several challenges related to usability, data management, ethics, and clinical integration.

6.1 Usability and Acceptance

User acceptance is critical for successful implementation. Neurological impairments such as cognitive dysfunction, tremor, and fatigue may limit the usability of wearable devices. Device design must prioritize comfort, simplicity, and minimal setup requirements (Patel et al., 2012).

Clinician acceptance is equally important. Wearable systems must provide clinically meaningful metrics that integrate seamlessly into existing workflows to avoid increasing documentation burden.

6.2 Data Management and Interpretation

Wearable technologies generate large volumes of data, raising challenges related to storage, processing, and interpretation. Transforming raw sensor data

into clinically actionable information requires robust algorithms and validation (Shull et al., 2014).

Standardized protocols and transparent algorithms are essential to ensure the reliability, reproducibility, and clinical interpretability of wearable-derived outcome measures (Patel et al., 2012; Stoppa & Chiolerio, 2014).

6.3 Ethical and Privacy Considerations

Continuous monitoring raises ethical concerns regarding data privacy, informed consent, and data ownership. Ensuring compliance with data protection regulations and implementing secure data transmission and storage systems are essential (Patel et al., 2012).

Clinicians must also consider the psychological impact of continuous monitoring, particularly in progressive neurological conditions.

7. Future Directions and Emerging Trends

The future of wearable technologies in neurorehabilitation lies in increased integration with artificial intelligence, multimodal sensing, and personalized rehabilitation frameworks.

7.1 Artificial Intelligence and Predictive Analytics

Machine learning approaches enable the identification of complex movement patterns and prediction of clinical outcomes based on wearable data. These tools support early detection of deterioration and optimization of intervention strategies (Shull et al., 2014).

7.2 Multimodal and Smart Wearables

Advances in smart textiles and multimodal wearables allow seamless integration of motion, physiological, and contextual data into everyday clothing. These innovations enhance comfort and long-term usability (Stoppa & Chiolerio, 2014).

7.3 Toward Precision Neurorehabilitation

Wearable technologies are key enablers of precision neurorehabilitation, supporting individualized interventions based on objective performance data, patient preferences, and contextual factors (Del Din et al., 2022).

8. Conclusion

Wearable technologies have transformed neurorehabilitation by enabling objective assessment, personalized intervention, and long-term monitoring across

diverse neurological conditions. Their ability to capture real-world performance and support home-based rehabilitation aligns with contemporary models of patient-centered and value-based care.

While challenges related to usability, data management, and ethics remain, ongoing technological advancements and growing clinical evidence support the integration of wearable technologies into routine neurorehabilitation practice. Future research should focus on standardization, clinical validation, and implementation strategies to maximize the impact of wearable technologies on functional recovery and quality of life.

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