CONTEMPORARY DEBATES AND INTERDISCIPLINARY APPROACHES IN HEALTH SCIENCES

Editor

Prof. Dr. Hülya ÇELİK

Contemporary Debates and Interdisciplinary Approaches in Health Sciences

Editor
Prof. Dr. Hülya ÇELİK

Editor in chief: Berkan Balpetek
Cover and Page Design: Duvar Design
Printing : May-2026
Publisher Certificate No: 49837
E-ISBN: 978-625-8756-73-9

853 Sokak No:13 P.10 Kemeraltı-Konak/İzmir
Tel: 0 232 484 88 68
www.duvaryayinlari.com
duvarkitabevi@gmail.com

TABLE OF CONTENTS

Chapter 1

Oxidative Stress and Antioxidant Systems in Bronchiectasis: From Clinical Biomarkers to Therapeutic Targets

Neslihan TAS[1], Tugba AGBEKTAS[2], Cemile ZONTUL[3]

INTRODUCTION

Bronchiectasis is a heterogeneous chronic airway disease characterized by irreversible and permanent dilation of the bronchial wall, with chronic infection and inflammation at the forefront. The "vicious cycle" model has long been the main paradigm for the pathogenesis of the disease; impaired mucociliary clearance, microbial colonization, persistent inflammation, and progressive tissue damage are defined as interrelated processes that perpetuate each other. However, recent studies have shown that bronchiectasis is not solely a disease of infection and inflammation, but is also associated with a marked imbalance in the oxidant–antioxidant system. In particular, increased production of reactive oxygen species (ROS) resulting from predominantly neutrophilic inflammation, along with insufficient antioxidant defense systems, deepens airway epithelial damage and contributes to disease progression.

In bronchiectasis, oxidative stress causes destructive molecular effects such as lipid peroxidation, protein oxidation, and DNA damage, while also activating NF-κB, the NLRP3 inflammasome, and various proinflammatory cytokine pathways, thereby perpetuating the inflammatory response. Although antioxidant defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) attempt to limit this oxidative burden, they are often insufficient under chronic inflammatory conditions. This suggests that oxidative stress in bronchiectasis is not merely a secondary biochemical event but one of the fundamental pathophysiological mechanisms driving disease progression.

Today, the assessment of oxidative stress biomarkers and antioxidant defense systems has become an important area of research for determining disease severity, predicting prognosis, and developing new therapeutic strategies. This review comprehensively addresses the basic mechanisms of oxidative stress in

1
2
3

bronchiectasis, antioxidant defense systems, oxidative damage biomarkers, and current antioxidant therapeutic approaches.

General Mechanisms of Oxidative Stress in Bronchiectasis

Bronchiectasis is a heterogeneous lung disease characterized by permanent and irreversible bronchial dilatation, accompanied by a cycle of chronic infection and inflammation. The "vicious cycle" model (infection–inflammation–tissue damage–impaired clearance) is fundamental in understanding its pathogenesis, with oxidative stress as an important biochemical component of this process. Oxidative stress arises from a shift in the balance between reactive oxygen species (ROS) and antioxidant defense systems in favor of ROS and plays a central role in both sustaining inflammation and progressive tissue damage in bronchiectasis (Chalmers et al., 2017; Polverino et al., 2017).

The main source of oxidative stress in bronchiectasis is overly activated neutrophils and macrophages. Chronic bacterial colonization (especially Pseudomonas aeruginosa) continuously stimulates these cells, increasing NADPH oxidase activity via the "respiratory burst" mechanism, leading to the production of strong oxidants such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) (Stockley et al., 2013). During neutrophil degranulation, myeloperoxidase (MPO) is released, which exerts direct toxic effects on epithelial cells and the extracellular matrix, particularly via hypochlorous acid production. This process accelerates structural destruction in the airway wall and contributes to the progression of bronchiectasis (Aliberti et al., 2016).

Additionally, neutrophil extracellular traps (NETs) are a significant source of oxidative stress in bronchiectasis. During NET formation, histones, proteases, and oxidant enzymes are released extracellularly. The uncontrolled accumulation of these structures impairs pathogen clearance and damages the epithelial barrier, deepening chronic inflammation. NET-associated DNA and protein complexes also increase mucus viscosity, further impairing mucociliary clearance (Chalmers et al., 2017).

Oxidative stress in bronchiectasis is related not only to increased ROS production but also to insufficient antioxidant defense systems. Decreases in the activity of systems such as glutathione (GSH), superoxide dismutase (SOD), catalase, and glutathione peroxidase have been reported. This imbalance, especially in epithelial cells, increases lipid peroxidation, protein oxidation, and DNA damage. Lipid peroxidation products (e.g., malondialdehyde, 4-HNE) alter cellular signaling pathways, activate proinflammatory transcription factors such as NF-κB, and enhance cytokine production (Polverino et al., 2017).

NF-κB activation is one of the most critical cellular signaling pathways linking oxidative stress and inflammation. ROS inactivate the inhibitor protein complex (mainly IκBα) that retains NF-κB in the cytoplasm via phosphorylation and subsequent proteasomal degradation. The degradation of IκB allows NF-κB (especially the p65/p50 dimer) to translocate into the nucleus and initiate the transcription of proinflammatory genes, including IL-8 (CXCL8), TNF-α, and IL-1β, which are strong proinflammatory and neutrophil chemotactic cytokines. IL-8, in particular, increases neutrophil migration and airway wall infiltration, further amplifying oxidative stress and perpetuating inflammation (Lawrence, 2009; Mittal et al., 2014).

Airway epithelial cells are also important targets of oxidative stress. ROS disrupt tight junction proteins, increasing epithelial permeability and facilitating deeper tissue invasion by pathogens. In addition, ROS inhibit ciliary function, reducing mucociliary clearance and favoring persistent bacterial colonization, thereby supporting the chronicity of the disease (Stockley et al., 2013).

Finally, oxidative stress has systemic effects in bronchiectasis. Increased oxidant load in the systemic circulation is associated with endothelial dysfunction, oxidative DNA damage, and elevated inflammatory markers, contributing to the risk of cardiovascular comorbidities in patients with bronchiectasis (Aliberti et al., 2016).

In summary, oxidative stress in bronchiectasis is one of the principal pathophysiological mechanisms perpetuating disease progression through infection-induced neutrophilic inflammation, ROS production, antioxidant defense deficiency, and a cycle of cellular damage. Therefore, targeting antioxidant systems and reducing oxidative stress are regarded as important research areas for future therapeutic approaches.

Antioxidant Defense System in Bronchiectasis

The antioxidant defense system in bronchiectasis is the main biological mechanism attempting to balance the increased load of reactive oxygen species (ROS) resulting from chronic inflammation and recurrent infections. However, the marked increase in oxidant production alongside insufficient antioxidant capacity creates an "oxidant–antioxidant imbalance," promoting airway epithelial damage, mucociliary dysfunction, and the chronicity of inflammation. The literature emphasizes that oxidative stress in bronchiectasis is not merely a byproduct but an active driver of disease progression, and that antioxidant systems act as a critical "protective barrier" in this process (Chalmers et al., 2017; Polverino et al., 2017).

The antioxidant defense system in bronchiectasis consists of both enzymatic and non-enzymatic components. The most important enzymatic antioxidants include superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). SOD controls the initial step of the ROS chain reaction by converting superoxide anion into hydrogen peroxide. Subsequently, catalase and GPx convert hydrogen peroxide into water and oxygen, preventing the spread of toxic effects. Studies have shown that, particularly due to neutrophil-dominant inflammation, SOD and GPx activities are decreased, while the oxidant load is increased in patients with bronchiectasis (King et al., 2005; Stockley et al., 2013).

The most important non-enzymatic antioxidant is glutathione (GSH). GSH serves as a direct ROS scavenger and also as a substrate for GPx. Chronic inflammation and recurrent infections deplete the GSH pool, rendering epithelial cells vulnerable to oxidative damage. Furthermore, increased oxidative stress in bronchiectasis disrupts the GSH/GSSG (reduced/oxidized glutathione) ratio, shifting cellular redox balance toward a pro-oxidant state, which indirectly enhances inflammatory signaling pathways such as NF-κB activation (Mittal et al., 2014).

The weakening of the antioxidant defense system in bronchiectasis is due not only to decreased enzyme activity but also to the overactivation of inflammatory cells. Notably, myeloperoxidase (MPO) released from neutrophils generates strong oxidants, creating a burden that exceeds endogenous antioxidant capacity. Moreover, histones and proteases within NETs increase oxidative damage while limiting the effect of local antioxidant molecules. This creates a self-perpetuating cycle of inflammation and oxidative stress in bronchiectasis (Chalmers et al., 2017).

Airway epithelial cells also constitute a significant part of antioxidant defense. Normally, epithelial cells possess both enzymatic systems limiting ROS production and detoxification mechanisms. However, epithelial barrier damage in bronchiectasis weakens this cellular defense, making it easier for external pathogens to colonize. Oxidative damage in epithelial cells impairs tight junction proteins (e.g., occludin and claudin), further compromising barrier function (Polverino et al., 2017).

Recent studies have shown that not only local airway inflammation but also the systemic oxidative stress response is increased in bronchiectasis. Elevated serum and plasma levels of oxidative stress markers—especially malondialdehyde (MDA) and F2-isoprostanes such as 8-isoprostane—have been reported, along with decreased total antioxidant capacity. These changes have been shown to correlate with disease severity and radiological extent, suggesting that bronchiectasis is not merely a localized airway disease but a spectrum

involving systemic inflammation and oxidative stress components (Stockley et al., 2013; Mittal et al., 2014).

From a therapeutic perspective, strengthening antioxidant systems is considered a potential target in the management of bronchiectasis. Studies are ongoing on glutathione precursors such as N-acetylcysteine (NAC), ROS scavenging molecules, and inhibitors of inflammatory pathways. However, current guidelines state that antioxidant therapies have not yet become part of standard treatment algorithms, and larger randomized controlled trials are needed for this purpose (Polverino et al., 2017; Hill et al., 2019).

Superoxide Dismutase (SOD) and Clinical Importance

Superoxide dismutase (SOD) is one of the fundamental antioxidant enzymes involved in the control of oxidative stress, converting superoxide anion ($O_2^{\bullet -}$) into hydrogen peroxide (H_2O_2) and thus into less reactive products. This mechanism forms the first line of defense protecting cells from the harmful effects of reactive oxygen species (ROS) (Mittal et al., 2014). There are three main isoforms in humans: SOD1 (cytoplasmic Cu/Zn-SOD), SOD2 (mitochondrial Mn-SOD), and SOD3 (extracellular SOD). These isoforms function in different cellular compartments, controlling both intracellular and extracellular oxidant load (Chalmers et al., 2017).

Due to high oxygen exposure, lung tissue is particularly susceptible to oxidative stress. Accordingly, SOD limits epithelial cell damage, lipid peroxidation, and inflammatory signaling activation by scavenging superoxide radicals, especially during inflammatory cell activation. Decreased SOD activity has been associated with increased oxidative damage in chronic lung diseases (Stockley et al., 2013).

Clinically, the importance of SOD is especially pronounced in chronic inflammatory lung diseases. In diseases such as COPD, asthma, and bronchiectasis, an increase in oxidative stress is accompanied by imbalances in SOD activity. In particular, extracellular SOD (EC-SOD) protects the airway matrix from oxidative damage, limiting the spread of inflammation (Chalmers et al., 2017). Experimental studies have shown that decreased SOD activity leads to the accumulation of superoxide, increasing the formation of more toxic oxidants (e.g., peroxynitrite) and thereby exacerbating tissue damage. Conversely, increased SOD activity reduces inflammatory cytokine production and suppresses oxidative tissue destruction (Mittal et al., 2014).

Recently, recombinant SOD therapies are being investigated as potential antioxidant strategies. However, limitations such as short half-life and

insufficient tissue distribution have been noted in clinical use (Stockley et al., 2013).

Catalase (CAT) Activity and Oxidative Damage

Catalase (CAT) is one of the most important antioxidant enzymes controlling oxidative stress in cells, and it catalyzes the conversion of hydrogen peroxide (H_2O_2) to water and oxygen, preventing the accumulation of toxic oxidants. This reaction is one of the fundamental defense mechanisms preventing cellular damage by reactive oxygen species (ROS) and is especially significant in tissues with high oxygen exposure (lung, erythrocytes, liver) (Mittal et al., 2014).

Although hydrogen peroxide is more stable than superoxide anion, it can react with transition metals such as iron and copper within the cell (via the Fenton reaction) to generate highly reactive and damaging hydroxyl radicals (•OH). Thus, catalase not only removes H_2O_2 but also indirectly prevents the formation of the most toxic ROS species, making it a "critical control point" in oxidative stress (Barnes, 2020).

In lung tissue, catalase activity plays a critical role in detoxifying ROS, especially during inflammatory cell infiltration. ROS produced by neutrophils and macrophages can cause lipid peroxidation, protein oxidation, and DNA damage via hydrogen peroxide. When catalase is insufficient, the accumulation of H_2O_2 increases and, through the Fenton reaction, facilitates the formation of more reactive hydroxyl radicals, contributing to the progression of oxidative tissue damage and the persistence of chronic inflammation (Mittal et al., 2014; Barnes, 2020).

In chronic airway diseases, particularly bronchiectasis, asthma, and COPD, imbalances in antioxidant enzyme systems have been reported. Reduced catalase activity in these diseases correlates with increased oxidative stress load and has been associated with disease severity. Catalase deficiency leads to H_2O_2 accumulation, which can trigger NF-κB activation and increase the expression of proinflammatory cytokines such as IL-8, TNF-α, and IL-1β (Barnes, 2020; Mittal et al., 2014).

Chronic bacterial colonization and persistent neutrophilic inflammation in bronchiectasis lead to high ROS production, overwhelming catalase capacity and resulting in "oxidative stress overload." The presence of pathogens such as Pseudomonas aeruginosa increases inflammatory cell activation, elevates H_2O_2 production, and challenges catalase detoxification capacity (Polverino et al., 2017).

Catalase also acts synergistically with other antioxidant enzymes (SOD and glutathione peroxidase). While SOD converts superoxide to H_2O_2, catalase

rapidly detoxifies this product. When this coordinated system is disrupted, H_2O_2 accumulation increases oxidative chain reactions, leading to lipid peroxidation in cell membranes and mitochondrial dysfunction (Mittal et al., 2014).

Recent studies have shown that catalase is not only a "scavenger enzyme" but also plays a regulatory role in redox signaling. Low levels of H_2O_2 have physiological roles in cellular signaling, while catalase maintains homeostasis by preventing levels from reaching a toxic threshold. Thus, imbalance in catalase activity has a dual pathological effect, contributing to both oxidative damage and inflammatory response (Barnes, 2020).

Glutathione (GSH) Levels and Cellular Protection

Glutathione (GSH) is one of the most important low molecular weight antioxidants, playing a central role in maintaining cellular redox balance. As a tripeptide (γ-glutamyl-cysteinyl-glycine), GSH not only directly neutralizes reactive oxygen species (ROS) but also serves as a key substrate for glutathione peroxidase (GPx), playing a critical role in the detoxification of hydrogen peroxide and lipid peroxides. This dual effect makes GSH one of the most important "intracellular buffer" systems protecting the cell from oxidative damage (Forman et al., 2019).

On a cellular level, the GSH/GSSG (reduced/oxidized glutathione) ratio is used as an indicator of redox status. Under normal physiological conditions, this ratio is kept high, but during oxidative stress, GSH is consumed and GSSG accumulates. This imbalance not only increases oxidative damage but also facilitates the activation of inflammatory signaling pathways such as NF-κB and MAPK, strengthening the inflammatory response (Sies & Jones, 2020).

In lung tissue, GSH is found at high concentrations, particularly in the airway epithelium and alveolar surface fluid, reflecting a primary defense mechanism against continuous oxygen exposure and environmental oxidants. However, in chronic inflammatory lung diseases, GSH levels decrease, leading to disruption of epithelial barrier integrity and increased oxidative damage (Rahman & Adcock, 2019).

In chronic airway diseases such as bronchiectasis, increased ROS production due to persistent neutrophilic inflammation and bacterial colonization accelerates GSH consumption and depletes cellular antioxidant capacity. Chronic infections, especially with Pseudomonas aeruginosa, further deplete the GSH pool by increasing inflammatory cell activation. GSH depletion increases lipid peroxidation and protein oxidation, contributing to mucociliary dysfunction (Polverino et al., 2017).

GSH also plays an important role in cellular detoxification processes. Through the glutathione-S-transferase (GST) enzyme family, it facilitates the conjugation and excretion of toxic electrophilic compounds, which is especially important for protecting epithelial cells in the lung, where oxidative stress is intense. Reduced GSH levels weaken this detoxification capacity, increasing cellular damage (Forman et al., 2019).

Recent studies have demonstrated that the GSH system is involved not only in antioxidant defense but also in the regulation of inflammatory signaling and control of cell death (apoptosis). GSH deficiency may accelerate cellular aging via mitochondrial dysfunction and oxidative DNA damage. Therefore, GSH is considered both a protective factor and a biomarker of disease progression in chronic lung diseases (Sies & Jones, 2020).

Malondialdehyde (MDA) and Lipid Peroxidation

Malondialdehyde (MDA) is one of the most important end products of the free radical-mediated oxidation of polyunsaturated fatty acids (PUFAs) in cell membranes. The chain reaction of lipid peroxidation initiated by increased ROS disrupts membrane integrity, increases permeability, inactivates enzymes, and causes loss of cellular function. MDA is considered both a "damage marker" and, due to its reactive aldehyde structure, a biologically active molecule that can induce secondary cellular damage (Ayala et al., 2014).

Lipid peroxidation occurs in three stages: initiation, propagation, and termination. During initiation, strong ROS species such as hydroxyl radicals abstract hydrogen atoms from membrane lipids, creating lipid radicals. In the propagation phase, these radicals react with oxygen to generate lipid peroxyl radicals, continuing the chain reaction. Termination occurs via antioxidant systems or radical–radical interactions. If antioxidant defense is insufficient, the process proceeds uncontrollably, increasing the accumulation of toxic aldehydes such as MDA (Ayala et al., 2014).

MDA increases cellular toxicity by forming covalent bonds with proteins and DNA and can cause mutagenesis by reacting with DNA bases. For this reason, MDA is regarded not only as an indicator of oxidative stress but also as a biological effector of oxidative damage (Ayala et al., 2014).

In chronic lung diseases such as bronchiectasis, COPD, and asthma, levels of lipid peroxidation and MDA have been shown to increase, correlating with disease severity. In these diseases, predominant neutrophilic inflammation increases ROS production via MPO and NADPH oxidase, damaging membrane lipids. This results in structural deterioration of the airway epithelium, ciliary dysfunction, and reduced mucociliary clearance (Rahman & Adcock, 2019).

Chronic bacterial colonization and persistent inflammatory cell activation in bronchiectasis make lipid peroxidation continuous. Especially in Pseudomonas aeruginosa infection, a strong neutrophilic response increases ROS production and accelerates MDA accumulation, disrupting epithelial barrier integrity and perpetuating the infection cycle (Polverino et al., 2017).

Recent studies have shown that MDA is not only a passive oxidative stress marker but can also activate inflammatory signaling pathways such as NF-κB, increasing the proinflammatory response. Therefore, lipid peroxidation forms a bidirectional amplification loop between oxidative stress and inflammation (Sies & Jones, 2020).

Protein Carbonyl Derivatives and Protein Oxidation

Protein oxidation is an important molecular damage mechanism characterized by the structural and functional impairment of proteins by reactive oxygen species (ROS) and reactive nitrogen species (RNS). In this process, the side chains of amino acids (especially proline, arginine, lysine, and threonine) undergo oxidative modification, forming carbonyl derivatives. Protein carbonyl content is considered one of the most stable and reliable biomarkers of oxidative protein damage (Dalle-Donne et al., 2003; Stadtman & Levine, 2003).

Protein carbonyl formation can occur both directly via ROS-mediated oxidation and indirectly via lipid peroxidation products (especially reactive aldehydes such as MDA and 4-hydroxynonenal). These reactive aldehydes form covalent bonds with proteins, creating advanced glycation and oxidation end products that permanently impair protein function (Dalle-Donne et al., 2003).

Oxidative protein damage results in decreased enzyme activity, impaired receptor function, and disrupted intracellular signal transduction at the cellular level. Additionally, the failure to adequately remove oxidized proteins via the proteasome system leads to the accumulation of toxic protein aggregates, an important mechanism of cellular dysfunction, especially in chronic inflammatory diseases (Stadtman & Levine, 2003).

The lung is particularly susceptible to protein oxidation due to high oxygen exposure and continuous contact with environmental oxidants (cigarette smoke, infections, particulate matter). Increased ROS production in chronic airway diseases elevates protein carbonyl levels in airway epithelial cells, exacerbating cellular damage, impairing mucociliary clearance, weakening epithelial barrier integrity, and contributing to the chronicity of the inflammatory response (Barnes, 2020).

In diseases such as bronchiectasis, where chronic neutrophilic inflammation predominates, increases in protein oxidation products—particularly protein

carbonyl levels—have been associated with disease severity. ROS generated by MPO and NADPH oxidase from neutrophils cause direct oxidative modification of proteins and indirectly increase carbonyl formation via lipid peroxidation products. These mechanisms create a self-sustaining cycle between oxidative stress and inflammation, promoting the progression of airway tissue damage (Dalle-Donne et al., 2003; Mittal et al., 2014; Barnes, 2020).

An increase in protein carbonyl derivatives is not only a marker of damage but also an indicator of loss of cellular function. Oxidized proteins may lose enzymatic activity, disrupt cytoskeletal structure, and activate apoptotic signals. Therefore, protein carbonyl levels are considered the biochemical "signature" of oxidative stress in chronic lung diseases (Dalle-Donne et al., 2003).

Recent studies have shown that protein oxidation has both reversible and irreversible forms. Oxidation at methionine and cysteine residues can be reversed by certain redox enzymes, while carbonyl formation is generally considered irreversible. Thus, protein carbonyl levels reflect the chronic and progressive aspect of oxidative stress (Sies & Jones, 2020).

Clinical Use of Oxidative Stress Biomarkers

Oxidative stress biomarkers are molecular indicators used to assess the imbalance between reactive oxygen species (ROS) and antioxidant defense systems. In clinical practice, these biomarkers are increasingly important for evaluating disease activity and predicting prognosis. In chronic inflammatory diseases, oxidative stress has been shown to be not merely a byproduct but an active process affecting disease progression (Sies & Jones, 2020; Pizzino et al., 2017).

Biomarkers used to assess oxidative stress are generally divided into three main groups: lipid peroxidation products (MDA, 4-HNE, 8-isoprostane), protein oxidation products (protein carbonyls), and DNA oxidation products (8-OHdG). Among these, 8-isoprostane is accepted as a stable and reliable indicator of lipid peroxidation in vivo, while malondialdehyde (MDA) is more easily measured but considered a more variable parameter (Ayala et al., 2014; Halliwell & Gutteridge, 2015).

The increase in oxidative stress biomarkers has been associated with disease severity in chronic airway diseases such as COPD, asthma, and bronchiectasis. In these diseases, increased neutrophilic inflammation and MPO activity increase ROS production, leading to lipid, protein, and DNA damage. Thus, oxidative stress biomarkers serve as potential markers reflecting both disease activity and the risk of exacerbations (Barnes, 2020; Rahman & Adcock, 2019).

Specifically in bronchiectasis, the clinical use of oxidative stress biomarkers is increasingly being investigated. Increased serum and sputum levels of MDA and protein carbonyls, and decreased antioxidant capacity, have been associated with disease severity, radiological extent, and frequent exacerbations. Therefore, the oxidative stress panel is considered a potential tool for phenotyping and risk stratification in bronchiectasis (Polverino et al., 2017).

Protein carbonyl levels are preferred as a stable indicator of oxidative damage, especially in chronic diseases. Similarly, 8-isoprostane, as a reliable indicator of lipid peroxidation in vivo, has been correlated with the severity of airway inflammation. However, the most important limitation of these biomarkers in clinical use is the lack of well-established reference ranges and heterogeneity in measurement methods (Halliwell & Gutteridge, 2015).

Current literature emphasizes that oxidative stress biomarkers should be evaluated together with antioxidant capacity measurements (total antioxidant capacity, SOD, catalase, glutathione levels), rather than alone. This holistic approach provides a more accurate interpretation of redox balance and more meaningful information in clinical decision-making (Sies & Jones, 2020).

Assessment of the Oxidant–Antioxidant Balance in Bronchiectasis

Bronchiectasis is a progressive chronic airway disease characterized by irreversible bronchial dilatation, chronic infection, and persistent inflammation. The "vicious vortex" model underpins its pathogenesis, in which impaired mucociliary clearance, recurrent infections, and neutrophil-dominated inflammation perpetuate structural lung damage (Chalmers et al., 2025). Recent years have shown that bronchiectasis is not only a disease based on infection and inflammation but also associated with a marked oxidant–antioxidant imbalance. The disruption of the balance between reactive oxygen/nitrogen species (ROS/RNS) and antioxidant defense systems plays a significant role in both the onset and progression of the disease (Qin et al., 2026).

The main source of oxidant load in bronchiectasis is neutrophil activation. Neutrophils produce high amounts of ROS via NADPH oxidase and myeloperoxidase during infection and inflammation, which becomes even more pronounced in the presence of chronic bacterial colonization. Especially pathogens like Pseudomonas aeruginosa increase the oxidative stress response in epithelial cells, perpetuate inflammation, and support biofilm formation. In addition, macrophages and epithelial cells increase oxidant production via proinflammatory cytokines such as TNF-α and IL-8, deepening tissue damage.

The antioxidant defense system consists of both enzymatic and non-enzymatic components. Enzymatic antioxidants such as superoxide dismutase (SOD),

catalase, and glutathione peroxidase (GPx) neutralize ROS and attempt to reduce cellular damage. However, decreased activity of these enzymes has been demonstrated in bronchiectasis. Non-enzymatic antioxidants, including glutathione, vitamin C, and vitamin E, are also generally depleted, further increasing oxidative stress (Demirci-Çekiç et al., 2022).

The fundamental mechanism of oxidant–antioxidant imbalance is explained by increased ROS production and insufficient antioxidant capacity. Increased ROS disrupts the protease–antiprotease balance, enhances neutrophil elastase activity, and causes bronchial wall destruction. Additionally, ROS activates the NLRP3 inflammasome, increasing IL-1β production and triggering mucus hypersecretion, further impairing mucociliary clearance and perpetuating infections. Oxidative stress also causes mitochondrial dysfunction, reducing cellular energy production and creating a vicious cycle that triggers additional ROS formation.

Various biomarkers are used to assess oxidative stress in bronchiectasis. These include 8-hydroxy-2-deoxyguanosine (8-OHdG) as a DNA oxidation product, malondialdehyde (MDA) and F2-isoprostanes as indicators of lipid peroxidation, and measurements of total antioxidant capacity (TAC), SOD, and GPx activities to assess the antioxidant system. Clinical studies show that oxidative stress biomarkers correlate with disease severity, exacerbation frequency, and quality of life (Bayraktar et al., 2024).

The current literature emphasizes that oxidative stress in bronchiectasis is not just a biochemical finding, but a fundamental mechanism directing disease progression. In particular, patients with high oxidative stress levels have been reported to have more frequent exacerbations, worse radiological scores, and lower quality of life. Thus, supporting the antioxidant system is becoming increasingly important in therapeutic approaches. The use of glutathione precursors such as N-acetylcysteine and anti-inflammatory agents are considered potential strategies for restoring redox balance (Chalmers et al., 2025).

Antioxidant Therapeutic Approaches and New Targets

Bronchiectasis is a progressive airway disease in which chronic infection, neutrophil-dominated inflammation, and oxidative stress perpetuate one another. In recent years, management of the disease has focused not only on infection control and airway clearance but also on the restoration of redox balance. In this context, antioxidant therapeutic approaches are being increasingly investigated for their potential to reduce the inflammatory load and limit tissue damage (Chalmers et al., 2025).

One of the most studied antioxidant agents is N-acetylcysteine (NAC). As a precursor of glutathione (GSH), NAC increases intracellular antioxidant capacity and neutralizes ROS. Clinical studies report that NAC reduces sputum viscosity, improves mucociliary clearance, and lowers oxidative stress markers. Notably, reductions in 8-OHdG and lipid peroxidation products indicate that NAC may decrease systemic oxidative load (Bayraktar et al., 2024).

Additionally, non-enzymatic antioxidants such as vitamins C and E can directly scavenge free radicals and reduce cellular damage. However, their use alone in bronchiectasis has shown limited clinical efficacy. Therefore, the current approach favors combined antioxidant strategies or their use alongside anti-inflammatory agents rather than single antioxidants (Demirci-Çekiç et al., 2022).

A prominent new target in antioxidant therapy is the suppression of neutrophil-derived oxidative burst. In this regard, studies are ongoing on NADPH oxidase inhibitors and myeloperoxidase (MPO)-targeted agents. Inhibition of these enzymes can directly reduce ROS production and limit tissue destruction. In addition, dipeptidyl peptidase-1 (DPP-1) inhibitors, by reducing neutrophil elastase activity, have the potential to lower both protease burden and oxidative damage (Chalmers et al., 2025).

Another important research area is targeting mitochondrial dysfunction. Chronic inflammation is known to impair mitochondria, leading to additional ROS production. Thus, mitochondrial protective agents and mitochondrial antioxidants (such as MitoQ-like compounds) are considered important future therapeutic options (Qin et al., 2026).

Furthermore, the NLRP3 inflammasome is a critical pathway activated by oxidative stress in bronchiectasis. Activation of the inflammasome by ROS increases IL-1β production, exacerbating mucus hypersecretion and inflammation. Therefore, inflammasome inhibitors and IL-1 pathway blockade are also considered potential targets (Qin et al., 2026).

Finally, the future of antioxidant therapy in bronchiectasis is shaped by personalized medicine approaches. Phenotyping based on oxidative stress biomarkers (8-OHdG, MDA, total antioxidant capacity) can help identify which patients will benefit most from antioxidant therapy, moving treatment from a standard approach to one tailored to the individual (Tzortzaki et al., 2012).

Overall, antioxidant therapeutic approaches in bronchiectasis are evolving from merely supportive strategies to important research areas targeting the disease pathogenesis. Suppression of oxidant production, preservation of mitochondrial function, and control of inflammasome activation will form the basis of future therapeutic strategies (Chalmers et al., 2025).

Relationship Between Oxidative Stress and Disease Severity

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms, is a fundamental pathophysiological process involved in the progression of many chronic diseases. In chronic airway diseases such as bronchiectasis, increased oxidative stress is increasingly recognized not just as a consequence of inflammation but also as an active driver of disease severity (Rahman & Adcock, 2015).

In bronchiectasis, the relationship between disease severity and oxidative stress parallels the intensity of neutrophilic inflammation. Reactive molecules produced by activated neutrophils, such as superoxide anion, hydrogen peroxide, and hypochlorous acid, damage epithelial cells and accelerate airway structural deterioration. Clinically, this is associated with more frequent exacerbations, increased sputum production, and progressive airway obstruction (Polverino et al., 2017).

A key biomarker supporting the relationship between oxidative stress and disease severity is 8-hydroxy-2-deoxyguanosine (8-OHdG), a DNA oxidation product. Increased levels of this molecule in serum and sputum are associated with a more severe clinical phenotype, lower FEV1 values, and higher radiological extent scores (Mittal et al., 2014). Similarly, increased levels of lipid peroxidation products such as malondialdehyde (MDA) and F2-isoprostanes are biochemical indicators of disease severity.

Chronic oxidative burden also contributes to impaired mucociliary clearance, perpetuating infections. This is particularly relevant in patients with Pseudomonas aeruginosa colonization, leading to a more severe disease phenotype. Increased ROS also disrupts the protease–antiprotease balance, accelerating elastin and extracellular matrix destruction and explaining the structural progression of bronchiectasis (Stockley et al., 2013).

Oxidative stress is not limited to local lung damage; it is also associated with systemic inflammation. High oxidative stress correlates positively with serum C-reactive protein (CRP) and interleukin-6 (IL-6) levels, suggesting that the systemic effects of the disease are shaped by oxidative mechanisms as well (Barnes, 2017).

In conclusion, oxidative stress is both a biochemical and clinical indicator of disease severity in bronchiectasis. The interaction between increased ROS production, decreased antioxidant capacity, and DNA/lipid/protein damage accelerates disease progression and worsens the clinical phenotype. Thus, oxidative stress markers are considered potential biomarkers for both prognostic assessment and monitoring therapeutic response.

Therapeutic Strategies and Molecular Targets

Future therapeutic strategies in bronchiectasis are increasingly focusing on molecular and endotype-based approaches, moving beyond a purely infection-control-based clinical model. Recent research shows that the heterogeneous nature of bronchiectasis comprises interrelated pathophysiological processes, including chronic neutrophilic inflammation, protease–antiprotease imbalance, epithelial barrier damage, microbial colonization, and oxidative stress. Consequently, future therapeutic targets will focus not only on symptom control but also on direct modulation of the underlying biological mechanisms (Polverino et al., 2017).

One of the main research areas is the selective suppression of neutrophilic inflammation. Neutrophil migration and activation are regulated by the CXCR2 receptor and its ligands, particularly ELR+ CXC chemokines (e.g., CXCL8/IL-8). Inhibition of the CXCR2 signaling pathway has been shown to reduce neutrophil infiltration and limit tissue damage in experimental and clinical studies (Belperio et al., 2005; Johnston et al., 2005). However, complete suppression of neutrophils could increase the risk of infection, particularly in patients prone to chronic infections, thus current strategies focus on "functional modulation" of the inflammatory response rather than its total elimination (Stockley et al., 2013).

Another important target is the control of protease activity. In bronchiectasis, increased neutrophil elastase is directly associated with epithelial destruction and mucus hypersecretion. Protease inhibitors, especially those suppressing neutrophil granule enzymes via dipeptidyl peptidase-1 (DPP-1), have the potential to slow the structural progression of the disease and are among the strongest pharmacological approaches for preserving lung tissue (Pham et al., 2018).

Control of microbial colonization is also central to future therapies. The development of antibiotic resistance by biofilm-forming bacteria reduces the effectiveness of classical treatments. Thus, biofilm-disrupting agents, optimized inhaled antibiotics, and bacteriophage therapy are being increasingly explored. Additionally, re-balancing the lung microbiota represents a new paradigm focusing not only on pathogen eradication but also on restoring the microbial ecosystem (Huang & Lynch, 2011).

Oxidative stress and redox imbalance are also important components of future therapeutic strategies. Excessive ROS production is associated with epithelial damage and persistent inflammation. Accordingly, agents preserving mitochondrial function, NADPH oxidase inhibitors, and therapies enhancing endogenous antioxidant systems are being investigated, aiming to break the cycle between inflammation and tissue destruction (Barnes, 2017).

Targeting inflammatory signaling pathways is also gaining importance. The NLRP3 inflammasome and IL-1β axis play critical roles in mucus production and

chronic inflammation in bronchiectasis. Thus, inflammasome inhibitors and cytokine-targeted biological therapies are being considered as more selective and effective treatment options (Dinarello, 2018).

Finally, the most important future approach in bronchiectasis is personalized medicine. Classifying patients based on their inflammatory profile, microbiological features, and biomarker levels will optimize therapeutic response, allowing for the development of more effective and safer molecularly targeted therapies for each patient.

CONCLUSION

Bronchiectasis is a complex disease that cannot be adequately explained by chronic infection and inflammation alone; oxidative stress is a central pathophysiological mechanism in this process. Increased reactive oxygen species resulting from chronic bacterial colonization and neutrophilic inflammation cause lipid, protein, and DNA damage in the airway epithelium, accelerating structural destruction in the bronchial wall. Conversely, the insufficiency of antioxidant defense systems such as superoxide dismutase, catalase, and glutathione further deepens the oxidant–antioxidant imbalance and contributes to the persistence of inflammation.

Biomarkers used to assess oxidative stress in bronchiectasis—such as MDA, protein carbonyls, 8-OHdG, and total antioxidant capacity—have been shown to correlate with disease severity, frequency of exacerbations, and radiological extent. These findings indicate that oxidative stress is not merely a biochemical marker but also an important process affecting clinical prognosis. Furthermore, the bidirectional relationship between oxidative stress and inflammatory pathways is one of the main mechanisms perpetuating the chronicity of the disease.

In recent years, antioxidant therapeutic strategies have emerged as a promising area of research in the management of bronchiectasis. Glutathione precursors such as N-acetylcysteine, NADPH oxidase and myeloperoxidase inhibitors, inflammasome-targeted therapies, and mitochondrial antioxidants have the potential to control inflammation by reducing oxidative load. However, current evidence indicates that larger-scale and longer-term randomized controlled trials are needed before these therapies become part of routine clinical practice.

In summary, oxidative stress is a multifaceted mechanism closely related to the onset, progression, and clinical severity of bronchiectasis. In the future, the development of personalized treatment approaches based on oxidative stress biomarkers may enable more effective and targeted strategies in the management of bronchiectasis.

REFERENCES

Aliberti, S., Goeminne, P. C., O'Donnell, A. E., et al. (2016). Bronchiectasis in adults: A review of clinical features and pathogenesis. *The Lancet Respiratory Medicine*, 4(10), 847–856.

Polverino, E., Goeminne, P. C., McDonnell, M. J., et al. (2017). European Respiratory Society guidelines for the management of adult bronchiectasis. *European Respiratory Journal*, 50(3), 1700629.

Stockley, R. A., Turner, A. M., & Hill, A. T. (2013). Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD and bronchiectasis. *Chest*, 144(4), 1219–1227.

Lawrence, T. (2009). The nuclear factor NF-κB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology*, 1(6), a001651.

Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P., & Malik, A. B. (2014). Reactive oxygen species in inflammation and tissue injury. *Antioxidants & Redox Signaling*, 20(7), 1126–1167.

Hill, A. T., Sullivan, A. L., Chalmers, J. D., et al. (2019). British Thoracic Society guideline for bronchiectasis in adults. *Thorax*, 74(Suppl 1), 1–69.

King, P. T. (2005). The role of antioxidants in chronic lung disease. *Proceedings of the American Thoracic Society*, 2(4), 373–378.

Chalmers, J. D., Chang, A. B., Chotirmall, S. H., & Dhar, R. (2017). *Bronchiectasis*. Nature Reviews Disease Primers.

Barnes, P. J. (2020). Oxidative stress-based chronic lung inflammation. *Free Radical Biology and Medicine*.

Forman, H. J., Zhang, H., & Rinna, A. (2019). Glutathione: Overview of its protective roles. *Molecular Aspects of Medicine*, 30(1-2), 1–12.

Rahman, I., & Adcock, I. M. (2019). Oxidative stress and redox regulation in chronic lung diseases. *Free Radical Biology and Medicine*, 137, 48–63.

Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, 21, 363–383.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms. *Oxidative Medicine and Cellular Longevity*, 2014, 360438.

Dalle-Donne, I., Rossi, R., Giustarini, D., Milzani, A., & Colombo, R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta*, 329(1–2), 23–38.

Stadtman, E. R., & Levine, R. L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids*, 25, 207–218.

Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine*. Oxford University Press.

Pizzino, G., Irrera, N., Cucinotta, M., et al. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763.

Bayraktar, N., Badem, K., Turan, H., & Bayraktar, M. (2024). N-asetilsistein tedavisinin bronşektazi hastalarında oksidatif stres belirteçlerine etkisi. *Harran Üniversitesi Tıp Fakültesi Dergisi, 21*(3), 476–482. https://doi.org/10.35440/hutfd.1537146

Demirci-Çekiç, S., et al. (2022). Oxidative stress and antioxidant defense systems in chronic disease. *Antioxidants, 11*(2), 1–20.

Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews, 281*(1), 8–27. https://doi.org/10.1111/imr.12621

Huang, Y. J., & Lynch, S. V. (2011). The emerging relationship between the airway microbiota and chronic respiratory disease. *Journal of Allergy and Clinical Immunology, 129*(2), 570–578. https://doi.org/10.1016/j.jaci.2011.11.001

Pham, T. H., et al. (2018). Dipeptidyl peptidase 1 inhibition as a novel anti-inflammatory strategy targeting neutrophil serine proteases. *Nature Reviews Drug Discovery, 17*(5), 321–338.

Watz, H., et al. (2020). Anti-inflammatory effects of CXCR2 antagonism in neutrophilic airway disease. *European Respiratory Journal, 55*(2), 1902017.

Qin, Y., et al. (2026). Redox signaling in chronic airway diseases: pathogenic mechanisms and therapeutic implications. *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2026.1734890

Tzortzaki, E. G., et al. (2012). Biomarkers of oxidative stress in airway diseases. *Respiratory Medicine, 106*, 1–10.

Chalmers, J. D., Metersky, M., Aliberti, S., & Morgan, L. (2025). Neutrophilic inflammation in bronchiectasis. *European Respiratory Journal / PMC*.

Barnes, P. J. (2017). Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clinics in Chest Medicine, 35*(1), 71–86.

Rahman, I., & Adcock, I. M. (2015). Oxidative stress and redox regulation of lung inflammation in COPD. *European Respiratory Journal, 45*(6), 1713–1726.

Stockley, R. A., Parr, D. G., Piitulainen, E., Stolk, J., Stoel, B. C., Dirksen, A., ... & Janes, S. M. (2013). Therapeutic efficacy of alpha-1 antitrypsin augmentation therapy on emphysema progression. *Thorax, 68*(12), 1125–1132.

Chapter 2

The Relationship between the Microbiota and Agmatine Levels

Cemile ZONTUL[1], Tugba AGBEKTAS[2], Cemre GURLEYUK[3]

INTRODUCTION

The gut microbiota refers to the diverse community of microorganisms primarily bacteria, fungi, viruses, and other microorganisms that inhabit the human gut. The gut microbiota is a key component of the complex human microecosystem and plays a vital role in regulating metabolism, protecting the intestinal barrier, and modulating the immune system (Kamada et al., 2013; Kuziel & Rakoff-Nahoum, 2022).

Agmatine is a cationic polyamine produced from L-arginine via a reaction catalyzed by the enzyme arginine decarboxylase (Valverde et al., 2021). Polyamines are low-molecular-weight, linear aliphatic compounds containing multiple amino groups that form a polycation under physiological conditions (Bekebrede et al., 2020; Killiny & Nehela, 2020). Due to their positive charges, polyamines easily bind to and modulate negatively charged macromolecules, including DNA, RNA, membrane phospholipids, and acidic proteins (Chang et al., 2021; Zahedi et al., 2022). Intestinal polyamine levels are controlled by a tightly regulated balance between endogenous synthesis, dietary intake, and microbial metabolism (Mafe & Büsselberg, 2026).

For this reason, the reciprocal interaction between the gut microbiota and polyamine metabolism is gaining increasing importance, not only in terms of the physiological functioning of the gastrointestinal system but also due to its effects on systemic health. In particular, microbiota-associated polyamines such as agmatine have been shown to play a role in maintaining intestinal epithelial integrity, regulating cellular proliferation, controlling oxidative stress, and modulating inflammatory responses. Additionally, polyamines are thought to play a regulatory role in immune cell activation, cytokine production, and mucosal barrier functions within the intestinal mucosa.

1
2
3

Recent studies have revealed that changes in the gut microbiota (dysbiosis) may influence polyamine metabolism and be associated with inflammatory bowel diseases, metabolic syndrome, neurodegenerative diseases, and various chronic inflammatory processes. In particular, the effects of microbiota-derived metabolites on host cell signaling are of significant interest in terms of the gut-brain axis and gut-immune system interactions. Given agmatine's anti-inflammatory, antioxidant, and neuromodulatory properties, a better understanding of this molecule's interaction with the gut microbiota is considered important for both elucidating disease mechanisms and identifying new therapeutic targets.

Gut Microbiota

The gut microbiota is mainly composed of bacterial communities and accounts for more than 90% of the total intestinal microbiota (Abdallhi-Rodasz et al., 2016). Based on their functions, intestinal bacteria can generally be grouped as probiotics, opportunistic pathogens, and pathogenic bacteria. Probiotics support host health by contributing to several physiological processes, including the production of short-chain fatty acids, vitamin synthesis, and the regulation of immune responses (Ventura et al., 2012). In contrast, opportunistic pathogens are normally harmless under balanced conditions; however, when immune defenses are weakened or environmental conditions favor their excessive growth, they may lead to intestinal infections, inflammation, and toxin release (Qiu et al., 2022).

Although beneficial and opportunistic microorganisms constitute a large proportion of the gut microbiota, approximately 10% of this microbial community consists of pathogenic bacteria. These harmful bacteria may contribute to gastrointestinal disorders and tumor development through the secretion of toxic metabolites, disruption of the intestinal mucosal barrier, and suppression of immune cell functions (Rubinstein et al., 2013; Shin et al., 2015). Therefore, maintaining a balanced gut microbiota is essential for protecting intestinal health and reducing the risk of gut-related diseases (He et al., 2023).

In conclusion, the bacteria found in the gut microbiota are important microorganisms that play a fundamental role in maintaining the host organism's physiological balance. A balanced microbial composition, in which beneficial bacteria predominate, is of critical importance for maintaining intestinal barrier function, regulating the immune system, and keeping harmful microorganisms under control. Conversely, disruption of microbial balance can lead to the proliferation of pathogenic bacteria and the triggering of inflammatory processes, thereby contributing to the development of various gastrointestinal and systemic diseases.

Today, a better understanding of the gut microbiota's effects on health and disease has increased interest in microbiota-based therapeutic approaches. In particular, reshaping the gut microbial composition through probiotics, prebiotics, and dietary modifications is considered a promising strategy for maintaining gut homeostasis and reducing disease risk. Therefore, a detailed examination of the structure and functions of the gut microbiota will provide significant contributions to the development of preventive and targeted therapeutic approaches in the future.

Agmatine

Agmatine is a polyamine generated through the decarboxylation of L-arginine by arginine decarboxylase (ADC), and it functions as an important regulatory molecule within the polyamine pathway (Haenisch et al., 2008). Polyamines such as putrescine, spermidine, and spermine are essential for cell proliferation (Isome et al., 2007). In the polyamine biosynthetic pathway, ornithine decarboxylase (ODC) acts as the rate-limiting enzyme, catalyzing the conversion of ornithine into putrescine (Wu et al., 2017). Increased polyamine production is commonly observed in rapidly dividing cells, including tumor cells (Pegg et al., 1995). Agmatine has been reported to inhibit cell growth by reducing intracellular polyamine levels, which are required for cellular proliferation, and by suppressing ODC activity (Dudkowska et al., 2003; Gardini et al., 2003; Molder et al., 2005; Satriano et al., 1998).

In addition, agmatine contributes to the maintenance of homeostasis in the brain, heart, and surrounding vascular structures by regulating calcium concentrations (Popolo et al., 2014; Raasch et al., 1995; Raghavan & Dikshit, 2004). It influences several cellular processes in the central nervous system, including apoptosis, inflammatory regulation, and neural edema. Agmatine has also been shown to suppress the formation of advanced glycation end products (AGEs), which are known to damage extracellular proteins and contribute to the development of various neurodegenerative disorders (Kosonen et al., 2021). Furthermore, agmatine supports the proliferation and differentiation of neural progenitor cells in the adult hippocampus by modulating regulatory factors involved in neurogenesis, such as nitric oxide synthase (NOS) stress and glutamate levels (Y. F. Li et al., 2006; Wang et al., 2010).

The major biological actions of agmatine in mammals are thought to occur through direct or indirect modulation of functions related to cell membrane and cytoplasmic targets, including neurotransmitters, N-methyl-D-aspartate (NMDA) receptors, ion channels, and nitric oxide (NO) (Berkels et al., 2004; G. Li et al., 1994; Piletz et al., 2013; Reis & Regunathan, 1999).

Consequently, agmatine is not merely an intermediate product of polyamine metabolism but also an important biomolecule exhibiting multifaceted biological effects in the regulation of cellular homeostasis. Its effects, such as the control of cell proliferation, the regulation of polyamine synthesis via ornithine decarboxylase inhibition, and the modulation of apoptotic mechanisms, demonstrate that agmatine is a particularly noteworthy molecule in the context of rapidly proliferating cells and tumor biology. In addition, its properties such as the suppression of inflammation, reduction of oxidative stress, and inhibition of the formation of advanced glycation end products support agmatine's cellular protective effects.

Agmatine's effects on the central nervous system have also been intensively studied in recent years. It has been reported that NMDA receptors play a significant role in neurotransmission, neurogenesis, and neuronal differentiation processes due to their regulatory effects on ion channels and nitric oxide signaling pathways. Furthermore, its influence on the proliferation and differentiation of hippocampal neural progenitor cells suggests that agmatine holds potential therapeutic importance in the context of neurodegenerative diseases and neuroregenerative therapies.

The Relationship Between Microbiota and Agmatine Levels

Polyamines, including putrescine, spermidine, spermine, and agmatine, are endogenously controlled metabolites that play crucial roles in cellular proliferation and the maintenance of intestinal homeostasis. By contrast, biogenic amines such as cadaverine, histamine, and tyramine are mainly produced through bacterial decarboxylation of amino acids in foods or under dysbiotic conditions. When these compounds accumulate excessively, they may indicate disrupted metabolic regulation and may be associated with adverse health effects. In foods and beverages, the production of biogenic amines is primarily influenced by the presence of proteins and free amino acids, which provide substrates for microbial or endogenous enzymes with decarboxylation or amination activity (Doeun et al., 2017; Mafe & Büsselberg, 2026).

Arginine serves as a major precursor for several polyamines, including agmatine, putrescine, spermidine, and spermine (Saha et al., 2023). Polyamine biosynthesis proceeds mainly through two alternative routes. In the first route, arginine is converted into ornithine by arginase 1 (ARG1). Ornithine is then decarboxylated by ornithine decarboxylase (ODC), the rate-limiting enzyme of polyamine biosynthesis, resulting in the formation of putrescine. In the second route, arginine-derived agmatine is converted into putrescine through the action of agmatinase. Subsequently, putrescine acts as the precursor for the synthesis of

spermidine and spermine, which are generated by spermidine synthase (SRM) and spermine synthase (SMS), respectively.

During this process, decarboxylated S-adenosyl-L-methionine (dcSAM), generated by adenosyl-L-methionine decarboxylase (AMD1), provides the aminopropyl groups required for the conversion of putrescine into spermidine and spermine. However, polyamine metabolism involves not only biosynthetic reactions but also reversible catabolic pathways. Within this catabolic network, spermine can be oxidized by spermine oxidase (SMOX), leading to the production of spermidine. Additionally, the N1-acetyl-SPD and N1-acetyl-SPM molecules formed by spermidine/spermine N1-acetyltransferase (SSAT) are subjected to oxidative degradation by N1-acetylpolyamine oxidase (PAOX), thereby facilitating the reformation of putrescine and spermidine, respectively. Thus, the polyamine pool is dynamically regulated at the cellular level (Nakanishi & Cleveland, 2021; Schibalski et al., 2024).

The high agmatine content in the gastrointestinal lumen originates from three sources. Agmatine is synthesized and released in high quantities by bacteria belonging to the physiological intestinal microbiota (E. coli, etc.) and by pathogenic microorganisms such as Helicobacter pylori. In addition, dietary sources may contain varying amounts of agmatine, suggesting that exogenous contributions may also be significant. Furthermore, luminal agmatine may also arise from the shedding of gastrointestinal epithelial cells. However, since the relative contribution of this source to the luminal polyamine pool is quite limited, it is considered physiologically insignificant (Benamouzig et al., 1997; Molderings et al., 1999, 2003; Tabor & Tabor, 1983). Agmatine is found in abundance in the stomach, intestines, aorta, and throughout the brain and spinal cord (Raasch et al., 1995). The highest concentration of agmatine has been reported in the gastrointestinal system. Since ADC has relatively low expression in mammalian tissues, agmatine primarily originates from dietary intake and local synthesis by the intestinal microbiota (Benamouzig et al., 1997; Osborne & Seidel, 1990; Saha et al., 2023). The gut microbiota is considered the primary factor responsible for polyamine levels in the lower small intestine (Matsumoto & Benno, 2007). Dietary intake, the primary source of polyamines in the intestinal lumen, is absorbed in the upper intestines for most of these compounds to support growth processes in the body and enters the bloodstream via the colonic mucosa (Milovic, 2001; Tofalo et al., 2019).

Intracellular polyamine levels are regulated by endogenous biosynthesis, degradation, and exogenous transport. Polyamines are taken up from the intestine or extracellular environments by specific membrane transporters and are subsequently redistributed to cellular organelles or the cytosol. Both endocytic

and solute-dependent mechanisms have been identified for polyamine uptake in the intestinal lumen (Sugiyama et al., 2017; Tao et al., 2026; Tofalo et al., 2019). Polyamine transport is important for the regulation of cellular polyamine levels (Abdulhussein & Wallace, 2013). Polyamine transporters include members of the solute carrier (SLC) and p-type ATPase families. Multiple members of the organic cation transporter (OCT/SLC22A) family, such as SLC22A1-3, play a role in the transport of putrescine and agmatine (Zahedi et al., 2022b). Additionally, human SLC family transporters hOCT (1/2/3) and hMATE (multiple drug and toxin efflux transporter 1/2/2K) are potential transporter systems that may play a role in the transport of agmatine and polyamines. OCTs mediate the facilitated diffusion or membrane potential-driven transport of a wide variety of both toxic and therapeutic cations and are predominantly expressed in rodent and human liver (OCT1/OCT3), kidney (OCT2), and placenta/heart (OCT3) (Koepsell et al., 2007). MATE transporters (MATE1/2/2K) are recently discovered H+/cation antiporters that mediate the electrically neutral tubular and canalicular efflux of intracellular cations in the kidney and liver (Otsuka et al.,2005). ATP13A2 and ATP13A3, members of the P5-type ATPase family, have recently been identified as key transporters mediating the import and export of polyamines in lysosomes (van Veen et al., 2020).

The microbiota–gut–brain axis is defined as a dynamic, bidirectional communication network linking gut microbial activity to central nervous system physiology. The gut microbiota can influence fundamental neurobiological processes such as neuroinflammation, blood-brain barrier permeability, and neurotransmitter synthesis through metabolic, immune, endocrine, and neural pathways. It has been demonstrated that microbial dysbiosis is associated with Alzheimer's disease, Parkinson's disease, and other neurodegenerative disorders, and it has been proposed that modulating the microbiome could serve as a potential therapeutic approach to alter the progression of these diseases (Dinan & Cryan, 2017).

Polyamines are among the key biomolecules integrated into this axis. Polyamines, which can be synthesized by both the host organism and the gut microbiota, play a role in processes such as cellular proliferation, synaptic plasticity, and neuroprotection. Polyamine levels in the central nervous system are tightly regulated through biosynthesis, catabolism, and transport mechanisms. It is known that the dysregulation observed in polyamine metabolism in Alzheimer's and Parkinson's diseases may be associated with increased oxidative stress, impaired autophagy processes, and mitochondrial dysfunction (Adhikari & Saha, 2026).

In conclusion, the relationship between the gut microbiota and agmatine and other polyamines is of great importance for the maintenance of gastrointestinal homeostasis and the regulation of systemic physiological processes. Agmatine is a dynamic metabolite shaped by both host metabolism and gut microorganisms, playing a role in numerous biological processes such as polyamine biosynthesis, cellular proliferation, inflammation control, and neuroprotection. The levels of polyamines synthesized or modified by the gut microbiota in the intestinal lumen are regulated by complex interactions between diet, microbial composition, and cellular metabolic activities.

It has been reported that changes in the gut microbiota can directly affect polyamine metabolism, and that increased production of biogenic amines particularly in dysbiotic conditions is associated with inflammation, oxidative stress, and intestinal barrier dysfunction. In addition, polyamine transport systems and catabolic mechanisms play a critical role in maintaining intracellular polyamine homeostasis, thereby determining the biological effects of agmatine and other polyamines. In particular, the roles of OCT, MATE, and ATP13A family transporters in polyamine transport highlight the importance of these molecules in cellular signaling and metabolic regulation processes.

In recent years, studies on the microbiota–gut–brain axis have shown that agmatine and polyamines play a role not only in gut physiology but also in central nervous system function. It is thought that abnormalities in polyamine metabolism may be associated with neurodegenerative diseases such as Alzheimer's and Parkinson's disease through mechanisms involving neuroinflammation, mitochondrial dysfunction, and oxidative stress. Therefore, a better understanding of the relationship between the gut microbiota and agmatine levels represents an important area of research for elucidating the pathogenesis of both gastrointestinal and neurological diseases and for developing new microbiota-based treatment strategies.

CONCLUSION

The relationship between the gut microbiota and agmatine forms a broad network of interactions shaped by polyamine metabolism, extending from gastrointestinal homeostasis to systemic physiological processes. The gut microbiota plays a central role not only in digestion and nutrient absorption but also in the regulation of host metabolism, the modulation of immune responses, and the influence on neurological signaling pathways. In this context, agmatine is considered a key biomolecular mediator of microbiota–host interactions due to its ability to be derived from both endogenous and microbial sources and its regulatory role in polyamine metabolism.

The data obtained indicate that agmatine levels may vary depending on diet, host enzymatic activity, and, in particular, the composition of the gut microbiota. Amino acid decarboxylation processes carried out by the microbiota are a decisive factor in the formation of the polyamine pool in the intestinal lumen, and alterations in these processes during dysbiosis can lead to disruptions in biogenic amine balance. This condition is not limited to local intestinal effects but is associated with increased inflammatory responses, impaired mucosal barrier integrity, and systemic metabolic dysregulation.

Given agmatine's regulatory effects on nitric oxide metabolism, cellular proliferation, inflammatory signaling pathways, and neurotransmission, it is thought that this molecule may play a significant role as a neuromodulator within the gut–brain axis. Particularly when considering the effects of microbiota-derived metabolites on the central nervous system, it has been proposed that agmatine could serve as a potential mediator in the pathophysiology of psychiatric and neurodegenerative diseases. Furthermore, its anti-inflammatory and antioxidant properties position agmatine as a potential biomarker and therapeutic target in microbiota-based treatment strategies.

Transporter systems belonging to the SLC and P-type ATPase families, which regulate polyamine transport and cellular distribution, play a critical role in the systemic regulation of agmatine and related metabolites. It is believed that changes in the expression and function of these transporters may affect both local intestinal metabolism and polyamine balance in peripheral organ systems. Therefore, the microbiota–agmatine axis must be evaluated holistically, considering not only production levels but also transport and catabolism processes. The gut microbiota functions as a fundamental regulatory system linking metabolic, immune, and neurological processes through the microbiota–gut–brain axis; it influences central nervous system functions by modulating processes such as neuroinflammation, blood-brain barrier permeability, and neurotransmitter synthesis.

In conclusion, the interaction between the gut microbiota and agmatine influences a broad biological spectrum ranging from gastrointestinal physiology to neurological processes. However, to fully understand this relationship at the molecular level, advanced metabolomic, microbiomic, and clinical studies are needed regarding the agmatine production capacity of different bacterial species, their interaction with host enzyme systems, and the role of transport mechanisms. Future studies are expected to contribute to personalized medicine by providing a clearer understanding of the role of the microbiota–agmatine axis in disease pathogenesis.

REFERENCES

Abdollahi-Roodsaz, S., Abramson, S. B., & Scher, J. U. (2016). The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nature Reviews Rheumatology 2016 12:8*, *12*(8), 446–455. https://doi.org/10.1038/nrrheum.2016.68

Abdulhussein, A. A., & Wallace, H. M. (2013). Polyamines and membrane transporters. *Amino Acids 2013 46:3*, *46*(3), 655–660. https://doi.org/10.1007/S00726-013-1553-6

Adhikari, T., & Saha, P. (2026). Probiotic and microbial modulation of polyamine production: an emerging avenue for neuroprotection in neurodegenerative disorders. Amino Acids 2026 58:1, 58(1), 15-. https://doi.org/10.1007/S00726-026-03505-5

Bekebrede, A. F., Keijer, J., Gerrits, W. J. J., & de Boer, V. C. J. (2020). The Molecular and Physiological Effects of Protein-Derived Polyamines in the Intestine. *Nutrients 2020, Vol. 12, Page 197*, *12*(1), 197. https://doi.org/10.3390/NU12010197

Benamouzig, R., Mahé, S., Luengo, C., Rautureau, J., & Tomé, D. (1997). Fasting and postprandial polyamine concentrations in the human digestive lumen. *The American Journal of Clinical Nutrition*, *65*(3), 766–770. https://doi.org/10.1093/AJCN/65.3.766

Chang, L., Li, Z., Guo, H., Zhang, W., Lan, W., Wang, J., Shen, G., Xia, Q., & Zhao, P. (2021). Function of polyamines in regulating cell cycle progression of cultured silkworm cells. *Insects*, *12*(7), 624. https://doi.org/10.3390/INSECTS12070624/S1

Dinan, T. G., & Cryan, J. F. (2017). The Microbiome-Gut-Brain Axis in Health and Disease. Gastroenterology Clinics of North America, 46(1), 77–89. https://doi.org/10.1016/j.gtc.2016.09.007

Doeun, D., Davaatseren, M., & Chung, M. S. (2017). Biogenic amines in foods. *Food Science and Biotechnology 2017 26:6*, *26*(6), 1463–1474. https://doi.org/10.1007/S10068-017-0239-3

Dudkowska, M., Lai, J., Gardini, G., Stachurska, A., Grzelakowska-Sztabert, B., Colombatto, S., & Manteuffel-Cymborowska, M. (2003). Agmatine modulates the in vivo biosynthesis and interconversion of polyamines and cell proliferation. *Biochimica et Biophysica Acta - General Subjects*, *1619*(2), 159–166. https://doi.org/10.1016/S0304-4165(02)00476-2

Gardini, G., Cravanzola, C., Autelli, R., Testore, G., Cesa, R., Morando, L., Solinas, S. P., Muzio, G., Grillo, M. A., & Colombatto, S. (2003). Agmatine inhibits the proliferation of rat hepatoma cells by modulation of polyamine

metabolism. *Journal of Hepatology*, *39*(5), 793–799. https://doi.org/10.1016/S0168-8278(03)00386-6

Haenisch, B., Von Kügelgen, I., Bönisch, H., Göthert, M., Sauerbruch, T., Schepke, M., Marklein, G., Höfling, K., Schröder, D., & Molderings, G. J. (2008). Regulatory mechanisms underlying agmatine homeostasis in humans. *Https://Doi.Org/10.1152/Ajpgi.90374.2008*, *295*(5). https://doi.org/10.1152/AJPGI.90374.2008

He, J., Li, H., Jia, J., Liu, Y., Zhang, N., Wang, R., Qu, W., Liu, Y., & Jia, L. (2023). Mechanisms by which the intestinal microbiota affects gastrointestinal tumours and therapeutic effects. *Molecular Biomedicine 2023 4:1*, *4*(1), 45-. https://doi.org/10.1186/S43556-023-00157-9

Isome, M., Lortie, M. J., Murakami, Y., Parisi, E., Matsufuji, S., & Satriano, J. (2007). The antiproliferative effects of agmatine correlate with the rate of cellular proliferation. *Https://Doi.Org/10.1152/Ajpcell.00084.2007*, *293*(2). https://doi.org/10.1152/AJPCELL.00084.2007

Kamada, N., Chen, G. Y., Inohara, N., & Núñez, G. (2013). Control of pathogens and pathobionts by the gut microbiota. *Nature Immunology 2013 14:7*, *14*(7), 685–690. https://doi.org/10.1038/ni.2608

Killiny, N., & Nehela, Y. (2020). Citrus Polyamines: Structure, Biosynthesis, and Physiological Functions. *Plants 2020, Vol. 9, Page 426*, *9*(4), 426. https://doi.org/10.3390/PLANTS9040426

Koepsell, H., Lips, K., & Volk, C. (2007). Polyspecific Organic Cation Transporters: Structure, Function, Physiological Roles, and Biopharmaceutical Implications. *Pharmaceutical Research 2007 24:7*, *24*(7), 1227–1251. https://doi.org/10.1007/S11095-007-9254-Z

Kuziel, G. A., & Rakoff-Nahoum, S. (2022). The gut microbiome. *Current Biology*, *32*(6), R257–R264. https://doi.org/10.1016/j.cub.2022.02.023

Mafe, A. N., & Büsselberg, D. (2026). The Diet–Microbiota–Polyamine Axis in Intestinal Aging: Microbial Pathways, Functional Foods, and Physiological Implications. *Nutrients 2026, Vol. 18, Page 578*, *18*(4), 578. https://doi.org/10.3390/NU18040578

Matsumoto, M., & Benno, Y. (2007). The Relationship between Microbiota and Polyamine Concentration in the Human Intestine: A Pilot Study. *Microbiology and Immunology*, *51*(1), 25–35. https://doi.org/10.1111/J.1348-0421.2007.TB03887.X

Mayeur, C., Veuillet, G., Michaud, M., Raul, F., Blottière, H. M., & Blachier, F. (2005). Effects of agmatine accumulation in human colon carcinoma cells on polyamine metabolism, DNA synthesis and the cell cycle. *Biochimica et*

Biophysica Acta - Molecular Cell Research, *1745*(1), 111–123. https://doi.org/10.1016/j.bbamcr.2004.12.004

Milovic, V. (2001). Polyamines in the gut lumen: bioavailability and biodistribution. *European Journal of Gastroenterology & Hepatology*, *13*(9). https://journals.lww.com/eurojgh/fulltext/2001/09000/polyamines_in_the_gut_lumen__bioavailability_and.4.aspx

Molderings, G. J., Burian, M., Homann, Jü., Nilius, M., & Göthert, M. (1999). Potential relevance of agmatine as a virulence factor of Helicobacter pylori. Digestive Diseases and Sciences, 44(12), 2397–2404. https://doi.org/10.1023/A:1026662316750/METRICS

Molderings, G. J., Heinen, A., Menzel, S., Lübbecke, F., Homann, J., & Göthert, M. (2003). Gastrointestinal Uptake of Agmatine: Distribution in Tissues and Organs and Pathophysiologic Relevance. Annals of the New York Academy of Sciences, 1009(1), 44–51. https://doi.org/10.1196/ANNALS.1304.005;WGROUP:STRING:PUBLICATION

Nakanishi, S., & Cleveland, J. L. (2021). Polyamine Homeostasis in Development and Disease. *Medical Sciences 2021, Vol. 9, Page 28*, *9*(2), 28. https://doi.org/10.3390/MEDSCI9020028

Osborne, D. L., & Seidel, E. R. (1990). Gastrointestinal luminal polyamines: cellular accumulation and enterohepatic circulation. *Https://Doi.Org/10.1152/Ajpgi.1990.258.4.G576*, *258*(4 21-4). https://doi.org/10.1152/AJPGI.1990.258.4.G576

Otsuka, M., Matsumoto, T., Morimoto, R., Arioka, S., Omote, H., & Moriyama, Y. (2005). A human transporter protein that mediates the final excretion step for toxic organic cations. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(50), 17923–17928. https://doi.org/10.1073/PNAS.0506483102;WGROUP:STRING:PUBLICATION

Pegg, A. E., Shantz, L. M., & Coleman, C. S. (1995). Ornithine decarboxylase as a target for chemoprevention. *Journal of Cellular Biochemistry*, *59*(22 S), 132–138. https://doi.org/10.1002/JCB.240590817;SUBPAGE:STRING:ABSTRACT;WEBSITE:WEBSITE:PERICLES;ISSUE:ISSUE:DOI

Qiu, P., Ishimoto, T., Fu, L., Zhang, J., Zhang, Z., & Liu, Y. (2022). The Gut Microbiota in Inflammatory Bowel Disease. *Frontiers in Cellular and Infection Microbiology*, *12*, 733992. https://doi.org/10.3389/FCIMB.2022.733992/FULL

Raasch, W., Regunathan, S., Li, G., & Reis, D. J. (1995). Agmatine, the bacterial amine, is widely distributed in mammalian tissues. *Life Sciences*, *56*(26), 2319–2330. https://doi.org/10.1016/0024-3205(95)00226-V

Rubinstein, M. R., Wang, X., Liu, W., Hao, Y., Cai, G., & Han, Y. W. (2013). Fusobacterium nucleatum Promotes Colorectal Carcinogenesis by Modulating E-Cadherin/β-Catenin Signaling via its FadA Adhesin. *Cell Host and Microbe*, *14*(2), 195–206. https://doi.org/10.1016/j.chom.2013.07.012

Saha, P., Panda, S., Holkar, A., Vashishth, R., Rana, S. S., Arumugam, M., Ashraf, G. M., Haque, S., & Ahmad, F. (2023). Neuroprotection by agmatine: Possible involvement of the gut microbiome? *Ageing Research Reviews*, *91*, 102056. https://doi.org/10.1016/J.ARR.2023.102056

Satriano, J., Matsufuji, S., Murakami, Y., Lortie, M. J., Schwartz, D., Kelly, C. J., Hayashi, S. I., & Blantz, R. C. (1998). Agmatine suppresses proliferation by frameshift induction of antizyme and attenuation of cellular polyamine levels. *Journal of Biological Chemistry*, *273*(25), 15313–15316. https://doi.org/10.1074/jbc.273.25.15313

Schibalski, R. S., Shulha, A. S., Tsao, B. P., Palygin, O., & Ilatovskaya, D. V. (2024). The role of polyamine metabolism in cellular function and physiology. *Https://Doi.Org/10.1152/Ajpcell.00074.2024*, *327*(2), C341–C356. https://doi.org/10.1152/AJPCELL.00074.2024

Shin, N. R., Whon, T. W., & Bae, J. W. (2015). Proteobacteria: Microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*, *33*(9), 496–503. https://doi.org/10.1016/j.tibtech.2015.06.011

Sugiyama, Y., Nara, M., Sakanaka, M., Gotoh, A., Kitakata, A., Okuda, S., & Kurihara, S. (2017). Comprehensive analysis of polyamine transport and biosynthesis in the dominant human gut bacteria: Potential presence of novel polyamine metabolism and transport genes. *The International Journal of Biochemistry & Cell Biology*, *93*, 52–61. https://doi.org/10.1016/J.BIOCEL.2017.10.015

Tabor, C. W., & Tabor, H. (1983). Polyamines. Annual Review of Biochemistry, VOL. 53(Volume 53, 1984), 749–790. https://doi.org/10.1146/ANNUREV.BI.53.070184.003533/CITE/REFWORKS

Tao, X., Nassuna, T., & Zhai, R. G. (2026). Polyamine Metabolism in Brain Health and Disease. *Neuropharmacology and Therapy*, *3*, 49–62. https://doi.org/10.15212/NPT-2025-0028

Tofalo, R., Cocchi, S., & Suzzi, G. (2019). Polyamines and Gut Microbiota. *Frontiers in Nutrition*, *Volume 6-2019*. https://doi.org/10.3389/fnut.2019.00016

Valverde, A. P., Camargo, A., & Rodrigues, A. L. S. (2021). Agmatine as a novel candidate for rapid-onset antidepressant response. *World Journal of Psychiatry*, *11*(11), 981. https://doi.org/10.5498/WJP.V11.I11.981

van Veen, S., Martin, S., Van den Haute, C., Benoy, V., Lyons, J., Vanhoutte, R., Kahler, J. P., Decuypere, J. P., Gelders, G., Lambie, E., Zielich, J., Swinnen, J. V., Annaert, W., Agostinis, P., Ghesquière, B., Verhelst, S., Baekelandt, V., Eggermont, J., & Vangheluwe, P. (2020). ATP13A2 deficiency disrupts lysosomal polyamine export. *Nature*, *578*(7795), 419–424. https://doi.org/10.1038/S41586-020-1968-7;TECHMETA

Ventura, M., Turroni, F., Motherway, M. O. C., MacSharry, J., & van Sinderen, D. (2012). Host-microbe interactions that facilitate gut colonization by commensal bifidobacteria. *Trends in Microbiology*, *20*(10), 467–476. https://doi.org/10.1016/j.tim.2012.07.002

Wu, C. G., Tian, J. L., Liu, R., Cao, P. F., Zhang, T. J., Ren, A., Shi, L., & Zhao, M. W. (2017). Ornithine Decarboxylase-Mediated Production of Putrescine Influences Ganoderic Acid Biosynthesis by Regulating Reactive Oxygen Species in Ganoderma lucidum. *Applied and Environmental Microbiology*, *83*(20), 1289–1306. https://doi.org/10.1128/AEM.01289-17

Zahedi, K., Barone, S., & Soleimani, M. (2022). Polyamines and Their Metabolism: From the Maintenance of Physiological Homeostasis to the Mediation of Disease. *Medical Sciences 2022, Vol. 10, Page 38*, *10*(3), 38. https://doi.org/10.3390/MEDSCI10030038

Chapter 3

A Multidisciplinary Approach to Osteoarthritis in Primary Care

Murathan GULER[1], Çiğdem GEREKLIOĞLU[2]

1. Introduction and Burden of Osteoarthritis

1.1. Definition and Epidemiology

Osteoarthritis (OA) is a degenerative joint disease affecting joint cartilage, ligaments, and the underlying bone. Cartilage degradation, bone remodeling, and osteophyte formation lead to pain, stiffness, swelling, and restricted joint movement over time. While it was previously considered a natural consequence of aging, it is now believed to result from the interaction of various factors, including joint structure, genetics, mechanical forces, and other intra-articular ligament injuries (1,2).

Osteoarthritis (OA) is among the most common musculoskeletal disorder affecting mainly middle-aged and elderly populations with an estimated prevalence of 9.6% among males and 18% among females above 60 years of age. Global Burden of Diseases data show that 242 million individuals have symptoms of knee and hip OA. While the global prevalence was in 1990, it is seen to increase by 132.2% by the year 2020. The Clinical Practice Research Datalink from the UK reports an incidence of OA 40.5 per 1000 persons in primary care. Its incidence may vary according to the regions. The estimated prevalence was reported to be 10–17%in Europe, 12–21% in North America, 2–4% in South America, 16–29% in Asia, Africa, and Middle-Eastern countries. Prevalence of symptomatic knee, hip, and hand OA are reported to be 3.8%, 0.85%, and 7-8%, respectively. Prevalence of radiographic OA was reported higher as compared to symptomatic OA. As OA is a rapidly increasing non-communicable diseases causing disability, it is expected to become an important economic and public health problem in the aging population (3,4,5,6,7).

[1] Dr., Sağlık Bilimleri Üniversitesi, Gaziosmanpaşa Eğitim ve Araştırma Hastanesi
murathanguler@gmail.com, ORCID: 0000-0001-9543-1564
[2] Doç. Dr., Çukurova Üniversitesi Tıp Fakültesi Aile Hekimliği AD
gereklioglucigdem@hotmail.com, ORCID: 0000-0003-3556-9865

1.2. Classification

OA may be primary (idiopathic) or secondary. While ethnicity, genetic factors, biomechanical factors, and age-related physiological changes are accused in primary OA; posttraumatic, dysplastic, infectious, inflammatory, or biochemical factors are suggested to be responsible for secondary OA. (8)

The Kellgren-Lawrence system is the standard for classifying knee, hip, and hand OA severity through imaging

Grade 0 (none): definite absence of x-ray changes of osteoarthritis

Grade 1 (doubtful): doubtful joint space narrowing and possible osteophytic lipping

Grade 2 (minimal): definite osteophytes and possible joint space narrowing

Grade 3 (moderate): moderate multiple osteophytes, definite narrowing of joint space, some sclerosis and possible deformity of bone ends

Grade 4 (severe): large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends (9)

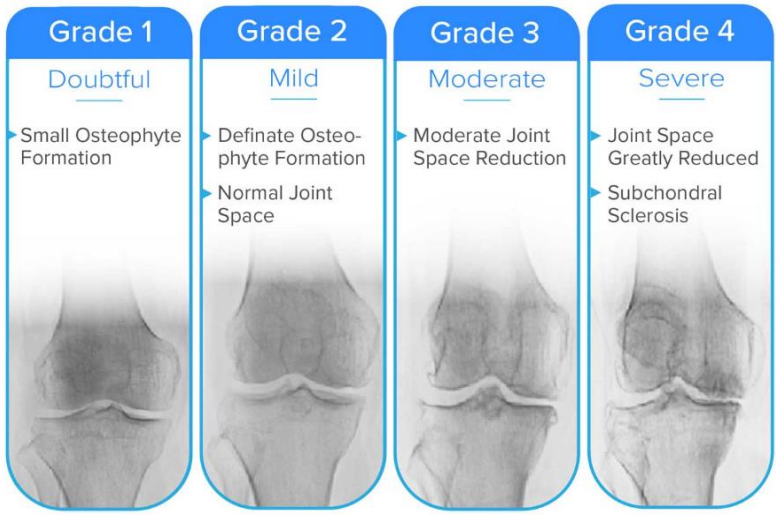


Figure 1. Kellgren-Lawrence Classification System (Radiographic Stages) (10)

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is another tool designed to evaluate pain, stiffness, and physical function in patients with hip or knee OA. It can be used for practice and research. The questionnaire is self-administered and has 24 items that is divided into three subscales, lower scores indicate a better health status. The subscales are as follows:

Pain (5 items): Assesses discomfort during walking, using stairs, in bed, sitting/lying, and standing.

Stiffness (2 items): Assesses severity of stiffness after waking and later in the day.

Physical Function (17 items): Measures limitations in daily activities such as using stairs, rising from sitting, bending, and shopping (11)

1.3. Burden of Osteoarthritis

As being the 15th highest cause of years lived with disability globally, OA brings a huge individual and socioeconomic burden. For example, data from the USA showed that they spent about $80 billion for osteoarthritis in 2016 and data Hong Kong showed costs of 11,690–40,180 HKD per person annually, with indirect costs ranging from 3,300–6,640 HKD per person annually. In the Clinical Practice Research Datalink from UK, the incidence of OA was found to be 40.5 per 1000 persons in primary care (2,5,7,12).

As a chronic health condition, OA may also influence mental health, sleep, work participation, and even mortality (2). Early detection and effective management of OA in primary care may improve patient outcomes and reduce healthcare system burden (12). Given that this chronic disorder should be addressed via the hollistic approach, a multi-disciplinary management and meeting multiple demands and needs of the patients may yield better patient outcomes and improve quality of life.

2. Pathophysiology and Risk Factors

2.1. Pathophysiology

Osteoarthritis was formerly addressed as a degenerative cartilage disease, however modern imaging techniques have indicated that disease pathogenesis involves the structural changes in the cartilage and the whole joint and also a low-grade, chronic inflammation (6,13,14).

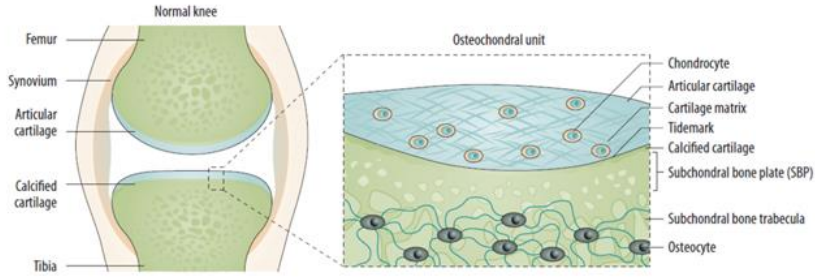


Figure 2. Schematic representation of a normal joint showing two articulating bones (e.g., the femur and tibia in the knee joint), the articular cartilage, and the synovial lining of the joint cavity (6).

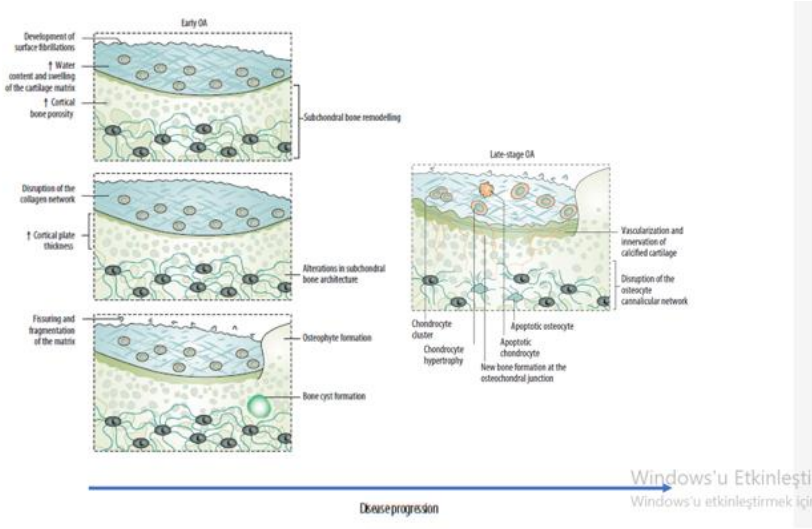


Figure 3. Schematic representation of sequential osteochondral changes during osteoarthritis progression. **(Left)** Early alterations involve remodeling of the subchondral bone plate accompanied by increased cortical plate thickness. **(Right)** Progressive expansion of the calcified cartilage extends into the adjacent hyaline articular cartilage (6).

2.2. Risk Factors

The risk factors for OA can be divided into individual susceptibility features (increasing age, obesity, female sex, joint biomechanics, genetic factors) and joints-related factors (injury, repetitive joint use through occupation or leisure, and joint malalignment (15,16,17). Risk factors can also be classified as modifiable (local muscle weakness, traumatic joint injury, overweight/obesity,

altered joint shape and malalignment, high-impact sports, high occupational loading) and non-modifiable ones (structural changes, female sex, older age, genetic predisposition) (18).

2.2.1. Modifiable Risk Factors

Obesity was reported to be well correlated with knee OA, but less correlated with hip OA. On the other hand, the Framingham study revealed that weight loss decreased the risk for knee OA in. Obesity may increase the risk for knee OA due increasing the mechanical loading of the knee. (19,20,21,22,23,24,25,26,27,28,29,30).

Research have indicated that the ***increased bone mineral density*** (BMD) is associated with OA of the hip, knee, hand and spine (31,32,33,34,35,36,37,38). Although the underlying mechanism is still unclear, genetic mediators of bone turnover and local biomechanical factors have been accused (40,41).

The influence of certain dietary modifications on the prevention and development of OA is also unclear, it may be suggested that a healthy balanced diet may hinder obesity and thereby prevent OA, further studies are required to make a comment on the direct effect of supplements (42).

Several studies have indicated a reduced risk of OA in smokers, although the mechanism is unclear, it may be proposed that smokers may generally have a lower BMI than nonsmokers (43, 44, 45).

A certain mechanism between the OA and ***alcohol consumption*** could not be enlightened (17).

Evidence is available suggesting that individuals who do moderate levels of ***physical activity*** are less likely to develop hip or knee OA. Although muscle weakness has been identified as a risk factor for OA, further large scale studies are needed to clarify the role of exercise in the primary prevention of knee OA (17)

While ***diabetes and impaired glucose tolerance*** were found to be an independent risk factor for OA, more recent studies did not find such an association (20).

2.2.2. Non-modifiable Risk Factors

Female sex was found to be strongly correlated with knee OA (17, 19).

The incidence of OA increases with ***age*** probably due to the age-related musculo-sceletal changes and long-term exposure to risk factors (20).

The role of ***genetics*** in OA was reported to be between 40% and 80%, mostly for hand and hip OA. While rare mutations in monogenetic disorders can lead to early-onset OA, common DNA variants can lead to late-onset OA (17, 47, 48).

In twin studies, genetic factors were shown to be able to be responsible for 40% of hand OA in women, for 65% of knee OA, for 70% of hip OA, and for 70% of spine OA (49,50)

2.2.3. Joint-Related Factors

Structural abnormalities of the joint may be a factor that increases OA risk. For incident hip and knee OA, femoral head shape variation , knee malalignment and knee extensor muscle weakness were shown to be risk factors (6,52).

Traumatic joint injury is among the well-known risk factors for OA (21), particularly knee injuries are highly prevalent (53,54) and associated with a 6-fold greater risk of structural knee OA by 11 years. Trauma-related OA prevalence was found to be 12% among all symptomatic OA in the US. Trauma causes long-term structural changes in the joint and leads to biomechanical alterations. Injury type is a determinant for the risk. For example, anterior cruciate ligament tears, meniscal tears, and intra-articular fractures may cause a higher risk as compared to collateral ligament tears (55,56,57).

Heavy work activities, especially requiring heavy lifting and kneeling, working in the building trade or ranching were shown to be risk factors for OA of the hip and the knee (19).

While several studies have shown that ***high-impact sports*** including football, handball, hockey, wrestling, weight-lifting, and long-distance running increase the risk of hip or knee OA in a dose- dependent manner (58,59,60), a community-based study conducted with middle-aged and older adults did not find an association between moderate–vigorous physical activity and incident knee OA (61). For those who do sports and develop knee OA, the accused risk factor is knee injuries; for those with hip OA, the risk may be cam impingement (24).

In the developing world, the association between hand OA and the ***increasing use of technological tools*** including computers, and smartphones may be a novel issue to investigate. Current evidence is not sufficient to indicate a direct relationship, however, it deserves further investigation (62).

3. Diagnosis and Differential Diagnosis of Osteoarthritis

3.1. Signs and Symptoms

The symptoms of osteoarthritis often begin slowly or start quickly after an injury or strain, typically affecting one or a few joints. Knees, hips, spine, and small joints in the hands are the most affected. Muscles and tissue around the joint are also affected. It is chronic and progressive. While pain is more prominent with movements and may improve with rest, in severe cases, patients may feel pain even during rest. Joint stiffness, usually lasting less than 30

minutes, is a common symptom that often occurs in the morning or after a period of rest, accompanied by limited joint movement, swelling in and around the joint, and a feeling that the joint is loose or unstable. As a result of exposure to chronic pain, reduced movement, losing muscle strength, the quality of life may be impaired, being physically less active may lead to obesity, diabetes, or cardiovascular disorders. Reduced participation in homeworks and social life may cause impaired sleep and mental problems (63,64).
Table 1 summarizes the signs and symptoms of osteoarthritis according to localization and Table 2 summarizes the signs and symptoms of knee according to disease stage.

Table 1. Signs and symptoms of osteoarthritis (65)

Hand	**Hip**
Pain on range of motion	Pain on range of motion
Hypertrophic changes at distal and proximal interphalangeal joints (Heberden nodes and Bouchard nodes; Figure 1)	Pain in buttock
Tenderness over carpometacarpal joint of thumb	Limitation of range of motion, especially internal rotation
Shoulder	**Foot**
Pain on range of motion	Pain on ambulation, especially at first metatarsophalangeal joint
Limitation of range of motion, especially external rotation	Limited range of motion of first metatarsophalangeal joint, hallux rigidus
Crepitus on range of motion	Hallux valgus deformity
Knee	**Spine**
Pain on range of motion	Pain on range of motion
Joint effusion	Limitation of range of motion
Crepitus on range of motion	Lower extremity sensory loss, reflex loss, motor weakness caused by nerve root impingement
Presence of popliteal cyst (Baker cyst)	Pseudoclaudication caused by spinal stenosis
Lateral instability	
Valgus or varus deformity	

Table 2. Signs and symptoms of knee osteoarthritis according to disease stage (66)

Stage of knee osteoarthritis	Symptoms	Radiography results
Early	• Sporadic but predictable pain • Minimal impact on daily activities	• Normal joint space • Osteophyte formation
Moderate	• Unpredictable pain • Locking or buckling of the knees • Affects daily activities, such as walking, bending, squatting, and climbing stairs	• Moderate reduction in joint space • Multiple osteophytes
Advanced	• Constant but mostly dull pain • Occasional episodes of high-intensity pain • Limited joint motion • Reduced ability to ambulate • Muscle weakness • Fatigue • Sleep impairment • Depression	• Considerable reduction in joint space • Subchondral sclerosis • Large osteophytes • Bone end deformity

3.2. Physical Examination

The first step of the physical examination is inspection. Surgical marks, scars, erythema, ecchymosis, , or any other skin disorders should all be noted. Lower limb alignment and gait should be tested. Both knees are examined in standing position for deformities like genu varum or genu valgum, that indicate a more severe osteoarthritis. The presence of swelling in the popliteal region may be indicative for a Baker cyst. Knee examination is continued in supine position. A quadriceps muscle atrophy suggests a chronic or more advanced pathology (66).

With the knee maintained at 90 degrees of flexion, the joint lines are palpated for tenderness and osteophyte formation. Patellar crepitation can be detected during passive flexion–extension movements of the knee while the patella is held in position. Mediolateral assessment of patellar movement may help identify patellofemoral osteoarthritis when movement is painful or restricted (66).

Range of motion and muscle strength assessment should be included as part of the knee examination. To evaluate active knee flexion, the patient is asked to bring the heel toward the buttock, with flexion greater than 130 degrees generally considered normal. When active movement is limited, passive range of motion should also be assessed. Knee flexion strength may be examined using isometric testing with the patient in the supine position by stabilizing the knee at 90 degrees and asking the patient to resist extension force. Knee extension should then be assessed by instructing the patient to fully straighten the leg, with full extension reaching approximately 180 degrees. The presence of any flexion contracture is considered abnormal. Extension strength can be evaluated by applying resistance against the leg while the patient attempts to maintain extension. In addition, to

evaluate the referring pain to the knee from the hip or lower back, it is important to examine the ipsilateral hip and lumbar spine (66).

Fluid effusions may be detected by special tests (Figures 4,5,6) (66)

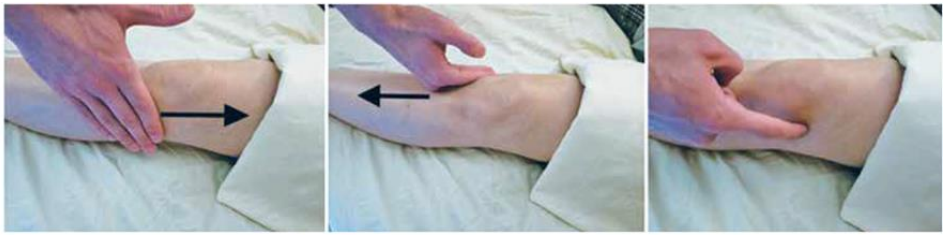


Figure 4. ***The milking test*** is used to detect small knee effusions. Firm pressure is applied to the medial aspect of the knee while the hand is moved proximally along the medial side, and synovial fluid is displaced into the lateral suprapatellar recess. The presence of a fluid wave or bulge sign suggests a small joint effusion (66).

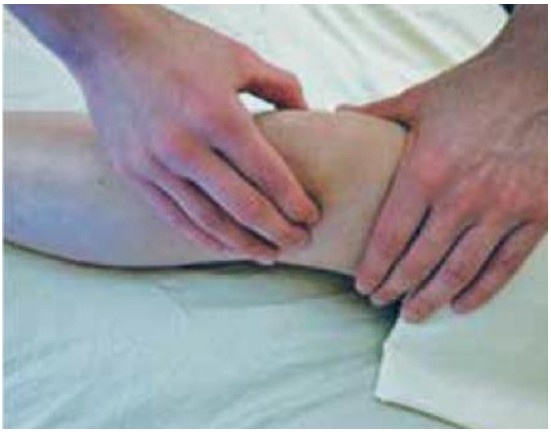


Figure 5. The ballottement test is performed to detect moderate to large effusions. The thumb and fingers of one hand on either side of the patella are cupped and the other hand is placed on the suprapatellar recess (66)

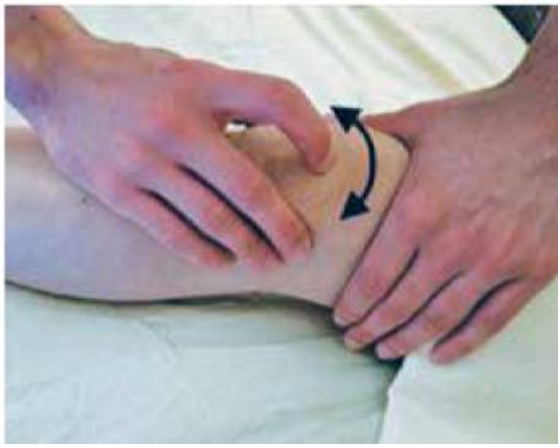


Figure 6. ***The patellar tap*** is performed to detect large effusions. One hand is placed over the suprapatellar recess and pressure is applied to displace fluid under the patella (66)

3.3. ***Laboratory examination***

Laboratory investigations are generally not necessary for establishing the diagnosis. In most patients, inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels remain within normal limits. Immunological tests, like antinuclear antibody (ANA) and rheumatoid factor (RF), are not routinely indicated unless clinical findings suggest joint inflammation or synovitis. Measurement of serum uric acid levels should be considered only when gout is suspected. Unnecessary laboratory testing may lead to diagnostic uncertainty, as false-positive findings can occur and potentially result in misinterpretation (65,67).

3.4. Imaging Tests

The diagnosis of OA can be made based on physical examination findings and clinical signs and symptoms. Plain radiography can reveal the diagnosis and rule out other diagnoses. Magnetic resonance imaging or computed tomography are usually not required if a doubtful diagnosis or another condition like a meniscal injury is not present (65).

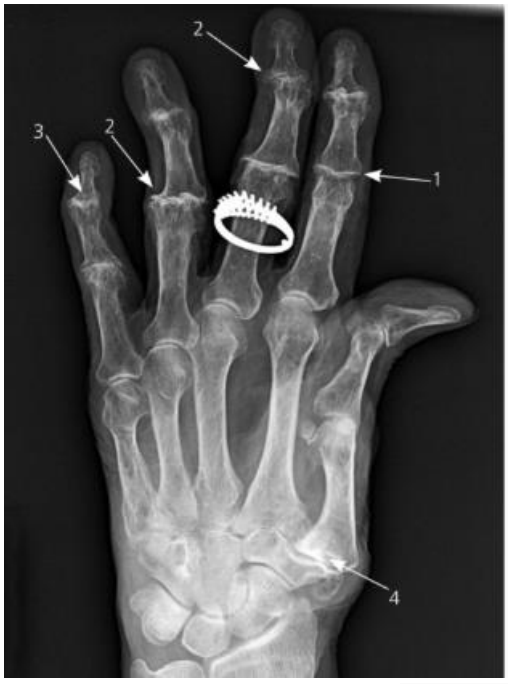


Figure 7. Radiographic image of hand OA displaying (1) joint space narrowing (1), osteophytes (2), joint destruction (3), and carpometacarpal joint changes (4) (65)

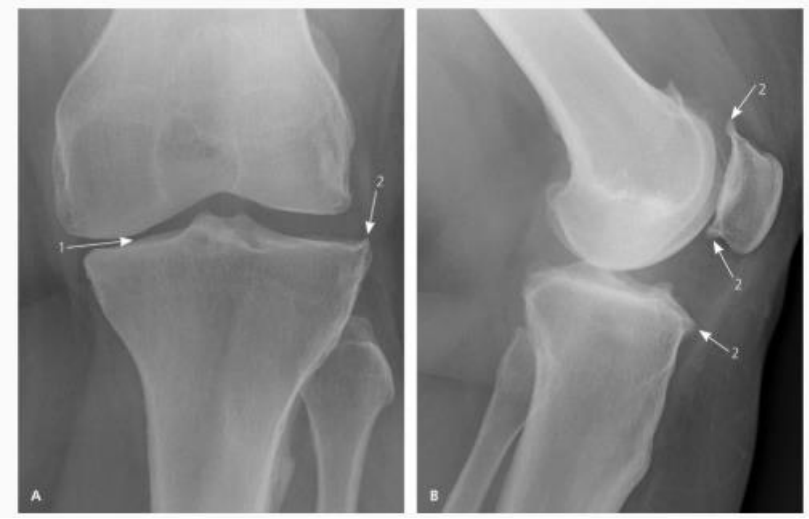


Figure 8. Radiographic image of the knee in anteroposterior (A) and lateral (B) views. Joint space narrowing (1) and osteophyte formation (2) in knee OA (67).

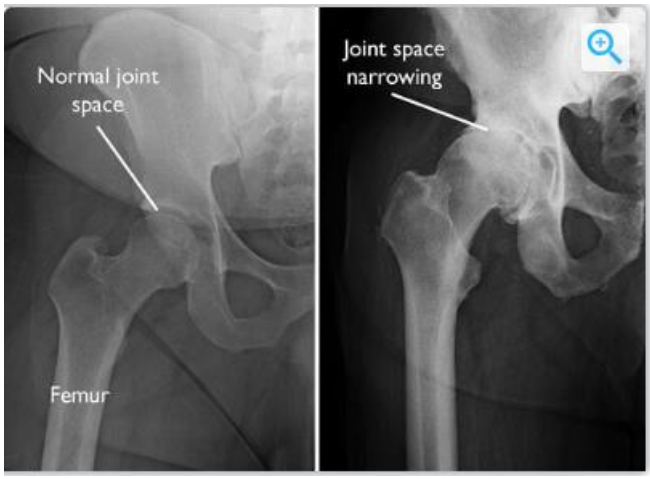


Figure 9. Radiographic image of a normal hip and hip with OA (67)

3.4. Referral

Patients presenting with clinical findings suggestive of inflammatory arthritis should be referred to a rheumatologist. Indications for referral include polyarticular involvement affecting small and large joints, constitutional symptoms like fatigue and unexplained weight loss, and extra-articular manifestations including rash, psoriasis, or inflammatory bowel disease (66).

Patients should be referred to an orthopedic surgeon when pain control remains insufficient, joint mobility is significantly impaired and interferes with activities of daily living, or when medical treatment and rehabilitation approaches fail to provide adequate symptom relief (66).

4. Treatment

No curative treatment is available for OA, however many treatment options may be used for pain management and for maintaining the activity of the patients (67). A stepped-care approach is recommended for management of OA (65).

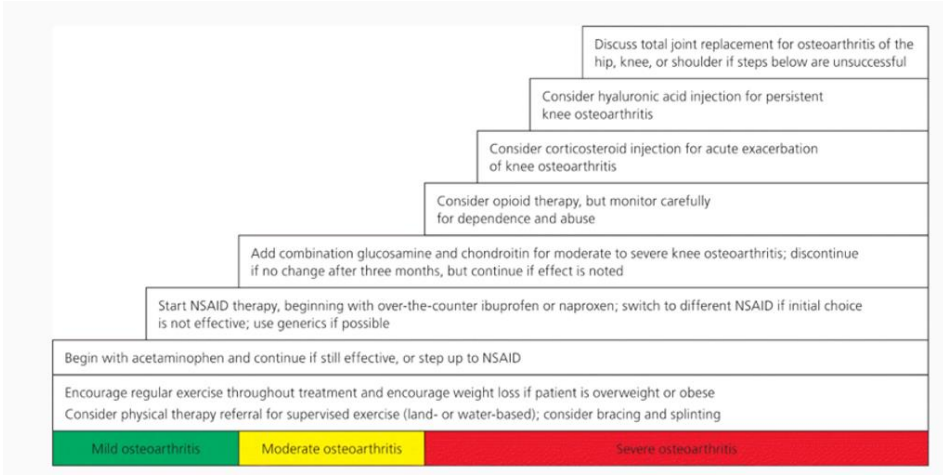


Figure 10. Stepped-care approach for the treatment of OA (65)

The least invasive and the safest treatment modalities should be preferred first, more invasive and/or more expensive options should be selected thereafter. It is recommended to administer one or more options from the first two categories to all patients. For patients irresponsive to life style changes and medications, surgery may be considered (65).

Joint mobility may be maintained, muscle strength may be improved, and pain may be relieved through early non-surgical treatment. A combination of lifestyle modifications, medication, and physical therapy is usually preferred (67).

4.1. Non-surgical treatment

4.1.1. Lifestyle Changes

Patients with OA may benefit from lifestyle changes including weight loss (especially if spine, hip, knee, or ankle are affected), work activity modifications, discontinuation or reduction of high-impact activities like aerobics, running, jumping, or competitive sports, instead preferring low-impact exercises like stretching, walking, swimming, or cycling) (65,67).

4.1.2. Medications

Various medications including acetaminophen, NSAIDs, tramadol, and duloxetine may be used for the treatment of patients with OA.

Acetaminophen may be useful for patients with mild-moderate pain. It must be remembered that excessive doses may lead to hepatic dysfunction (68).

Nonsteroidal anti-inflammatory drugs (NSAIDs) may usually be sufficient to relieve OA-related pain and they are the most common medications for OA. Patients should be warned about adverse effects, including gastrointestinal and

cardiovascular problems, coagulation disorders, and hepatic and renal injury. And a risk profile should be considered (69). While the Osteoarthritis Research Society International (OARSI) recommends oral NSAIDs for individuals with knee, hip, or polyarticular OA without comorbidities, (70). The American College of Rheumatology (ACR)/Arthritis Foundation and the American Academy of Orthopedic Surgeons recommend oral NSAIDs for the treatment of knee, hip, or hand OA (71,72,73). While the European Alliance of Associations for Rheumatology recommended oral NSAIDs for a limited duration for hand OA (74), the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases strongly recommended oral NSAIDs for patients with persistent symptomatic knee OA (75).

Topical NSAIDs are preferred due to similar pain relieveing effect and less side effects as compared to peroral preparations (71,72,75). OARSI strongly recommended topical NSAIDs for patients with knee OA, especially for patients with gastrointestinal, cardiovascular, or frailty morbidities (70).

Tramadol that is a synthetic opioid and serotonin/norepinephrine reuptake-inhibitor is conditionally recommended by ACR for patients with knee, hip, or hand OA (71).

Duloxetine that is originally an antidepressant is also approved to treat chronic pain, including osteoarthritis pain (68,69).

4.1.3. Physical therapy

Selected patients may benefit from regular exercises for relieving pain, improving muscle strength, and increasing flexibility. Patients may do these exercises by their own, such as swimming or walking, they can be both effective (67,68).

4.1.4. Other non-surgical treatments

- Some patients may benefit from ***supportive or assistive devices*** (such as a brace, splint, elastic bandage, cane, crutches, or walker) (67).
- ***Occupational therapy*** can be helpful to do daily activities by decreasing the stress on the painful joint. For a patient with hand OA, a toothbrush with a large grip may be preferred to facilitate tooth brushing. For a patient with knee OA, the pain of standing may be reduced by placing a bench in the bath (68).
- ***Transcutaneous electrical nerve stimulation (TENS)*** that uses a low-voltage electrical current may relieve pain in the short-term in patients with knee and hip osteoarthritis (68).

- ***Intra-articular corticosteroids (IACS)*** may relieve pain relief for a short time (<4 weeks) (69).
- ***Lubrication injections*** **o**f hyaluronic acid, that is normally found in the joint space, may provide cushioning in the knee and thereby relieve pain. Despite the presence of several studies suggesting that lubrication injections are not more effective than a placebo (68), OARSI conditionally recommended intra-articular hyaluronic acid injections for patients with knee OA in all comorbidity groups (69).

4.2. Surgical treatment

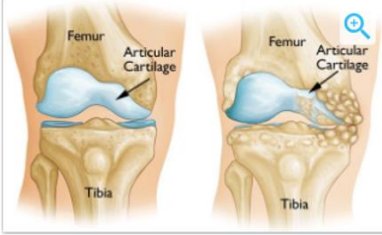
When pharmacological treatments are not sufficient to relieve symptoms, surgery may come in the froe. The decision for surgery depends on the patient's age and functional status, the degree of joint damage, and the degree of disease (67).

4.2.1. Arthroscopy

Arthroscopy is a common procedure for the diagnosis and treatment of many knee problems (67).Partial meniscectomy, repair of a torn meniscus or damaged articular cartilage, removal of loose fragments of bone or cartilage, inflamed synovial tissue, treatment of patella problems, and knee sepsis are the most common area of use of this procedure. Hip arthroscopy may be used for the relief of the problems damaging the labrum, articular cartilage, or other soft tissues surrounding the joint (67).

4.2.2. Osteotomy

The long bones of the arm or leg are realigned to take pressure off the affected portion of the joint. Knee osteotomy is used for early-stage OA that has damaged just one side of the knee joint. By shifting weight off of the damaged side of the joint, an osteotomy can relieve pain and significantly improve function in an arthritic knee (67).



(Left) A normal knee joint with healthy cartilage. **(Right)** Osteoarthritis that has damaged just one side of the knee joint.

Figure 11. Appearance of a normal knee and with OA (67)

4.2.3. In ***joint fusion*** the ends of bone are brought and fastened together with the help of pins, plates, screws, or rods. This procedure eliminates the joint's flexibility (67).

4.2.4. Joint replacement

Total joint replacement refers to a surgical procedure in which diseased or damaged joint surfaces are replaced with prosthetic components intended to mimic the function and mobility of a normal joint. While hip and knee replacements represent the most common forms of arthroplasty, the procedure can also be performed for other joints such as the ankle, shoulder, wrist, and elbow (67).

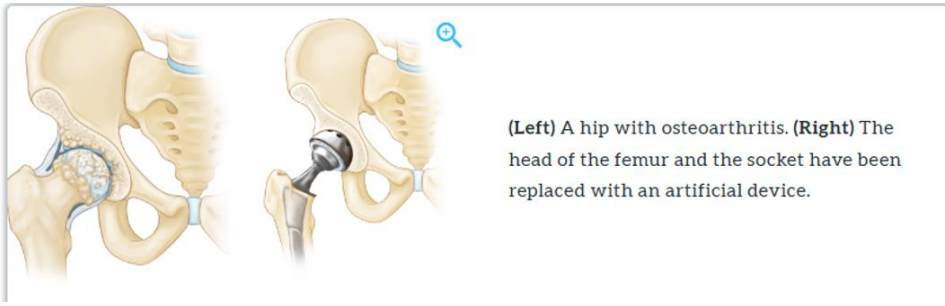


Figure 12. Joint replacement for the hip (67)

References

1. T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü (n.d.). *Osteoartrit*. Retrieved May 13, 2026, from https://hsgm.saglik.gov.tr/tr/kronik-hastaliklar/kas-iskelet-sistemi-hastaliklari/osteoartrit.html
2. Allen, K. D., Thoma, L. M., & Golightly, Y. M. (2022). Epidemiology of osteoarthritis. *Osteoarthritis and Cartilage, 30*(2), 184–195. https://doi.org/10.1016/j.joca.2021.04.020
3. Yalıman, A. (2020). Osteoartrit epidemiyolojisi ve klasifikasyonu. In A. S. Hepgüler (Ed.), *Osteoartrit* (1st ed., pp. 1–8). Ankara, Türkiye: Türkiye Klinikleri.
4. Xie, X., Zhang, K., Li, Y., Li, Y., Li, X., Lin, Y., Huang, L., & Tian, G. (2025). Global, regional, and national burden of osteoarthritis from 1990 to 2021 and projections to 2035: A cross-sectional study for the Global Burden of Disease Study 2021. *PLoS ONE, 20*(5), e0324296. https://doi.org/10.1371/journal.pone.0324296
5. Dieleman, J. L., Baral, R., Birger, M., Bui, A. L., Bulchis, A., & Chapin, A. (2016). US spending on personal health care and public health, 1996–2013. *JAMA, 316*(24), 2627–2646.
6. Coaccioli, S., Sarzi-Puttini, P., Zis, P., Rinonapoli, G., & Varrassi, G. (2022). Osteoarthritis: New insight on its pathophysiology. *Journal of Clinical Medicine, 11*, 6013. https://doi.org/10.3390/jcm11206013
7. Woo, J., Lau, E., Lau, C. S., Lee, P., Zhang, J., Kwok, T., et al. (2003). Socioeconomic impact of osteoarthritis in Hong Kong: Utilization of health and social services, and direct and indirect costs. *Arthritis & Rheumatism, 49*(4), 526–534. https://doi.org/10.1002/art.11198
8. Kohn, M. D., Sassoon, A. A., & Fernando, N. D. (2016). Classifications in brief: Kellgren-Lawrence classification of osteoarthritis. *Clinical Orthopaedics and Related Research, 474*(8), 1886–1893. https://doi.org/10.1007/s11999-016-4732-4
9. Radiopaedia (n.d.). *Kellgren and Lawrence system for classification of osteoarthritis*. Retrieved May 12, 2026, from https://radiopaedia.org/articles/kellgren-and-lawrence-system-for-classification-of-osteoarthritis
10. Spring Loaded Technology (n.d.). *Guide to severe knee osteoarthritis*. Retrieved May 12, 2026, from https://springloadedtechnology.com/guide-to-severe-knee-osteoarthritis/
11. McConnell, S., Kolopack, P., & Davis, A. M. (2001). The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC): A review of its utility and measurement properties. *Arthritis & Rheumatism, 45*, 453–

461. https://doi.org/10.1002/1529-0131(200110)45:5<453::AID-ART365>3.0.CO;2-W

12. Cunningham, J., Doyle, F., Ryan, J. M., Clyne, B., Cadogan, C., Cottrell, E., Murphy, P., Smith, S. M., & French, H. P. (2023). Primary care-based models of care for osteoarthritis: A scoping review. *Seminars in Arthritis and Rheumatism, 61*, 152221. https://doi.org/10.1016/j.semarthrit.2023.152221
13. Fusco, M., Skaper, S. D., Coaccioli, S., Varrassi, G., & Paladini, A. (2017). Degenerative joint diseases and neuroinflammation. *Pain Practice, 17*, 522–532.
14. Geraghty, T., Winter, D. R., Miller, R. J., Miller, R. E., & Malfait, A. M. (2021). Neuroimmune interactions and osteoarthritis pain: Focus on macrophages. *Pain Reports, 6*, e892.
15. He, Y., Li, Z., Alexander, P. G., Ocasio-Nieves, B. D., Yocum, L., Lin, H., & Tuan, R. S. (2020). Pathogenesis of osteoarthritis: Risk factors, regulatory pathways in chondrocytes, and experimental models. *Biology, 9*, 194.
16. Martel-Pelletier, J., Barr, A. J., Cicuttini, F. M., Conaghan, P. G., Cooper, C., Goldring, M. B., Goldring, S. R., Jones, G., Teichtahl, A. J., & Pelletier, J. P. (2016). Osteoarthritis. *Nature Reviews Disease Primers, 2*, 16072.
17. O'Neill, T. W., McCabe, P. S., & McBeth, J. (2018). Update on the epidemiology, risk factors and disease outcomes of osteoarthritis. *Best Practice & Research Clinical Rheumatology, 32*, 312–326.
18. Whittaker, J. L., Runhaar, J., Bierma-Zeinstra, S., & Roos, E. M. (2021). A lifespan approach to osteoarthritis prevention. *Osteoarthritis and Cartilage, 29*(12), 1638–1653. https://doi.org/10.1016/j.joca.2021.06.015
19. Georgiev, T., & Angelov, A. K. (2019). Modifiable risk factors in knee osteoarthritis: Treatment implications. *Rheumatology International, 39*, 1145–1157.
20. Dong, Y., Yan, Y., Zhou, J., Zhou, Q., & Wei, H. (2023). Evidence on risk factors for knee osteoarthritis in middle-older aged: A systematic review and meta-analysis. *Journal of Orthopaedic Surgery and Research, 18*(1), 634. https://doi.org/10.1186/s13018-023-04089-6
21. Tan, G. S., Zhang, C., Oo, W. M., Fu, K., Risberg, M. A., Bierma-Zeinstra, S. M., Neogi, T., Atukorala, I., Malfait, A. M., Ding, C., & Hunter, D. J. (2025). Osteoarthritis. *Nature Reviews Disease Primers, 11*(1), 10. https://doi.org/10.1038/s41572-025-00594-6
22. Szilagyi, I. A., Waarsing, J. H., van Meurs, J. B. J., Bierma-Zeinstra, S. M. A., & Schiphof, D. (2023). A systematic review of the sex differences in

risk factors for knee osteoarthritis. *Rheumatology, 62*(6), 2037–2047. https://doi.org/10.1093/rheumatology/keac688

23. Blümel, J. E., Aedo, S., Arteaga, E., Vallejo, M. S., & Chedraui, P. (2022). Factores de riesgo de artrosis de rodilla, cadera o ambas en mujeres chilenas de mediana edad: Un estudio de cohorte de tres décadas [Risk factors for the development of osteoarthritis in middle-aged women]. *Revista Médica de Chile, 150*(1), 46–53. https://doi.org/10.4067/S0034-98872022000100046
24. Hunter, D. J., & Bierma-Zeinstra, S. (2019). Osteoarthritis. *The Lancet, 393*, 1745–1759.
25. Ho, J., Mak, C. C. H., Sharma, V., To, K., & Khan, W. (2022). Mendelian randomization studies of lifestyle-related risk factors for osteoarthritis: A PRISMA review and meta-analysis. *International Journal of Molecular Sciences, 23*(19), 11906. https://doi.org/10.3390/ijms231911906
26. Silverwood, V., Blagojevic-Bucknall, M., Jinks, C., Jordan, J. L., Protheroe, J., & Jordan, K. P. (2015). Current evidence on risk factors for knee osteoarthritis in older adults: A systematic review and meta-analysis. *Osteoarthritis and Cartilage, 23*, 507–515.
27. Felson, D. T., Zhang, Y., Anthony, J. M., Naimark, A., & Anderson, J. J. (1992). Weight loss reduces the risk for symptomatic knee osteoarthritis in women: The Framingham Study. *Annals of Internal Medicine, 116*, 535–539.
28. Reijman, M., Pols, H. A., Bergink, A. P., Hazes, J. M., Belo, J. N., Lievense, A. M., et al. (2007). Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: The Rotterdam Study. *Annals of the Rheumatic Diseases, 66*, 158–162.
29. Gelber, A. C., Hochberg, M. C., Mead, L. A., Wang, N. Y., Wigley, F. M., & Klag, M. J. (1999). Body mass index in young men and the risk of subsequent knee and hip osteoarthritis. *American Journal of Medicine, 107*, 542–548.
30. Tepper, S., & Hochberg, M. C. (1993). Factors associated with hip osteoarthritis: Data from the first National Health and Nutrition Examination Survey (NHANES-I). *American Journal of Epidemiology, 137*, 1081–1088.
31. Nevitt, M. C., Lane, N. E., Scott, J. C., Hochberg, M. C., Pressman, A. R., Genant, H. K., et al. (1995). Radiographic osteoarthritis of the hip and bone mineral density: The Study of Osteoporotic Fractures Research Group. *Arthritis & Rheumatism, 38*, 907–916.

32. Hart, D. J., Mootoosamy, I., Doyle, D. V., & Spector, T. D. (1994). The relationship between osteoarthritis and osteoporosis in the general population: The Chingford Study. *Annals of the Rheumatic Diseases, 53*, 158–162.
33. Peel, N. F., Barrington, N. A., Blumsohn, A., Colwell, A., Hannon, R., & Eastell, R. (1995). Bone mineral density and bone turnover in spinal osteoarthrosis. *Annals of the Rheumatic Diseases, 54*, 867–871.
34. Burger, H., van Daele, P. L., Odding, E., Valkenburg, H. A., Hofman, A., Grobbee, D. E., et al. (1996). Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age: The Rotterdam Study. *Arthritis & Rheumatism, 39*, 81–86.
35. Sowers, M. F., Hochberg, M., Crabbe, J. P., Muhich, A., Crutchfield, M., & Updike, S. (1996). Association of bone mineral density and sex hormone levels with osteoarthritis of the hand and knee in premenopausal women. *American Journal of Epidemiology, 143*, 38–47.
36. Zhang, Y., Hannan, M. T., Chaisson, C. E., McAlindon, T. E., Evans, S. R., Aliabadi, P., et al. (2000). Bone mineral density and risk of incident and progressive radiographic knee osteoarthritis in women: The Framingham Study. *Journal of Rheumatology, 27*, 1032–1037.
37. Sowers, M., Lachance, L., Jamadar, D., Hochberg, M. C., Hollis, B., Crutchfield, M., et al. (1999). The associations of bone mineral density and bone turnover markers with osteoarthritis of the hand and knee in pre- and perimenopausal women. *Arthritis & Rheumatism, 42*, 483–489.
38. Hart, D. J., Cronin, C., Daniels, M., Worthy, T., Doyle, D. V., & Spector, T. D. (2002). The relationship of bone density and fracture to incident and progressive radiographic osteoarthritis of the knee: The Chingford Study. *Arthritis & Rheumatism, 46*, 92–99.
39. Hochberg, M. C. (2004). Do risk factors for incident hip osteoarthritis (OA) differ from those for progression of hip OA? *Journal of Rheumatology Supplement, 70*, 6–9.
40. Nevitt, M. C., Zhang, Y., Javaid, M. K., Neogi, T., Curtis, J. R., Niu, J., et al. (2010). High systemic bone mineral density increases the risk of incident knee OA and joint space narrowing, but not radiographic progression of existing knee OA: The MOST study. *Annals of the Rheumatic Diseases, 69*, 163–168.
41. Hardcastle, S. A., Dieppe, P., Gregson, C. L., Davey Smith, G., & Tobias, J. H. (2015). Osteoarthritis and bone mineral density: Are strong bones bad for joints? *BoneKEy Reports, 4*.

42. Thomas, S., Browne, H., Mobasheri, A., & Rayman, M. P. (2018). What is the evidence for a role for diet and nutrition in osteoarthritis? *Rheumatology, 57*, iv61–iv74.
43. Kong, L., Wang, L., Meng, F., Cao, J., & Shen, Y. (2017). Association between smoking and risk of knee osteoarthritis: A systematic review and meta-analysis. *Osteoarthritis and Cartilage, 25*, 809–816.
44. Hui, M., Doherty, M., & Zhang, W. (2011). Does smoking protect against osteoarthritis? Meta-analysis of observational studies. *Annals of the Rheumatic Diseases, 70*, 1231–1237.
45. Felson, D. T., & Zhang, Y. (2015). Smoking and osteoarthritis: A review of the evidence and its implications. *Osteoarthritis and Cartilage, 23*, 331–333.
46. van Meurs, J. B. (2017). Osteoarthritis year in review 2016: Genetics, genomics and epigenetics. *Osteoarthritis and Cartilage, 25*, 181–189.
47. Zhu, X., Chan, Y. T., Yung, P. S. H., Tuan, R. S., & Jiang, Y. (2020). Subchondral bone remodeling: A therapeutic target for osteoarthritis. *Frontiers in Cell and Developmental Biology, 8*, 607764.
48. Varela-Eirin, M., Loureiro, J., Fonseca, E., Corrochano, S., Caeiro, J. R., Collado, M., & Mayan, M. D. (2018). Cartilage regeneration and ageing: Targeting cellular plasticity in osteoarthritis. *Ageing Research Reviews, 42*, 56–71.
49. Valdes, A. M., & Spector, T. D. (2011). Genetic epidemiology of hip and knee osteoarthritis. *Nature Reviews Rheumatology, 7*, 23–32.
50. Spector, T. D., & MacGregor, A. J. (2004). Risk factors for osteoarthritis: Genetics. *Osteoarthritis and Cartilage, 12*(Suppl. A), S39–S44.
51. Gregory, J. S., Waarsing, J. H., Day, J., Pols, H. A., Reijman, M., Weinans, H., et al. (2007). Early identification of radiographic osteoarthritis of the hip using an active shape model to quantify changes in bone morphometric features: Can hip shape tell us anything about the progression of osteoarthritis? *Arthritis & Rheumatism, 56*, 3634–3643.
52. Neogi, T., Bowes, M. A., Niu, J., Souza, K. M., Vincent, G. R., Goggins, J., et al. (2013). Magnetic resonance imaging–based three-dimensional bone shape of the knee predicts onset of knee osteoarthritis: Data from the Osteoarthritis Initiative. *Arthritis & Rheumatism, 65*, 2048–2058.
53. Gage, B. E., McIlvain, N. M., Collins, C. L., Fields, S. K., & Comstock, R. D. (2012). Epidemiology of 6.6 million knee injuries presenting to United States emergency departments from 1999 through 2008. *Academic Emergency Medicine, 19*(4), 378–385. https://doi.org/10.1111/j.1553-2712.2012.01315.x

54. Gianotti, S. M., Marshall, S. W., Hume, P. A., & Bunt, L. (2009). Incidence of anterior cruciate ligament injury and other knee ligament injuries: A national population-based study. *Journal of Science and Medicine in Sport, 12*(6), 622–627. https://doi.org/10.1016/j.jsams.2008.07.005
55. Snoeker, B., Turkiewicz, A., Magnusson, K., Frobell, R., Yu, D., Peat, G., et al. (2019). Risk of knee osteoarthritis after different types of knee injuries in young adults: A population-based cohort study. *British Journal of Sports Medicine*. https://doi.org/10.1136/bjsports-2019-100959
56. Olson, S. A., & Guilak, F. (2015). *Post-traumatic arthritis: Pathogenesis, diagnosis and management*. New York, NY: Springer.
57. Brown, T. D., Johnston, R. C., Saltzman, C. L., Marsh, J. L., & Buckwalter, J. A. (2006). Posttraumatic osteoarthritis: A first estimate of incidence, prevalence, and burden of disease. *Journal of Orthopaedic Trauma, 20*, 739–744.
58. Thelin, N., Holmberg, S., & Thelin, A. (2006). Knee injuries account for the sports-related increased risk of knee osteoarthritis. *Scandinavian Journal of Medicine & Science in Sports, 16*, 329–333.
59. Vigdorchik, J. M., Nepple, J. J., Eftekhary, N., Leunig, M., & Clohisy, J. C. (2017). What is the association of elite sporting activities with the development of hip osteoarthritis? *American Journal of Sports Medicine, 45*, 961–964.
60. Driban, J. B., Hootman, J. M., Sitler, M. R., Harris, K. P., & Cattano, N. M. (2017). Is participation in certain sports associated with knee osteoarthritis? A systematic review. *Journal of Athletic Training, 52*, 497–506.
61. Qin, J., Barbour, K. E., Nevitt, M. C., Helmick, C. G., Hootman, J. M., Murphy, L. B., Cauley, J. A., & Dunlop, D. D. (2018). Objectively measured physical activity and risk of knee osteoarthritis. *Medicine & Science in Sports & Exercise, 50*, 277–283.
62. Abramoff, B., & Caldera, F. E. (2020). Osteoarthritis: Pathology, diagnosis, and treatment options. *Medical Clinics of North America, 104*, 293–311.
63. National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) (n.d.). *Osteoarthritis*. Retrieved May 18, 2026, from https://www.niams.nih.gov/health-topics/osteoarthritis
64. World Health Organization (WHO) Osteoarthritis Fact Sheet (n.d.). *Osteoarthritis*. Retrieved May 18, 2026, from https://www.who.int/news-room/fact-sheets/detail/osteoarthritis

65. Sinusas, K. (2012). Osteoarthritis: Diagnosis and treatment. *American Family Physician, 85*(1), 49–56.
66. Darabian, S., Badii, M., & Wade, S. D. (2024). How to approach the diagnosis of knee osteoarthritis. *British Columbia Medical Journal, 65*(4), 113–117.
67. American Academy of Orthopaedic Surgeons (AAOS) Hip Osteoarthritis Page (n.d.). *Osteoarthritis of the hip*. Retrieved May 18, 2026, from https://orthoinfo.aaos.org/en/diseases--conditions/osteoarthritis-of-the-hip/
68. Mayo Clinic Osteoarthritis Diagnosis and Treatment (n.d.). *Osteoarthritis: Diagnosis and treatment*. Retrieved May 18, 2026, from https://www.mayoclinic.org/diseases-conditions/osteoarthritis/diagnosis-treatment/drc-20351930
69. Richard, M. J., Driban, J. B., & McAlindon, T. E. (2023). Pharmaceutical treatment of osteoarthritis. *Osteoarthritis and Cartilage, 31*(4), 458–466. https://doi.org/10.1016/j.joca.2022.11.005
70. Bannuru, R. R., Osani, M. C., Vaysbrot, E. E., Arden, N. K., Bennell, K., Bierma-Zeinstra, S. M. A., et al. (2019). OARSI guidelines for the non-surgical management of knee, hip, and polyarticular osteoarthritis. *Osteoarthritis and Cartilage, 27*, 1578–1589.
71. Kolasinski, S. L., Neogi, T., Hochberg, M. C., Oatis, C., Guyatt, G., Block, J., et al. (2020). 2019 American College of Rheumatology/Arthritis Foundation guideline for the management of osteoarthritis of the hand, hip, and knee. *Arthritis Care & Research, 72*, 149–162.
72. American Academy of Orthopaedic Surgeons. (2021). *Management of osteoarthritis of the knee (non-arthroplasty): Evidence-based clinical practice guideline.*
73. American Academy of Orthopaedic Surgeons. (2017). *Management of osteoarthritis of the hip: Evidence-based clinical practice guideline.*
74. Kloppenburg, M., Kroon, F. P. B., Blanco, F. J., Doherty, M., Dziedzic, K. S., Greibrokk, E., et al. (2019). 2018 update of the EULAR recommendations for the management of hand osteoarthritis. *Annals of the Rheumatic Diseases, 78*, 16.
75. Bruyère, O., Honvo, G., Veronese, N., Arden, N. K., Branco, J., Curtis, E. M., et al. (2019). An updated algorithm recommendation for the management of knee osteoarthritis from the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). *Seminars in Arthritis and Rheumatism, 49*, 337–350.

Chapter 4

Microangiopathic Hemolytic Anemias

Hatice TERZİ[1]

Introduction

Microangiopathic hemolytic anemias (MAHA) are clinical-pathological syndromes characterized by intravascular hemolysis caused by erythrocyte fragmentation due to microvascular mechanical stress, endothelial dysfunction, and thrombus formation. The presence of schistocytes on peripheral smear is considered a key morphological marker of thrombotic microangiopathy (TMA) (1). Laboratory findings such as elevated lactate dehydrogenase, low haptoglobin, indirect hyperbilirubinemia, and reticulocytosis reflect ongoing hemolysis. Clinically, MAHA is often associated with thrombocytopenia and organ ischemia, making early diagnosis and treatment essential (2). MAHA is currently regarded as the hematological phenotype of the TMA spectrum rather than a distinct disease entity. Although TMAs share a common pathophysiological basis involving endothelial injury and platelet-rich microthrombi, the underlying mechanisms are heterogeneous and include immune-mediated processes, complement dysregulation, genetic abnormalities, malignancy-related endothelial injury, and drug toxicity (2). Therefore, modern hematology practice emphasizes rapid identification of the underlying mechanism.

Historically classified within the “TTP–HUS–DIC” framework, MAHA is now increasingly defined according to molecular mechanisms. The discovery of severe ADAMTS13 deficiency in thrombotic thrombocytopenic purpura (TTP) and complement dysregulation in certain forms of hemolytic uremic syndrome has led to a mechanism-based classification of TMA syndromes (1–3). Current international guidelines support an ADAMTS13-guided diagnostic and therapeutic approach in TTP, while recent advances, such as recombinant ADAMTS13, further reinforce targeted treatment strategies (3,4). Despite these developments, MAHA remains a hematologic emergency because delayed recognition may rapidly lead to irreversible organ damage and increased mortality. Accordingly, prompt recognition of the MAHA phenotype and rapid

[1] Assoc. Prof, MD

clarification of the underlying biological mechanism remain central to effective management (5).

2. Definition and Basic Hematological Characteristics

Microangiopathic hemolytic anemia (MAHA) refers to intravascular hemolysis caused by mechanical fragmentation of erythrocytes within the microcirculation. Clinically, it is characterized by hemolytic anemia, thrombocytopenia, and erythrocyte fragmentation in the peripheral smear. MAHA is generally considered the hematological manifestation of thrombotic microangiopathy (TMA), in which endothelial injury and platelet-rich microvascular thrombi constitute the underlying pathological basis (2). The hallmark biological feature of MAHA is intravascular hemolysis resulting from erythrocyte deformation and fragmentation while passing through partially obstructed or damaged microvessels. These fragmented erythrocytes, defined as schistocytes, are among the most important morphological markers of TMA. The International Council for Standardization in Hematology (ICSH) has emphasized the diagnostic importance of schistocyte identification and quantification in TMA syndromes (5). Laboratory findings typically include elevated lactate dehydrogenase (LDH), low or undetectable haptoglobin, indirect hyperbilirubinemia, and reticulocytosis, reflecting active intravascular hemolysis and compensatory erythropoiesis (6). Thrombocytopenia, resulting from platelet consumption during microvascular thrombus formation, is another characteristic feature and is particularly prominent in primary TMA syndromes such as thrombotic thrombocytopenic purpura (TTP) (2).

The main pathogenic mechanism involves erythrocyte fragmentation caused by high shear stress within a pathological microvascular environment containing fibrin-platelet aggregates, von Willebrand factor multimers, or endothelial injury. Inflammatory cytokines, complement activation, and coagulation pathway activation may further aggravate endothelial damage and hemolysis (6,7).

Although findings such as schistocytosis, hemolysis biomarkers, and thrombocytopenia are characteristic of MAHA, they are insufficient alone for etiological diagnosis. Clinical evaluation should integrate organ involvement, coagulation parameters, and molecular biomarkers such as ADAMTS13 activity and complement analyses. Therefore, MAHA should be regarded not merely as a laboratory abnormality but as a clinical syndrome requiring rapid identification of the underlying pathobiological mechanism.

3. Pathophysiology: Common Pathway

The pathophysiology underlying microangiopathic hemolytic anemias (MAHA) reflects a process of microvascular damage that progresses through a common final pathway despite different etiological triggers. At the center of this common pathological basis lies endothelial activation or damage, the formation of platelet-rich microthrombi, and the resulting microcirculatory flow disturbance. As erythrocytes pass through this pathological microvascular environment, they are exposed to increased mechanical stress and abnormal shear forces; consequently, membrane integrity is compromised and characteristic erythrocyte fragmentation occurs. Therefore, MAHA is the hematological end product of thrombotic or inflammatory processes developing at the microvascular level (2,7). The modern molecular approach demonstrates that MAHA pathogenesis largely begins through three main biological mechanisms: endothelial damage, ADAMTS13 deficiency, and complement-mediated endothelial dysfunction. Although each has different initial triggers, the ultimate common pathway is microthrombosis and microangiopathic hemolysis (2).

3.1 Endothelial Damage and Microvascular Activation

The endothelium is a dynamic organ that plays a central role in maintaining vascular homeostasis. It preserves physiological microcirculation through functions such as its antithrombotic surface properties, vasoregulation, and control of the inflammatory response. Disruption of endothelial integrity triggers a procoagulant phenotype, thereby promoting platelet adhesion and microvascular thrombus formation (2,8). Endothelial damage can develop in many clinical conditions:

- Systemic infections and sepsis
- Hyperinflammatory conditions accompanied by cytokine storms
- Drug toxicity (especially chemotherapy drugs and anti-VEGF agents)
- Malignancy-related endothelial dysfunction
- Severe immune activation conditions

One of the early consequences of endothelial activation is the release of high molecular weight von Willebrand factor (vWF) multimers from Weibel-Palade bodies. Increased vWF levels enhance platelet adhesion and aggregation, leading to the formation of platelet-rich thrombi in the microcirculation. This process causes narrowing of the vascular lumen, increased local shear forces, and mechanical fragmentation of erythrocytes. Thus, microangiopathic hemolysis develops, as observed in secondary forms of MAHA (9).

3.2 ADAMTS13 Deficiency and vWF-Mediated Microthrombosis

One of the best-defined molecular mechanisms of MAHA is ADAMTS13 activity deficiency. ADAMTS13 is a key metalloprotease that controls microvascular platelet aggregation by proteolytically cleaving circulating ultra-large vWF (UL-vWF) multimers. A significant reduction or loss of this enzyme's function leads to uncontrolled microthrombosis (9).

In the absence of ADAMTS13:

- UL-vWF multimers accumulate in circulation
- Platelet adhesion increases significantly
- Platelet-rich thrombi develop in capillaries and arterioles.
- Microcirculatory obstruction occurs

In this pathological environment, erythrocytes undergo fragmentation as they pass through narrowed microvessels, resulting in microangiopathic hemolytic anemia. This mechanism is particularly central to the pathogenesis of thrombotic thrombocytopenic purpura (TTP), and severe ADAMTS13 deficiency (<10%) is currently accepted as the molecular defining criterion for TTP (2). Current ISTH guidelines emphasize the central role of the ADAMTS13-based approach in the diagnosis and treatment of TTP and demonstrate that early treatment significantly reduces mortality (5).

3.3 Complement Activation and Endothelial Dysfunction

The third mechanism gaining increasing importance in the pathophysiology of MAHA is the uncontrolled activation of the complement system. In particular, genetic or acquired defects in proteins involved in the regulation of the alternative complement pathway lead to persistent complement activation and endothelial cell damage (10).

Complement-mediated microvascular damage:

- Alternative pathway hyperactivation develops
- C5b-9 membrane attack complex damages the endothelium
- The endothelium transforms into a procoagulant phenotype
- Microthrombus formation is triggered

This mechanism is particularly prominent in the pathogenesis of atypical hemolytic uremic syndrome (aHUS). Complement-mediated endothelial damage not only triggers thrombosis; it also contributes to the development of the MAHA phenotype by exacerbating inflammation, vascular permeability, and microcirculatory dysfunction. With a better understanding of complement

biology, complement-mediated TMA is now defined as a distinct biological group in modern classifications, and targeted complement inhibitors have become a fundamental approach in treatment (10).

3.4 Common Final Pathway: Microthrombosis and Erythrocyte Fragmentation

Although the initial mechanism may be endothelial damage, ADAMTS13 deficiency, or complement dysregulation, the ultimate pathological process is similar in all forms of MAHA:

- Endothelial activation or damage
- Platelet adhesion and microthrombus formation
- Narrowing of the microvascular lumen
- Increased shear stress
- Erythrocyte deformation and fragmentation
- Intravascular hemolysis and organ ischemia

This common pathway explains the emergence of MAHA with clinically similar hematological findings. However, since identifying the underlying pathobiological mechanism completely changes the treatment strategy, mechanism-based classification is of critical importance in the modern approach to hematology (2).

4. MAHA's Modern Classification

Microangiopathic hemolytic anemias (MAHA) exhibit significant heterogeneity in terms of underlying pathobiological mechanisms, despite presenting with a clinically and laboratory-based similar phenotype. In the current approach, MAHA is evaluated within a spectrum of thrombotic microangiopathy (TMA) based on molecular mechanisms, moving away from the classical clinical classification. The modern classification addresses TMA in two main groups: primary (intrinsic mechanism dominant) and secondary (resulting from other disease processes) (4).

A. Primary Thrombotic Microangiopathies

4.1 Immune Thrombotic Thrombocytopenic Purpura (iTTP)

Immune TTP is a life-threatening acute thrombotic microangiopathy characterized by severe ADAMTS13 deficiency (<10% activity) and the development of autoantibodies against it. The loss of ADAMTS13 activity results in the accumulation of ultra-large von Willebrand factor multimers in the

circulation, leading to uncontrolled platelet aggregation in the microcirculation. The result is widespread microthrombosis, organ ischemia, and marked microangiopathic hemolysis (4).

Basic biological characteristics:

- ADAMTS13 activity <10%
- Anti-ADAMTS13 autoantibodies
- Severe thrombocytopenia + MAHA
- Frequent neurological involvement

In current management, iTTP is considered an absolute hematologic emergency. The 2025 ISTH update supports the following combination approach while maintaining the main points of the 2020 recommendations: Therapeutic plasma exchange (TPE), corticosteroids, Rituximab, Caplacizumab (addition in the early stages is recommended). Although recent studies have shown that caplacizumab-based regimens may reduce the need for TPE, current guidelines do not recommend changes to the standard treatment algorithm (4). If iTTP is suspected, the ADAMTS13 result should not be awaited. Clinical suspicion is sufficient to initiate treatment; delay increases mortality.

4.2 Congenital Thrombotic Thrombocytopenic Purpura (cTTP)

Congenital TTP is a rare enzyme deficiency caused by inherited mutations in the ADAMTS13 gene. Clinical onset may occur during childhood or may appear in adulthood following infection, pregnancy, or inflammatory stress.

Key features:

- Genetic ADAMTS13 deficiency
- No autoantibodies
- Episodic microthrombotic attacks

The most significant change in the 2025 ISTH update is the strong recommendation for recombinant ADAMTS13 replacement therapy for cTTP. This approach has the potential to replace the chronic plasma infusion strategy that has been used for many years and may provide meaningful improvement in quality of life (4).

4.3 Complement-mediated hemolytic uremic syndrome (aHUS)

Complement-mediated TMA is characterized by endothelial damage resulting from uncontrolled activation of the alternative complement pathway. Genetic

complement regulation defects or acquired disorders may play a role in pathogenesis.

Pathobiological characteristics:

- Alternative complement pathway hyperactivation
- Endothelial damage and microthrombosis
- ADAMTS13 activity is usually normal

Clinical feature:

- Renal involvement is predominant
- Neurological findings may be less pronounced than in iTTP

Treatment:

- C5 inhibitors (eculizumab, ravulizumab)
- Early treatment is the most important factor determining long-term renal prognosis

A better understanding of complement biology has led to the identification of aHUS as a distinct biological group and dramatically improved prognosis with targeted therapies.

B. Secondary Thrombotic Microangiopathies

In secondary TMA, microvascular damage develops as a result of another systemic process. The mechanism is often endothelial activation and secondary stimulation of the coagulation system.

4.4 Disseminated Intravascular Coagulation (DIC)

Disseminated intravascular coagulation (DIC) is a widespread microvascular injury syndrome characterized by fibrin-rich microthrombus formation and consumption coagulopathy resulting from systemic coagulation activation. It usually develops secondary to underlying serious clinical conditions such as sepsis, malignancy, trauma, or obstetric complications. The fundamental mechanism in its pathogenesis is excessive thrombin production triggered by inflammation and widespread fibrin formation (11).

In DIC, microthrombosis is characterized by fibrin-rich thrombi rather than platelet-rich thrombi; in this respect, it differs from thrombotic thrombocytopenic purpura (TTP) associated with ADAMTS13 deficiency. Microvascular fibrin accumulation impairs organ perfusion, while the consumption of coagulation factors and platelets leads to a tendency to bleed.

Distinctive laboratory findings:

- PT and aPTT prolongation
- Decreased fibrinogen levels
- Elevated D-dimer
- Thrombocytopenia of varying degrees

The diagnosis is made based on a series of laboratory tests and clinical context rather than a single test. The current approach defines DIC as a dynamic process and emphasizes that early diagnosis is important for prognosis (12).

The most important point from a clinical perspective is the differential diagnosis from TTP: While coagulation tests are markedly impaired in DIC, they are often normal in TTP; this distinction directly determines the treatment approach.

4.5 Malignancy-Related TMA

Cancer-associated thrombotic microangiopathy (TMA) is a secondary cause of MAHA associated with poor prognosis, particularly seen in advanced solid tumors and metastatic adenocarcinomas. The pathogenesis is multifactorial, with tumor cells causing endothelial damage, proinflammatory cytokine release, and activation of the coagulation system playing key roles. Consequently, microvascular thrombus formation and microangiopathic hemolysis occur (13).

ADAMTS13 activity is generally not severely reduced in malignancy-associated TMA; this is an important biological feature that distinguishes the condition from immune TTP. Furthermore, bone marrow infiltration, particularly in metastatic adenocarcinomas, may contribute to both mechanical microvascular damage and hematopoietic dysfunction (13).

Clinical and hematological features:

- Association with advanced or metastatic malignancy
- Bone marrow infiltration may be present
- Severe MAHA and thrombocytopenia
- ADAMTS13 levels are usually normal or mildly decreased
- Limited response to plasma exchange

The prognostic significance of malignancy-associated TMA is substantial; in most cases, it is considered an indicator of aggressive tumor biology, and survival is often limited. Therefore, the cornerstone of treatment is effective control of the underlying malignancy rather than TTP-like immune therapies (13).

4.6 Drug-Related TMA

Drug-induced thrombotic microangiopathy (DITMA) is a secondary form of MAHA that develops as a result of various pharmacological agents causing microvascular thrombosis through endothelial damage, immune-mediated reactions, or dose-dependent toxicity. The pathogenesis is generally explained through two main mechanisms: (i) immune-mediated acute reactions and (ii) cumulative dose-related direct endothelial toxicity. This mechanistic heterogeneity leads to a variable clinical phenotype (14).

The most commonly associated agents are:

- Gemcitabine (the best-defined cause of chemotherapy-associated TMA)
- Calcineurin inhibitors (tacrolimus, cyclosporine)
- Anti-VEGF agents (bevacizumab and similar anti-angiogenic therapies)

The clinical presentation of TMA associated with chemotherapy or targeted therapy frequently manifests as hypertension, progressive renal dysfunction, and microangiopathic hemolysis. Particularly in gemcitabine-associated TMA, the presentation often develops subacutely, with renal involvement being predominant. Current data suggest that chemotherapy-related TMA may carry a more severe renal prognosis compared to primary TMA (15).

The most important step in diagnosing drug-induced TMA is to rule out alternative causes of TMA (particularly TTP and complement-mediated TMA). ADAMTS13 activity is usually normal, and the response to plasma exchange is limited in most cases. Therefore, the cornerstone of treatment is early discontinuation of the responsible drug.

Treatment approach:

- Immediate discontinuation of the suspected drug (first step)
- Supportive treatment and organ function monitoring
- Complement inhibition (especially eculizumab) may be beneficial in some cases of severe or refractory disease

Although there are positive case series in the literature regarding the use of eculizumab, current data are insufficient to establish a definitive standard; therefore, treatment should be individualized (15).

4.7 Pregnancy-Related TMA

Pregnancy and the postpartum period are high-risk periods for the development of thrombotic microangiopathy (TMA) due to physiological

changes in hemostasis, complement activity, and endothelial function. MAHA presentations during this period form a heterogeneous spectrum and may include both pregnancy-specific syndromes and primary TMA triggered by pregnancy (16).

Primary clinical presentations:

- HELLP syndrome / severe preeclampsia-associated TMA
- Pregnancy-triggered thrombotic thrombocytopenic purpura (TTP)
- Postpartum complement-mediated TMA (aHUS)

Although HELLP syndrome is the most common form of pregnancy-related TMA, TTP, and aHUS carry a higher risk of maternal mortality and kidney damage. Since these conditions can overlap significantly in clinical and laboratory findings, differential diagnosis is often difficult (17).

Clinically critical point: Postpartum persistent MAHA + renal failure

The most important clinical decision point in pregnancy-related TMA concerns the course of hematological and renal parameters after delivery. While improvement is generally expected within the first 48–72 hours after delivery in HELLP syndrome, TTP or especially postpartum aHUS should be considered in the presence of persistent MAHA and progressive acute kidney injury (16). This distinction is critically important because treatment strategies are entirely different:

- **TTP:** characterized by severe ADAMTS13 deficiency; requires urgent plasma exchange and immunosuppression.
- **aHUS:** complement-mediated endothelial damage is predominant; early C5 inhibitor therapy is the main factor determining renal prognosis (18).

Pathobiological and clinical clues:

- TTP can occur in any trimester of pregnancy and is characterized by severe thrombocytopenia.
- The vast majority of aHUS cases occur in the postpartum period and are dominated by renal involvement (19).
- HELLP syndrome usually develops in the late stages of pregnancy and tends to resolve spontaneously after delivery.

Systematic analyses conducted in recent years have shown that pregnancy-related aHUS carries a high risk of requiring dialysis and chronic kidney disease; therefore, early diagnosis and targeted treatment are considered critical (20).

4.8 Clinical Perspective: The Importance of Modern Classification

In current hematology practice, the classification of microangiopathic hemolytic anemias (MAHA) is not merely an academic approach but a critical clinical decision that directly determines treatment selection and patient prognosis. The approach that previously progressed through phenotypic classification (TTP–HUS–DIC) has now been replaced by a model based on the underlying pathobiological mechanism. This change, along with the development of targeted therapies, has significantly improved clinical outcomes (21).

In the modern approach, the first and most critical step is the rapid exclusion or confirmation of TTP associated with severe ADAMTS13 deficiency, as delayed diagnosis increases mortality. Current international guidelines recommend initiating plasma exchange and immunotherapy without waiting for ADAMTS13 results in cases of clinical suspicion. The 2025 ISTH update emphasized that the combination of plasma exchange, corticosteroids, rituximab, and caplacizumab remains the standard approach in the immunotherapy of TTP (4).

In contrast, the pathogenesis of complement-mediated TMA is primarily the uncontrolled activation of the alternative complement pathway. Since delayed diagnosis in these patients can lead to irreversible kidney damage, current algorithms recommend evaluating the complement-mediated mechanism early on and initiating treatment with C5 inhibitors in appropriate cases. This mechanism-based approach enables individualized treatment by reducing the use of unnecessary immunosuppression or plasma exchange (21).

In secondary TMA (malignancy, drug-related TMA, infection, DIC, etc.), the basis of treatment is controlling the underlying cause. Since the effect of primary TMA treatments may be limited in this group of patients, correct classification is critical to avoid unnecessary or potentially harmful treatments. Current multidisciplinary approaches emphasize the integration of hematology, nephrology, intensive care, and genetics expertise in TMA management (21).

In summary, modern classification is based on three main clinical axes that directly determine treatment decisions:

- **ADAMTS13 deficiency** → plasma exchange + immunosuppressive therapy
- **Complement-mediated process** → targeted therapy with C5 inhibitors
- **Secondary TMA** → control of underlying disease and supportive therapy

For this reason, MAHA assessment in current hematology practice is no longer based solely on phenotype but is now approached as a mechanism-based decision algorithm. Rapid diagnostic approach and accurate biological classification are

considered the most important clinical strategies for reducing mortality and preventing organ damage (21).

5. Diagnostic Approach

5.1 Initial Clinical Assessment

The diagnostic approach relies on rapid recognition of the MAHA phenotype and early assessment of the likelihood of TTP. The following questions should be systematically addressed in the initial evaluation:

- Are there any laboratory findings supporting hemolysis?

(Elevated LDH, low haptoglobin, increased indirect bilirubin, reticulocytosis)

- Is thrombocytopenia present?
- Are schistocytes seen in the peripheral smear?
- Are coagulation tests (PT/aPTT) normal?

→ This is critical for the differential diagnosis of DIC.

- What is the creatinine level?

→ The degree of renal involvement may be indicative of distinguishing TTP from aHUS.

- Are there any neurological findings or organ ischemia?

This early assessment is necessary to determine whether the patient is in the high-risk TTP group (22).

5.2 Risk Scores and Pre-test Probability

ADAMTS13 test results are available within hours or within days at most centers, making risk scores an important part of clinical decision-making. The two most commonly used clinical prediction tools today are:

PLASMIC Score

The PLASMIC score was developed to predict severe ADAMTS13 deficiency (<10%). The score is based on seven parameters: platelet count, hemolysis findings, creatinine level, INR, MCV, and clinical characteristics. A high score (6–7) indicates a high probability of severe ADAMTS13 deficiency. Various validation studies have reported that the PLASMIC score is highly sensitive for predicting severe ADAMTS13 deficiency (23).

French Score

The French score is a simpler clinical model and specifically assesses the likelihood of TTP based on the combination of platelet level and creatinine. Both the PLASMIC and French scores are tools to assist in the clinical decision-

making process; however, they are not a substitute for diagnosis. Although ISTH guidelines do not mandate a specific risk score, they acknowledge that these tools are useful in assessing the likelihood of pretest (22).

5.3 ADAMTS13 Test and Early Treatment Decision

The key biomarker for the diagnostic algorithm is ADAMTS13 activity. However, international guidelines emphasize an important principle:

The ADAMTS13 result should not delay treatment. The ISTH diagnostic guidelines recommend the following approach in patients with a high probability of TTP:

1. Blood sample collection for ADAMTS13
2. Initiation of plasma exchange (TPE) and corticosteroids without waiting for results
3. Consideration of early caplazumab in clinically appropriate cases

This approach is aimed at reducing early mortality and represents current standard practice (22).

5.4 Diagnostic Priority

The primary purpose of the diagnostic algorithm in a patient presenting with MAHA is as follows:

- First, rule out life-threatening but treatable TTP
- Then, evaluate complement-mediated TMA or secondary causes

Therefore, in the modern approach, diagnosis relies not only on laboratory confirmation but also on the ability to make early clinical decisions. Risk scores and ADAMTS13 measurement support the diagnosis; however, clinical suspicion remains decisive in initiating treatment.

6. Peripheral Blast: The Heart of the Diagnosis

Peripheral blood smear remains one of the most critical diagnostic tools in the diagnosis of microangiopathic hemolytic anemias (MAHA). Particularly in the thrombotic microangiopathy (TMA) spectrum, the morphological demonstration of erythrocyte fragmentation is a key finding that guides diagnosis. Current international standards recognize the presence of schistocytes as one of the most important morphological markers for TMA (5).

Schistosome and Its Diagnostic Significance

Schistocytes are fragments of erythrocytes formed as a result of mechanical damage in the microvascular circulation and are a direct morphological indicator

of microangiopathic hemolysis. According to the updated recommendations by the International Council for Standardization in Haematology (ICSH):

- The schistosome rate in healthy adults is generally ≤1%.
- A schistosome rate >1% should be considered suspicious for TMA.

For this reason, the quantitative assessment of schistosomes is considered not only as a qualitative observation but also as a diagnostic parameter (5). The main types of erythrocyte fragmentation seen in microangiopathic processes:

- Helmet cells
- Triangle cells
- Fragmented RBC / keratinocyte-like erythrocytes

The presence of these cells together, especially when accompanied by findings of thrombocytopenia and hemolysis, provides strong morphological support for TMA. ICSH recommendations suggest that these cell types be evaluated in a standard manner in schistocyte counts (5).

Diagnostic limitations and clinical correlation

Although the presence of schistocytes is quite important for TMA, it is not specific. Erythrocyte fragmentation can also be observed in the following conditions:

- Sepsis
- Mechanical heart valves
- Severe burns
- Disseminated intravascular coagulation (DIC)
- Severe hypertension

Therefore, peripheral smear findings must be evaluated in conjunction with the clinical context and laboratory parameters. Recent studies emphasize the importance of clinical-morphological correlation, particularly due to inter-observer variability (24).

7. TTP, aHUS, and DIC: Clinical-Pathobiological Comparison

Rapid differentiation between thrombotic thrombocytopenic purpura (TTP), complement-mediated TMA (aHUS), and disseminated intravascular coagulation (DIC) in patients presenting with microangiopathic hemolytic anemia (MAHA) is critical due to the completely different treatment approaches. The current approach emphasizes differentiation based on biological mechanisms rather than phenotype (22). The distinguishing features between TTP, aHUS, and DIC in

clinical practice are summarized in Table 1 to reflect a mechanism-based approach.

Table 1. Major Thrombotic Microangiopathy Phenotypes: Comparative Clinical and Pathobiologic Features

Feature	TTP	aHUS	DIC
Basic mechanism	ADAMTS13 deficiency → platelet-vWF microthrombosis	Activation of the alternative complement pathway → endothelial damage	Systemic activation of the coagulation cascade → fibrin clots
ADAMTS13 activity	<10% (diagnostic)	Usually normal	Usually normal Normal / slightly decreased
Kidney involvement	Mild to moderate	Generally heavy, prominent	Variable
Neurological finding	Frequent	Variable	Less
Coagulation tests	Usually normal	Usually normal	PTT/aPTT prolonged, fibrinogen low
Thrombus type	Platelet-rich	Endothelial/complement-associated	Fibrin-rich
Treatment approach	TPE + immunosuppression (+ caplacizumab)	C5 inhibitors	Management of the underlying cause
Urgency of treatment	Very high (within hours)	High (early renal protection)	Depending on the underlying condition

8. Treatment Approaches

In microangiopathic hemolytic anemias (MAHA), the treatment strategy is determined by the underlying pathobiological mechanism rather than the clinical phenotype. Just as early diagnosis is crucial, the prompt initiation of appropriate treatment is a key factor in determining the prognosis. In current hematology practice, the TMA spectrum is divided into three main biological categories based on a mechanism-driven approach: ADAMTS13-mediated TTP, complement-mediated TMA, and TMA syndromes secondary to underlying systemic processes (21).

8.1 TTP Treatment

Immune thrombotic thrombocytopenic purpura (iTTP) is considered a hematologic emergency, and delays in treatment significantly increase mortality. The standard approach in current international guidelines includes the following combination:

- Daily therapeutic plasma exchange (TPE)
- Corticosteroid therapy
- Rituximab (recommended for early-stage treatment)
- Caplacizumab (anti-vWF nanobody)

Early use of caplacizumab accelerates platelet recovery and shortens the duration of TPE (25). In recent years, real-world data have shown that the duration of caplacizumab treatment can be individualized based on ADAMTS13 activity levels. Discontinuing treatment earlier once ADAMTS13 activity begins to improve may reduce costs and the treatment burden, whereas extending treatment if low activity persists may reduce the risk of relapse (26). In cases of suspected TTP, initiating TPE without waiting for ADAMTS13 results is recommended; laboratory confirmation should not delay treatment.

8.2 Complement-Mediated TMA (aHUS/CM-TMA)

The cornerstone of treatment for complement-mediated TMA is the blockade of the alternative complement pathway. Clinically, complement-mediated TMA should be considered particularly in cases where:

Creatinine levels are elevated + MAHA + TTP have been ruled out.

Treatment principle:

- C5 inhibitors (eculizumab or ravulizumab)
- Early treatment → better renal outcomes

Current literature indicates that delays in treatment increase the risk of chronic kidney disease and that early complement inhibition improves renal survival (27).

8.3 Supportive Care

In the management of MAHA, appropriate supportive care is just as critical as specific treatments.

Red blood cell transfusion: It is used in cases of symptomatic anemia or a significant decrease in hemoglobin levels. As hemolysis is brought under control, the need decreases.

Platelet transfusion: TTP should generally be avoided. It is recommended only in cases of life-threatening active bleeding. Because platelet transfusions can increase the risk of microthrombosis and lead to clinical deterioration (28).

Antithrombotic therapy: It should generally be avoided when the platelet count is $<50 \times 10^9$/L. Decisions should be made on an individual basis based on the risk of thrombosis following platelet aggregation.

9. Prognosis and Long-Term Follow-Up

While immune thrombotic thrombocytopenic purpura (iTTP) has historically been associated with high mortality, the prognosis has improved dramatically with the introduction of therapeutic plasma exchange (TPE), immunosuppression, and targeted therapies such as caplacizumab. With modern treatment approaches, 30-day survival rates have risen above 90%, and mortality has dropped from the previously reported levels of 80–90% to less than 10% (29). However, despite the success of acute-phase treatment, iTTP is no longer considered merely an acute illness but rather a condition carrying a risk of chronic relapse that requires long-term follow-up.

9.1 Long-Term Outcomes and Late Complications

Although survival rates have improved following clinical remission, persistent morbidity has been reported in a significant proportion of patients during long-term follow-up. Neuropsychological complications such as cognitive dysfunction, depression and anxiety, chronic fatigue, and a decline in quality of life are being increasingly recognized. The likely mechanism underlying these findings is associated with microvascular cerebral damage and subclinical ischemic events that occur during an acute attack (29). For this reason, the modern approach in hematology aims not only for hematologic remission but also for long-term functional well-being.

9.2 Risk of relapse

Relapse remains a significant clinical issue in iTTP. Recent series report that approximately 15–20% of patients experience at least one clinical relapse (29). The primary factors increasing the risk of relapse have been identified as persistent low ADAMTS13 activity, the presence of anti-ADAMTS13 antibodies, and inadequate biochemical remission. For this reason, the concept of biochemical remission (normalization of ADAMTS13 levels) has come to the forefront alongside clinical remission (30).

9.3 ADAMTS13 Monitoring and Personalized Tracking

In recent years, the use of ADAMTS13 activity in long-term follow-up has become standard practice. Studies show that low ADAMTS13 activity during remission significantly increases the risk of relapse (31). In the modern approach, regular ADAMTS13 monitoring, close follow-up when activity levels are ≤20%, and preemptive rituximab when necessary are strategies aimed at reducing the risk of relapse. Recent studies suggest that monitoring ADAMTS13 conformation and biomarkers may aid in the early detection of subclinical disease activity (32).

9.4 Principles of Long-Term Monitoring

According to current recommendations, for patients in remission from iTTP:

- Regular clinical evaluation
- Periodic monitoring of ADAMTS13 activity
- Neurocognitive and psychosocial assessment
- An individualized immunomodulation approach based on relapse risk

is adopted. This allows for early intervention based on biomarker changes before a clinical relapse occurs.

References

1. Bommer M, Bloehdorn J. Microangiopathic Anemia. Transfus Med Hemother. 2025 Feb 13;52(4):259-270. doi: 10.1159/000544724. PMID: 40809448; PMCID: PMC12342704.
2. Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood. 2017 May 25;129(21):2836-2846. doi: 10.1182/blood-2016-10-709857.
3. Shaw, R. J., Abrams, S. T., Badu, S., Toh, C.-H., & Dutt, T. (2024). The Highs and Lows of ADAMTS13 Activity. Journal of Clinical Medicine, 13(17), 5152. https://doi.org/10.3390/jcm13175152
4. Zheng XL, Al-Housni Z, Cataland SR, Coppo P, Geldziler B, Germini F, Iorio A, Keepanasseril A, Masias C, Matsumoto M, McCrae KR, McIntyre J, Mustafa RA, Peyvandi F, Russell L, Tarawneh R, Vesely SK; International Society on Thrombosis and Haemostasis. 2025 focused update of the 2020 ISTH guidelines for management of thrombotic thrombocytopenic purpura. J Thromb Haemost. 2025 Nov;23(11):3711-3732. doi: 10.1016/j.jtha.2025.06.002.
5. Zini G, d'Onofrio G, Briggs C, Erber W, Jou JM, Lee SH, McFadden S, Vives-Corrons JL, Yutaka N, Lesesve JF; International Council for Standardization in Haematology (ICSH). ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes. Int J Lab Hematol. 2012 Apr;34(2):107-16. doi: 10.1111/j.1751-553X.2011.01380.x.
6. Barcellini W, Fattizzo B. Clinical Applications of Hemolytic Markers in the Differential Diagnosis and Management of Hemolytic Anemia. Dis Markers. 2015;2015:635670. doi: 10.1155/2015/635670. Epub 2015 Dec 27. PMID: 26819490; PMCID: PMC4706896.
7. Pande A, Kumar A, Krishnani H, Acharya S, Shukla S. Recent Advances in the Management of Microangiopathic Hemolytic Anemias (MAHA): A Narrative Review. Cureus. 2023 Oct 17;15(10):e47196. doi: 10.7759/cureus.47196. PMID: 38021690; PMCID: PMC10653637.
8. Obeagu EI. Pathogenic cytokines in thrombotic microangiopathies: molecular insights and therapeutic targets. Mol Med. 2025 Oct 24;31(1):316. doi: 10.1186/s10020-025-01331-1. PMID: 41136902; PMCID: PMC12551244.
9. Zheng XL. ADAMTS13 and von Willebrand factor in thrombotic thrombocytopenic purpura. Annu Rev Med. 2015;66:211-25. doi:

10.1146/annurev-med-061813-013241. PMID: 25587650; PMCID: PMC4599565.
10. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009 Oct 22;361(17):1676-87. doi: 10.1056/NEJMra0902814. PMID: 19846853.
11. Gong, F., Zheng, X., Zhao, S., Liu, H., Chen, E., Xie, R., ... & Chen, Y. (2025). Disseminated intravascular coagulation: cause, molecular mechanism, diagnosis, and therapy. MedComm, 6(2), e70058.
12. Iba T, Maier CL, Scarlatescu E, Levy JH. Introducing the New Definition and Diagnostic Criteria of Disseminated Intravascular Coagulation Released by the International Society on Thrombosis and Haemostasis in 2025. Semin Thromb Hemost. 2026 Feb;52(1):10-17. doi: 10.1055/a-2675-6068. Epub 2025 Aug 19. PMID: 40829630.
13. Posado-Domínguez L, Chamorro AJ, Del Barco-Morillo E, Martín-Galache M, Bueno-Sacristán D, Fonseca-Sánchez E, Olivares-Hernández A. Cancer-Associated Thrombotic Microangiopathy: Literature Review and Report of Five Cases. Life (Basel). 2024 Jul 10;14(7):865. doi: 10.3390/life14070865. PMID: 39063619; PMCID: PMC11278215.
14. aAl-Nouri ZL, Reese JA, Terrell DR, Vesely SK, George JN. Drug-induced thrombotic microangiopathy: a systematic review of published reports. Blood. 2015 Jan 22;125(4):616-8. doi: 10.1182/blood-2014-11-611335. Epub 2014 Nov 20. PMID: 25414441; PMCID: PMC4304106.
15. Aklilu AM, Shirali AC. Chemotherapy-Associated Thrombotic Microangiopathy. Kidney360. 2023 Mar 1;4(3):409-422. doi: 10.34067/KID.0000000000000061.
16. Fakhouri F, Scully M, Provôt F, Blasco M, Coppo P, Noris M, Paizis K, Kavanagh D, Pène F, Quezada S, Hertig A, Kissling S, O'Brien P, Delmas Y, Alberio L, Winer N, Veyradier A, Cataland S, Frémeaux-Bacchi V, Loirat C, Remuzzi G, Tsatsaris V. Management of thrombotic microangiopathy in pregnancy and postpartum: report from an international working group. Blood. 2020 Nov 5;136(19):2103-2117. doi: 10.1182/blood.2020005221. PMID: 32808006.
17. Camila Masias, Sumithira Vasu, Spero R. Cataland. None of the above: thrombotic microangiopathy beyond TTP and HUS. Blood (2017) 129 (21): 2857–2863. doi.org/10.1182/blood-2016-11-743104.
18. Che M, Moran SM, Smith RJ, Ren KYM, Smith GN, Shamseddin MK, Avila-Casado C, Garland JS. A case-based narrative review of pregnancy-associated atypical hemolytic uremic syndrome/complement-mediated

thrombotic microangiopathy. Kidney Int. 2024 May;105(5):960-970. doi: 10.1016/j.kint.2023.12.021. Epub 2024 Feb 24.

19. Gupta M, Govindappagari S, Burwick RM. Pregnancy-Associated Atypical Hemolytic Uremic Syndrome: A Systematic Review. Obstet Gynecol. 2020 Jan;135(1):46-58. doi: 10.1097/AOG.0000000000003554. PMID: 31809447; PMCID: PMC6922068.
20. Meena P, Gala R, Das RR, Bhargava V, Saivani Y, Panda S, Mantri A, Agrawaal KK. Kidney and pregnancy outcomes in pregnancy-associated atypical hemolytic uremic syndrome: A systematic review and meta-analysis. Medicine (Baltimore). 2025 Jan 31;104(5):e41403. doi: 10.1097/MD.0000000000041403. PMID: 39889176; PMCID: PMC11789862.
21. Doreille A, Rafat C, Rondeau E, Mesnard L. How I treat thrombotic microangiopathy in the era of rapid genomics. Blood. 2023 Jan 12;141(2):147-155. doi: 10.1182/blood.2022015583. PMID: 36347020.
22. Zheng XL, Vesely SK, Cataland SR, Coppo P, Geldziler B, Iorio A, Matsumoto M, Mustafa RA, Pai M, Rock G, Russell L, Tarawneh R, Valdes J, Peyvandi F. ISTH guidelines for the diagnosis of thrombotic thrombocytopenic purpura. J Thromb Haemost. 2020 Oct;18(10):2486-2495. doi: 10.1111/jth.15006. Epub 2020 Sep 11. Erratum in: J Thromb Haemost. 2021 May;19(5):1381. doi: 10.1111/jth.15304. PMID: 32914582; PMCID: PMC8146131.
23. Li A, Khalighi PR, Wu Q, Garcia DA. External validation of the PLASMIC score: a clinical prediction tool for thrombotic thrombocytopenic purpura diagnosis and treatment. J Thromb Haemost. 2018 Jan;16(1):164-169. doi: 10.1111/jth.13882. Epub 2017 Nov 16. PMID: 29064619; PMCID: PMC5760324.
24. Noutsos T, Laidman AY, Survela L, Arvanitis D, Segalla R, Brown SG, Isbister GK. An evaluation of existing manual blood film schistocyte quantitation guidelines and a new proposed method. Pathology. 2021 Oct;53(6):746-752. doi: 10.1016/j.pathol.2021.01.008. Epub 2021 Apr 15. PMID: 33863504.
25. Dierickx D, Connerotte T, Dallemagne J, De Becker A, Moors I, Snauwaert S, Sonet A, Theunissen K, Breems D, De Voeght A, Jaspers A, Lambert C, Heyrman B, Hautekiet J, Lyna S, Maertens C, Delrieu V. Real-world insights on the management of immune-mediated thrombotic thrombocytopenic purpura with caplacizumab. Blood Vessel Thromb

Hemost. 2024 May 3;1(2):100008. doi: 10.1016/j.bvth.2024.100008. PMID: 40766828; PMCID: PMC12320383.

26. Völker LA, Kaufeld J, Miesbach W, Brähler S, Reinhardt M, Kühne L, Mühlfeld A, Schreiber A, Gaedeke J, Tölle M, Jabs WJ, Özcan F, Markau S, Girndt M, Bauer F, Westhoff TH, Felten H, Hausberg M, Brand M, Gerth J, Bieringer M, Bommer M, Zschiedrich S, Schneider J, Elitok S, Gawlik A, Gäckler A, Kribben A, Schwenger V, Schoenermarck U, Roeder M, Radermacher J, Bramstedt J, Morgner A, Herbst R, Harth A, Potthoff SA, von Auer C, Wendt R, Christ H, Brinkkoetter PT, Menne J. ADAMTS13 and VWF activities guide individualized caplacizumab treatment in patients with aTTP. Blood Adv. 2020 Jul 14;4(13):3093-3101. doi: 10.1182/bloodadvances.2020001987. PMID: 32634237; PMCID: PMC7362349.
27. Angelucci, E., Artoni, A., Fianchi, L., Dovizio, M., Iacolare, B., Saragoni, S., & Esposti, L. D. (2024). Real-world data analysis of patients affected by immune-mediated thrombotic thrombocytopenic purpura in Italy. Journal of Clinical Medicine, 13(5), 1342.).
28. Riviere E, Saint-Léger M, James C, Delmas Y, Clouzeau B, Bui N, Vital A, Coppo P, Gruson D, Boyer A. Platelet transfusion and catheter insertion for plasma exchange in patients with thrombotic thrombocytopenic purpura and a low platelet count. Transfusion. 2015 Jul;55(7):1798-802. doi: 10.1111/trf.13041. Epub 2015 Feb 20. PMID: 25702952.
29. Pishko AM, Li A, Cuker A. Immune Thrombotic Thrombocytopenic Purpura: A Review. JAMA. 2025 Aug 12;334(6):517-529. doi: 10.1001/jama.2025.3807. PMID: 40388146.
30. Sui J, Cao W, Halkidis K, Abdelgawwad MS, Kocher NK, Guillory B, Williams LA, Gangaraju R, Marques MB, Zheng XL. Longitudinal assessments of plasma ADAMTS13 biomarkers predict recurrence of immune thrombotic thrombocytopenic purpura. Blood Adv. 2019 Dec 23;3(24):4177-4186. doi: 10.1182/bloodadvances.2019000939. PMID: 31856267; PMCID: PMC6929391.
31. Schieppati F, Russo L, Marchetti M, Barcella L, Cefis M, Gomez-Rosas P, Caldara G, Carpenedo M, D'Adda M, Rambaldi A, Savignano C, Billio A, Bruno Franco M, Toschi V, Falanga A. Low levels of ADAMTS-13 with high anti-ADAMTS-13 antibodies during remission of immune-mediated thrombotic thrombocytopenic purpura highly predict for disease relapse: A multi-institutional study. Am J Hematol. 2020 Aug;95(8):953-959. doi: 10.1002/ajh.25845. Epub 2020 May 21. PMID: 32350923.

32. Prasannan N, Dragunaite B, Subhan M, Thomas M, de Groot R, Singh D, Vanhoorelbeke K, Scully M. Peak ADAMTS13 activity to assess ADAMTS13 conformation and risk of relapse in immune-mediated thrombotic thrombocytopenic purpura. Blood. 2024 Jun 20;143(25):2644-2653. doi: 10.1182/blood.2023023269. PMID: 38502824.

Chapter 5

Next-Generation Metrics In Continuous Glucose Monitoring: Clinical Applications Of Time in Range, Glucose Management Indicator, and Glycemic Variability

İhsan BOYACI [1]

1. Introduction

Glycated hemoglobin (HbA1c) has long been the cornerstone of glycemic assessment in diabetes management and clinical research. Its established association with microvascular complication risk has positioned HbA1c as a surrogate marker of long-term glycemic exposure. (1) However, as an averaged measure reflecting approximately three months of glycemia, HbA1c does not capture short-term glucose fluctuations, hypoglycemia burden, or daily glycemic patterns that are increasingly recognized as clinically relevant. (2)

Continuous glucose monitoring (CGM) enables high-frequency assessment of interstitial glucose concentrations, allowing a dynamic and comprehensive evaluation of glycemic control. Although initially adopted primarily in type 1 diabetes, CGM use has expanded substantially in individuals with type 2 diabetes (T2D) and, more recently, across the spectrum of prediabetes and early dysglycemia. (3,4) Unlike laboratory-based metrics, CGM simultaneously captures glucose exposure, variability, and risk, providing clinically meaningful insights into hypoglycemia, postprandial hyperglycemia, and intraday glucose instability that are not reflected by HbA1c alone. (2)

These advances have driven a paradigm shift toward moving beyond HbA1c in both clinical practice and research. International consensus groups have proposed standardized CGM-derived metrics to complement traditional glycemic markers and to facilitate uniform interpretation of CGM data. (5,6) Among these metrics, time in range (TIR), glucose management indicator (GMI), and measures of glycemic variability such as the coefficient of variation (CV) have emerged as core components of CGM reporting. (1,7) Together, these parameters provide a multidimensional assessment of glycemic control that encompasses not only average glucose exposure but also stability and risk. The ambulatory glucose profile (AGP)

[1]*MD. Istanbul Medipol University Faculty of Medicine, Department of Internal Medicine, Istanbul, Türkiye. E-mail: iboyaci@medipol.edu.tr. ORCID: 0000-0001-7369-0426

further integrates these metrics into a standardized visual framework, supporting structured interpretation and clinical decision-making. (8)

2. Conceptual Framework of CGM-Derived Metrics

CGM-derived metrics capture complementary dimensions of glycemic control, including glucose exposure, stability, and risk (Table 1). Glucose exposure reflects the cumulative burden of hyperglycemia over time and is closely related to metrics such as mean glucose, TIR, and GMI. (1) In contrast, glycemic stability describes the degree of intraday and interday glucose fluctuation, which may predispose individuals to hypoglycemia and wide glucose excursions independent of average glucose levels. (9) Glycemic risk incorporates both hypoglycemic and marked hyperglycemic extremes, emphasizing the clinical consequences of time spent outside the target glucose range. (5)

No single CGM metric is sufficient to fully characterize the complexity of glucose regulation. Analyses of large CGM datasets have demonstrated that CGM metrics cluster into distinct yet interrelated domains associated with hyperglycemia and hypoglycemia risk, highlighting their non-redundant nature. (10) Accordingly, contemporary recommendations emphasize the integrated interpretation of multiple CGM-derived metrics to balance efficacy and safety in glycemic management. (5)

The AGP was developed to operationalize this integrative approach by providing a standardized visual summary of CGM data. AGP condenses extensive glucose datasets into a single, intuitive display that combines measures of central tendency with indices of variability across a representative 24-hour period. (8) By depicting the median glucose curve together with interquartile and percentile ranges, AGP facilitates identification of reproducible glycemic patterns, differentiation of random variability from systematic trends, and temporal localization of dysglycemia. (11)

3. Time in Range

3.1. Definition and Standard Targets

Time in range represents the proportion of time that sensor glucose values remain within a predefined target range during CGM. Complementary metrics, including time above range (TAR) and time below range (TBR), quantify exposure to hyperglycemia and hypoglycemia, respectively, thereby capturing glycemic extremes alongside overall glucose control. Together, these parameters provide a time-based framework that integrates both efficacy and safety of glycemic management. (5)

International consensus recommendations define the standard target glucose range for most nonpregnant adults with diabetes as 3.9–10.0 mmol/L (70–180 mg/dL). Within this framework, a TIR greater than 70% is generally recommended,

corresponding to approximately 16–17 hours per day spent within the target range. (5,6) TAR is stratified into level 1 (>10.0 mmol/L [>180 mg/dL]) and level 2 (>13.9 mmol/L [>250 mg/dL]), while TBR is divided into level 1 (3.0–3.8 mmol/L [54–69 mg/dL]) and level 2 (<3.0 mmol/L [<54 mg/dL]), with particular emphasis on minimizing clinically significant hypoglycemia. (5)

Target ranges should be individualized for specific populations. Less stringent TIR targets may be appropriate for older adults, individuals with long-standing diabetes, advanced comorbidities, or elevated hypoglycemia risk. (5) Conversely, selected individuals with early T2D and low hypoglycemia risk may benefit from higher TIR targets to optimize metabolic outcomes. (7)

At present, standardized TIR targets for prediabetes have not been formally established. However, CGM studies in individuals without diabetes suggest that early deviations in TIR and increases in TAR may precede diagnostic thresholds for diabetes. (12,13)

3.2. Clinical Significance of Time in Range

Time in range provides clinically relevant information that complements traditional measures of average glycemia such as HbA1c by capturing the temporal distribution of glucose values. While HbA1c reflects mean glucose exposure over time, TIR integrates both magnitude and time, offering a more patient-centered and actionable assessment of glycemic control that aligns closely with daily glucose patterns. (2,5)

Evidence indicates that TIR conveys prognostic information beyond its correlation with HbA1c. Individuals with comparable HbA1c levels may exhibit substantially different TIR profiles, reflecting heterogeneity in glucose variability and dysglycemic exposure not captured by average glycemia alone. (2) This discordance supports the clinical utility of TIR in refining risk stratification and guiding individualized therapeutic adjustments. (7)

A growing body of evidence has demonstrated a consistent association between lower TIR and an increased risk of microvascular complications in T2D. Studies using CGM-derived metrics have shown that reduced TIR is associated with higher prevalence and severity of diabetic retinopathy, nephropathy, and neuropathy. (12,14) Importantly, these associations persist after adjustment for HbA1c, suggesting that TIR captures clinically relevant aspects of glycemic exposure not fully reflected by conventional markers. (12)

Beyond microvascular outcomes, TIR has also been linked to macrovascular outcomes and mortality. Observational analyses have shown that reduced TIR is associated with increased all-cause and cardiovascular mortality in individuals with T2D. (15) Although causality cannot be inferred, the consistency of these

associations strengthens the role of TIR as an outcome-related CGM metric. (5,15,16)

3.3. Time in Range in Type 2 Diabetes Mellitus and Prediabetes

In individuals with T2D, TIR has emerged as a clinically relevant metric reflecting glycemic control in close association with long-term clinical outcomes. Evidence from CGM-based observational studies indicates that lower TIR is consistently associated with a higher burden of diabetes-related complications, independent of HbA1c. (12,14)

In addition to microvascular outcomes, accumulating evidence indicates a relationship between TIR and macrovascular risk in T2D. Studies examining time spent within narrower glucose ranges have demonstrated that reduced TIR is associated with increased all-cause and cardiovascular mortality. (15,16)

In individuals with prediabetes and early dysglycemia, CGM-based assessment of TIR provides a complementary approach for identifying early disturbances in glucose regulation not captured by conventional diagnostic criteria. CGM studies in individuals without diabetes have demonstrated that reductions in TIR and concomitant increases in time above range may appear early in the dysglycemic continuum, often preceding the development of overt diabetes. (13)

CGM-derived metrics also reveal marked heterogeneity within prediabetes. Analyses incorporating indices of glycemic variability have shown that individuals classified as having prediabetes may exhibit diverse glucose profiles, ranging from relatively stable patterns to pronounced postprandial dysglycemia. (17) Although standardized TIR targets for prediabetes have not yet been established, these findings support the exploratory use of TIR in early risk assessment.

4. Glucose Management Indicator

Glucose management indicator is a CGM-derived metric intended to estimate laboratory-measured HbA1c based on mean sensor glucose values over the monitoring period. By reflecting contemporaneous glucose exposure captured by CGM, GMI offers a practical reference point for interpreting CGM data within the framework of established HbA1c-based treatment targets. (18)

Although GMI and HbA1c are correlated at the population level, clinically relevant discordance between these metrics is frequently observed in individual patients. Recent studies have demonstrated substantial interindividual variability in the relationship between CGM-derived mean glucose and HbA1c, even among individuals with stable glucose profiles. (19) Such discordance may result in HbA1c values that either overestimate or underestimate actual glycemic exposure as reflected by CGM data. (18,19)

The clinical significance of GMI-HbA1c discordance lies in its ability to identify patients in whom HbA1c may not accurately represent current glycemic status. Individuals with higher HbA1c relative to GMI may be exposed to a disproportionate risk of hypoglycemia, whereas those with lower HbA1c relative to GMI may experience unrecognized hyperglycemia. (18,19)

Importantly, GMI should not be interpreted in isolation. Contemporary guidance emphasizes that GMI provides meaningful clinical insight only when considered alongside other CGM-derived metrics, particularly TIR and measures of glycemic variability. (20) Integrating GMI with complementary CGM metrics allows for a more comprehensive assessment of glycemic control that balances average exposure with variability and risk. (20)

In both clinical practice and research settings, GMI functions as a bridge between CGM-derived glucose data and traditional glycemic markers. Its primary value lies not in replacing HbA1c, but in enhancing the interpretation of CGM profiles, identifying metric discordance, and supporting individualized glycemic management strategies. (18,20)

4.1. Conceptual Basis of the Glucose Management Indicator

The glucose management indicator is a CGM-derived metric calculated from mean sensor glucose values and intended to provide an estimate conceptually aligned with laboratory-measured HbA1c. By summarizing time-weighted average glucose exposure captured during CGM wear, GMI offers a contemporaneous representation of glycemic status based exclusively on directly measured interstitial glucose data. (18,21)

Although GMI is conceptually related to HbA1c, it differs in its sensitivity to individual biological variability. HbA1c reflects long-term glycation processes influenced by factors such as erythrocyte lifespan, iron status, renal function, and interindividual differences in glycation rates. In contrast, GMI is independent of these biological modifiers and is derived solely from observed glucose concentrations. (19,21)

From a conceptual perspective, GMI should be regarded as a complementary indicator rather than a replacement for HbA1c. The combined interpretation of GMI and HbA1c allows clinicians to identify clinically meaningful discordance between laboratory-based and CGM-based assessments of glycemia and supports a more individualized understanding of glycemic control. (20,21)

4.2. Glucose Management Indicator – HbA1c Discordance

Clinically relevant discordance between GMI and HbA1c is a well-recognized phenomenon reflecting the combined influence of biological variability and methodological differences between laboratory-based and CGM-derived assessments of glycemia. HbA1c represents a cumulative glycation process affected by erythrocyte lifespan, iron status, renal function, and interindividual differences in glycation rates, whereas GMI is derived exclusively from contemporaneous CGM-measured glucose values. (9,18,22)

From a clinical standpoint, recognition of GMI-HbA1c discordance is essential to avoid misclassification of glycemic control. Exclusive reliance on HbA1c may lead to underestimation or overestimation of current glycemic exposure, whereas GMI provides complementary insight into recent glucose patterns. Therefore, discordance between these metrics should prompt a structured review of CGM profiles, assessment of data quality, and consideration of biological factors known to influence HbA1c. (18,20,22)

Rather than favoring one metric over the other, contemporary CGM-based approaches emphasize integrated interpretation of GMI, HbA1c, and additional CGM-derived metrics to support individualized and context-sensitive clinical decision-making. (20,22)

5. Glycemic Variability and Coefficient of Variation

Glycemic variability represents fluctuations in glucose levels over time and constitutes a key dimension of glycemic control not captured by metrics focused solely on average glucose exposure. Both intraday and interday variability contribute to metabolic instability and have been associated with increased oxidative stress, impaired counterregulatory responses, and a higher risk of hypoglycemia. (9,12)

Intraday variability reflects short-term glucose excursions driven by meals, physical activity, and pharmacologic interventions, whereas interday variability captures day-to-day inconsistency in glucose patterns. Elevated variability in either domain has been linked to reduced glucose predictability and increased susceptibility to hypoglycemia, particularly in individuals treated with insulin or insulin secretagogues. (9,12)

Among the available indices for quantifying glycemic variability, the coefficient of variation (CV) has emerged as a standardized and clinically practical metric. CV expresses glucose variability relative to mean glucose, allowing meaningful comparison across individuals with differing average glycemic levels. (9,23)

International consensus recommendations have identified CV as the preferred metric for routine clinical use, with a threshold of approximately 36% proposed to distinguish stable from unstable glucose profiles. Values exceeding this threshold

have been consistently associated with increased hypoglycemia risk and reduced TIR, independent of HbA1c. (5,9)

In clinical practice, CV should be interpreted in conjunction with other CGM-derived metrics rather than in isolation. Integrated assessment of CV together with TIR, time below range, and AGP patterns enables identification of individuals with excessive glucose instability and supports targeted therapeutic adjustments. (5,12,23)

5.1. Importance of Glycemic Variability

Glycemic variability refers to the magnitude, frequency, and duration of glucose fluctuations over time. Intraday variability reflects short-term glucose excursions occurring within a single day, whereas interday variability describes day-to-day inconsistency in glucose patterns. Both dimensions contribute to overall glucose instability and may persist even in individuals with similar average glycemic exposure. (9,12)

Intraday glucose fluctuations are primarily driven by meal-related responses, physical activity, and pharmacologic interventions, leading to pronounced postprandial peaks and rapid glucose declines. Interday variability reflects inconsistent glycemic responses to identical therapeutic regimens across different days and has been linked to impaired metabolic adaptability and reduced counterregulatory capacity. (9,12,24)

Evaluation of both intraday and interday glycemic variability provides essential insight into glucose stability and safety. Incorporation of variability assessment into routine CGM interpretation enables identification of individuals at heightened risk for hypoglycemia, thereby informing targeted therapeutic strategies. (9,12,23,24)

5.2. Role of the Coefficient of Variation

The coefficient of variation has emerged as the preferred standardized metric for assessing glycemic variability in clinical practice. By expressing glucose variability relative to mean glucose, CV provides a normalized measure enabling meaningful comparison across individuals with different average glycemic levels. This normalization distinguishes CV from absolute measures such as standard deviation. (9,23)

A threshold value of approximately 36% has been proposed to distinguish acceptable from excessive glycemic variability. CV values exceeding this threshold have been consistently associated with increased hypoglycemia risk and reduced glycemic stability, regardless of HbA1c level. (25)

In routine CGM interpretation, CV should be assessed in conjunction with complementary metrics such as TIR, time below range, and AGP patterns. Elevated

CV in the presence of acceptable mean glucose or HbA1c values signals clinically relevant instability and warrants targeted intervention. Improvement in CV may translate into enhanced glycemic safety even when changes in average glucose are modest. (9,23,25)

6. Ambulatory Glucose Profile

The ambulatory glucose profile is a standardized visual reporting tool developed to facilitate systematic interpretation of complex CGM datasets in routine clinical practice. By collapsing multiple days of glucose measurements into a single representative 24-hour profile, AGP enables simultaneous assessment of glucose exposure, variability, and temporal patterns of dysglycemia (Table 2). (8,9)

6.1. Structure of the Ambulatory Glucose Profile

The ambulatory glucose profile display is centered on the median glucose curve, which reflects the typical daily glucose pattern across the monitoring period. Surrounding this curve, interquartile and percentile bands depict the dispersion of glucose values at each time point, allowing differentiation between usual variability and more extreme excursions. Narrow bands indicate stable and predictable glucose control, whereas widening bands identify time-specific instability that may warrant targeted intervention. (8,9)

6.2. Clinical Advantages of the Ambulatory Glucose Profile

Ambulatory glucose profile facilitates rapid visual pattern recognition and structured CGM interpretation while simultaneously enhancing patient-clinician communication through intuitive visualization of numerical glucose data. Beyond international consensus statements, expert panel recommendations across diverse clinical settings emphasize AGP-centered interpretation as a key component of routine diabetes care. (8,26)

7. Continuous Glucose Monitoring Metrics and Clinical Outcomes

Accumulating evidence indicates that CGM-derived metrics are closely associated with clinically meaningful outcomes in diabetes. Time in range has been consistently linked to microvascular complications, with lower values associated with higher prevalence of diabetic retinopathy, nephropathy, and neuropathy in individuals with type 2 diabetes. These associations persist after adjustment for mean glucose and HbA1c. (14)

Beyond microvascular disease, CGM metrics also demonstrate prognostic relevance for cardiovascular outcomes and mortality. Reduced time spent within target glucose ranges has been associated with increased risks of all-cause and

cardiovascular mortality, independent of traditional risk factors and HbA1c. (15) Evidence from systematic reviews and meta-analyses has shown that greater long-term glycemic variability is associated with adverse cardiovascular outcomes and mortality. (27)

These findings highlight that CGM-derived metrics provide prognostic information beyond average glycemia. Their consistent associations with microvascular complications, cardiovascular outcomes, and mortality underscore the value of incorporating CGM metrics into clinical risk assessment frameworks. (14,15,27)

8. Continuous Glucose Monitoring in Prediabetes and Early Dysglycemia

The application of CGM in prediabetes and early dysglycemia is supported by its ability to identify dynamic glucose abnormalities not captured by conventional diagnostic markers. CGM can detect early reductions in time in range and exaggerated postprandial excursions that may precede the onset of overt diabetes. (13,17) Recent evidence has highlighted the potential value of CGM-derived metrics for characterizing early dysglycemia and informing individualized preventive strategies. (28) In addition, CGM-based behavioral feedback has been increasingly explored as a tool to support lifestyle modification and early risk stratification in prediabetes. (29) However, the absence of standardized CGM targets and limited prospective outcome data currently restrict routine clinical implementation in prediabetes.

9. Practical Integration into Clinical Practice

Clinical integration of CGM relies on structured interpretation of key metrics rather than isolated values. (30) Stepwise review of time in range, glycemic variability, and AGP patterns enables identification of recurrent dysglycemia and supports individualized treatment adjustment. Standardized CGM interpretation frameworks facilitate efficient clinical decision-making and improve patient-clinician communication during routine visits. (30,31)

10. Limitations and Future Directions

Despite the expanding use of CGM-derived metrics, the current evidence base remains predominantly observational, limiting causal inference regarding associations with clinical outcomes. Long-term prospective data linking CGM metrics to hard endpoints are still limited, particularly beyond microvascular complications. In addition, CGM-based targets for prediabetes and early dysglycemia have not yet been clearly defined, restricting routine clinical implementation in these populations. Future research should prioritize randomized

studies with extended follow-up and refinement of outcome-driven CGM targets to support broader clinical adoption. (21)

11. Conclusion

CGM-derived metrics provide complementary and non-redundant insights into glycemic control that extend well beyond conventional measures of average glycemia. Integrated interpretation of time in range, glucose management indicator, glycemic variability, and the ambulatory glucose profile enables a multidimensional assessment of glucose exposure, stability, and risk that cannot be captured by HbA1c alone. When applied together within a structured and standardized framework, these metrics enhance clinical interpretability, support individualized therapeutic decision-making, and facilitate more precise alignment between glycemic targets and patient-specific risk profiles.

Importantly, the clinical value of CGM lies not in any single metric, but in the synergistic use of complementary parameters that balance efficacy and safety across diverse clinical contexts, including type 2 diabetes and early dysglycemia. As CGM adoption continues to expand, standardized interpretation of these metrics will be essential to ensure consistent and meaningful translation of glucose data into clinical practice.

Looking forward, integration of CGM-derived metrics with digital decision-support tools, artificial intelligence-based pattern recognition, and personalized therapeutic algorithms is expected to further refine glycemic management. Such advances may enable earlier risk stratification, proactive intervention, and a shift toward more precision-oriented and preventive models of diabetes care.

References

1. Bergenstal RM, Beck RW, Close KL, Grunberger G, Sacks DB, Kowalski A, et al. Glucose management indicator (GMI): a new term for estimating A1C from continuous glucose monitoring. Diabetes Care. 2018;41(11):2275-80.
2. Vigersky RA, McMahon C. The relationship of hemoglobin A1c to time-in-range in patients with diabetes. Diabetes Technol Ther. 2019;21(2):81-5.
3. Vigersky RA, Fonda SJ, Chellappa M, Walker MS, Ehrhardt NM. Short- and long-term effects of real-time continuous glucose monitoring in patients with type 2 diabetes. Diabetes Care. 2012;35(1):32-8.
4. Battelino T, Lalic N, Hussain S, Ceriello A, Klobucar S, Davies SJ, et al. The use of continuous glucose monitoring in people living with obesity, intermediate hyperglycemia or type 2 diabetes. Diabetes Res Clin Pract. 2025;223:112111.
5. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the international consensus. Diabetes Care. 2019;42(8):1593-603.
6. American Diabetes Association Professional Practice Committee. Glycemic goals and hypoglycemia: standards of care in diabetes-2025. Diabetes Care. 2025;48(Suppl 1):S128-45.
7. Goshrani A, Lin R, O'Neal D, Ekinci EI. Time in range: a new gold standard in type 2 diabetes research? Diabetes Obes Metab. 2025;27(12):2342-62.
8. Doupis J, Horton ES. Utilizing the new glucometrics: a practical guide to ambulatory glucose profile interpretation. touchREV Endocrinol. 2022;18(1):20-6.
9. Rodbard D. Glucose variability: a review of clinical applications and research developments. Diabetes Technol Ther. 2018;20(Suppl 2):S25-33.
10. Beck RW, Bergenstal RM, Cheng P, Kollman C, Carlson A, Johnson ML, et al. The relationships between time in range, hyperglycemia metrics, and HbA1c. J Diabetes Sci Technol. 2019;13(4):614-26.
11. Czupryniak L, Dzida G, Fichna P, Jarosz-Chobot P, Gumprecht J, Klupa T, et al. Ambulatory glucose profile (AGP) report in daily care of patients with diabetes: practical tips and recommendations. Diabetes Ther. 2022;13(4):811-21.
12. Raj R, Mishra R, Jha N, Joshi V, Correa R, Kern PA. Time in range, as measured by continuous glucose monitor, as a predictor of microvascular complications in type 2 diabetes: a systematic review. BMJ Open Diabetes Res Care. 2022;10(1):e002573.

13. Klonoff DC, Nguyen KT, Xu NY, Gutierrez A, Espinoza JC, Vidmar AP. Use of continuous glucose monitors by people without diabetes: an idea whose time has come? J Diabetes Sci Technol. 2023;17(6):1686-97.
14. Lu J, Ma X, Zhou J, Zhang L, Mo Y, Ying L, et al. Association of time in range, as assessed by continuous glucose monitoring, with diabetic retinopathy in type 2 diabetes. Diabetes Care. 2018;41(11):2370-6.
15. Cai J, Liu J, Lu J, Ni J, Wang C, Chen L, et al. Impact of time in tight range on all-cause and cardiovascular mortality in type 2 diabetes: a prospective cohort study. Diabetes Obes Metab. 2025;27(4):2154-62.
16. Beck RW, Bergenstal RM, Riddlesworth TD, Kollman C, Li Z, Brown AS, et al. Validation of time in range as an outcome measure for diabetes clinical trials. Diabetes Care. 2019;42(3):400-5.
17. Cichosz SL, Kronborg T, Laugesen E, Hangaard S, Fleischer J, Hansen TK, et al. From stability to variability: classification of healthy individuals, prediabetes, and type 2 diabetes using glycemic variability indices from continuous glucose monitoring data. Diabetes Technol Ther. 2025;27(1):34-44.
18. Shah VN, Vigers T, Pyle L, Calhoun P, Bergenstal RM. Discordance between glucose management indicator and glycated hemoglobin in people without diabetes. Diabetes Technol Ther. 2023;25(5):324-8.
19. Fang M, Wang D, Rooney MR, Echouffo-Tcheugui JB, Coresh J, Aurora RN, et al. Performance of the glucose management indicator (GMI) in type 2 diabetes. Clin Chem. 2023;69(4):422-8.
20. Fellinger P, Rodewald K, Ferch M, Itariu B, Kautzky-Willer A, Winhofer Y. HbA1c and glucose management indicator discordance associated with obesity and type 2 diabetes in intermittent scanning glucose monitoring system. Biosensors (Basel). 2022;12(5):288.
21. American Diabetes Association Professional Practice Committee. Diagnosis and classification of diabetes: standards of care in diabetes-2025. Diabetes Care. 2025;48(Suppl 1):S23-43.
22. Selvin E. The glucose management indicator: time to change course? Diabetes Care. 2024;47(6):906-14.
23. Kohnert KD, Heinke P, Vogt L, Salzsieder E. Utility of different glycemic control metrics for optimizing management of diabetes. World J Diabetes. 2015;6(1):17-29.
24. Ceriello A, Monnier L, Owens D. Glycaemic variability in diabetes: clinical and therapeutic implications. Lancet Diabetes Endocrinol. 2019;7(3):221-30.

25. Monnier L, Colette C, Wojtusciszyn A, Dejager S, Renard E, Owens D, et al. Toward defining the threshold between low and high glucose variability in diabetes. Diabetes Care. 2017;40(7):832-8.
26. Dagdelen S, Deyneli O, Dinccag N, Yildiz BO, Alagozlu H, Erturk E, et al. Expert panel recommendations for use of standardized glucose reporting system based on standardized glucometrics plus visual ambulatory glucose profile (AGP) data in clinical practice. Front Endocrinol (Lausanne). 2022;12:663222.
27. Gorst C, Kwok CS, Aslam S, Buchan I, Kontopantelis E, Myint PK, et al. Long-term glycemic variability and risk of adverse outcomes: a systematic review and meta-analysis. Diabetes Care. 2015;38(12):2354-69.
28. Zahalka SJ, Galindo RJ, Shah VN, Low Wang CC. Continuous glucose monitoring for prediabetes: what are the best metrics? J Diabetes Sci Technol. 2024;18(4):835-46.
29. Black DS, Vidmar AP, Barnett B. Characterising the design and methods of continuous glucose monitoring used in behavioural interventions to inform future research in prediabetes. Lancet Digit Health. 2025;7(10):100904.
30. Fernando K, Alabraba V, Welsh JB, Evans M, Heller S, Evans ML, et al. Practical approaches to continuous glucose monitoring in primary care: a UK-based consensus opinion. Diabetes Ther. 2025;16(4):749-62.
31. Szmuilowicz ED, Aleppo G. Stepwise approach to continuous glucose monitoring interpretation for internists and family physicians. Postgrad Med. 2022;134(8):743-51.

Table 1. Core Continuous Glucose Monitoring-Derived Metrics: Definitions, Clinical Targets, and Practical Considerations

CGM Metric	Definition	Typical Clinical Targets*	Primary Clinical Utility	Key Considerations and Limitations
Time in Range (TIR)	% of time sensor glucose remains within target range	≥70% (most nonpregnant adults with T2DM)	Time-based glycemic assessment; associated with microvascular outcomes and mortality	Does not capture hypoglycemia or variability when used alone
Time Above Range (TAR)	% of time glucose exceeds target range	<25% (>10.0 mmol/L); <5% (>13.9 mmol/L)	Identifies hyperglycemia exposure and postprandial excursions	Reflects duration, not severity of hyperglycemia
Time Below Range (TBR)	% of time glucose falls below target range	<4% (<3.9 mmol/L); <1% (<3.0 mmol/L)	Quantifies hypoglycemia risk; supports safety assessment	Sensitive to sensor accuracy; requires data completeness
Glucose Management Indicator (GMI)	Estimate of laboratory HbA1c from mean CGM glucose	Interpreted alongside individualized HbA1c goals	Bridges CGM glucose data and HbA1c; highlights metric discordance	Does not replace laboratory HbA1c; depends on CGM duration and quality
Coefficient of Variation (CV)	Glucose variability expressed as SD/mean glucose (%)	≤36%	Standardized stability index; associated with hypoglycemia risk	Should be interpreted with mean glucose and TIR
Ambulatory Glucose Profile (AGP)	Standardized graphical CGM summary across a 24-hour period	Not target-based	Enables visual pattern recognition and structured clinical decision-making	Requires familiarity and clinical experience

* Clinical targets may require individualization based on patient characteristics, comorbidities, and hypoglycemia risk.

Table 2. Practical Stepwise Algorithm for Interpretation of the Ambulatory Glucose Profile (AGP)

Step	AGP Component Assessed	Key Questions	Clinical Interpretation	Potential Clinical Actions
1	Data sufficiency	Is CGM data ≥14 days with ≥70% wear time?	Ensures reliability and representativeness of AGP	Repeat CGM or extend monitoring if insufficient
2	Median glucose curve	Does the median line remain within target range?	Identifies overall glycemic exposure and timing of dysglycemia	Adjust basal therapy, meal timing, or treatment intensity
3	Interquartile range (IQR)	Is the IQR narrow or wide at specific times of day?	Reflects glucose stability and intraday variability	Target lifestyle factors, meal composition, or medication timing
4	Percentile bands (5th–95th)	Are there frequent excursions into hypoglycemia or marked hyperglycemia?	Highlights glycemic risk beyond averages	Modify therapy to reduce hypoglycemia or extreme hyperglycemia
5	Nocturnal profile	Are there overnight downward or upward trends?	Suggests nocturnal hypoglycemia or dawn phenomenon	Adjust evening medications, basal insulin, or bedtime snacks
6	Postprandial patterns	Do glucose rises occur consistently after meals?	Indicates postprandial dysglycemia	Optimize meal composition, prandial therapy, or physical activity
7	Integration with summary metrics	Are TIR, TBR, CV, and GMI concordant with visual patterns?	Confirms consistency between numeric and visual CGM data	Prioritize interventions targeting instability vs mean glucose

Interpretation should consider individual clinical context, including age, comorbidities, treatment modality, and hypoglycemia risk.

Chapter 6

Periodontal Status and Microbiota: The Relationship Between Oral and General Health

Ilknur Sidika Tayfun BUYUKEKEN [1], Elif MUTAFCILAR VELIOGLU[2]

1. INTRODUCTION

Medical and dental disciplines have traditionally been separated into systemic diseases and pathologies specific to the oral cavity. However, growing evidence demonstrates that oral health is not merely a local condition, but is also closely linked to systemic health. The relationship between oral and systemic health is increasingly recognized as bidirectional and interactive. Epidemiological and large-scale population studies, as well as clinical investigations and *in vitro* and *in vivo* experiments, have demonstrated the significant role of oral health on overall health. In this context, the strong association between chronic inflammatory periodontal diseases and systemic disorders has become increasingly evident [1] . Periodontal diseases have been shown to be associated with various systemic diseases, such as cardiovascular disease, diabetes mellitus, adverse pregnancy outcomes, and osteoporosis (Figure 1). This association is primarily driven by the chronic inflammation resulting from periodontal disease and the entry of bacteria and bacterial byproducts from the dysbiotic oral flora into the systemic circulation [2].

[1]DDS MSc, E., Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey
ORCID: 0000-0002-9939-6919
[2] DDS, MSc Selcuk University, Faculty of Dentistry, Department of Periodontology, Konya, Turkey
ORCID: 0000-0003-1882-553X

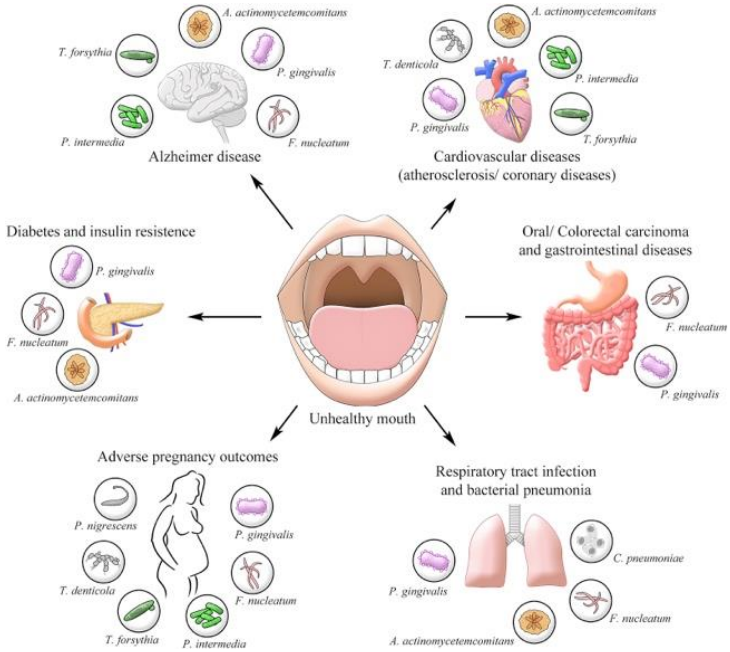


Figure 1. Schematic representation of the potential links between oral dysbiosis and periodontal pathogens and various systemic diseases [3].

Microbiota is an ecosystem composed of beneficial and harmful microorganisms living together in the human body [4]. The microbiota influences many physiological functions, particularly hematopoiesis, inflammation, and immunity [5]. In recent years, numerous studies have highlighted the link between the microbiota and diseases such as cancer, diabetes, and neurological disorders [4]. The demonstration of the microbiota's connection to human health and various diseases, including cancer, has spurred research in this field and led to the view that the microbiota should now be considered a new organ [6].

In this context, imbalance in the microbiota (dysbiosis) is not limited to the local environment where it occurs, but may also lead to systemic effects. Therefore, the composition and balance of the microbiota are considered important factors for overall systemic health.

2. PERIODONTAL DISEASE AND ORAL MICROBIOTA

Oral microbiota is one of the most important and complex microbial communities in the human body [7]. The oral microbiota contains more than 700 different bacterial species and is the second most diverse microbial community in the human body after the gut microbiota. It is a dynamic microbial ecosystem that changes continuously depending on factors such as diet, oral hygiene habits, oral hygiene practices, saliva flow, and the characteristics of saliva [8,9]. Eubiosis refers to a balanced and mutually beneficial relationship between the host and the microbiota, whereas dysbiosis is defined as alterations in the composition and functions of the microbiota that disrupt this balance [10]. The oral microbiota plays a critical role in maintaining oral health and supporting systemic health. A healthy oral microbiota helps prevent infections and supports the immune system by inhibiting the colonization of pathogens [11].

Gingivitis is considered a reversible periodontal disease representing the initial stage of inflammation in the periodontal tissues. However, if dental biofilm is not removed and the dysbiotic microbiota persists, the inflammatory process may progress and develop into periodontitis, which is characterized by destruction of the periodontal connective tissue and alveolar bone [12]. A disruption of the oral microbiome refers to a condition in which gram-negative bacteria become dominant in the periodontal microenvironment [13]. The retention of dysbiotic oral microbiota in periodontal tissues is considered the initial stage in the development of periodontitis [14]. Periodontitis is characterized by inflammatory processes, connective tissue destruction, and alveolar bone loss resulting from periodontal inflammation. Inflammatory cell infiltration is observed in the connective tissue adjacent to the periodontal pocket epithelium [15,16]. This inflammation is widely considered to have negative effects on overall health and may worsen the course of existing systemic diseases [17].

2.1. Periodontal Pathogens and Dysbiosis

Not all bacterial species in the periodontal microbiota exhibit the same pathogenic potential. Within the subgingival biofilm, microorganisms form different microbial complexes based on their interactions with one another. Among these complexes, the group with the highest periodontal pathogenic potential is known as the “red complex,” which consists of *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola* [18]. These three species show a strong association with advanced periodontal lesions; they are linked to increased pocket depth and bleeding. While the supragingival environment above the gum line primarily harbors aerobic bacteria, the anaerobic

and nutrient-rich nature of the periodontal pocket creates a favorable environment for these pathogens to colonize. As periodontitis progresses, the subgingival environment becomes more anaerobic, contributing to the worsening of the dysbiotic microbial profile [19].

Within the red complex, P. gingivalis in particular is currently defined as a "keystone pathogen." According to this concept, even if the bacterium is present in low numbers within the subgingival biofilm, it can cause the microbial community to become dysbiotic by affecting the host's immune and complement systems. This mechanism, explained by the analogy of a keystone pathogen in ecology, demonstrates that the bacterium disrupts the balance not through its numerical abundance but through its functional impact. It has been reported that this characteristic depends on the strain and environmental conditions and may partially explain differences in disease susceptibility among individuals [20].

3. THE RELATIONSHIP BETWEEN ORAL MICROBIOTA AND SYSTEMIC HEALTH

Disruption of the balance between the oral microbiota and periodontal tissues not only leads to local periodontal destruction, but may also cause systemic effects. Bacteria, endotoxins, and inflammatory mediators released during periodontal inflammation can enter the systemic circulation and affect distant organs and tissues [13,21]. In addition, the fact that periodontal pathogens and their virulence factors have been detected in systemic tissues supports the biological basis of the oral–systemic health relationship [3]. For this reason, understanding the effects of the oral microbiota and periodontal health on systemic health has become a major focus of interdisciplinary research today. In this section, the association between periodontal disease and cardiovascular diseases, respiratory diseases, diabetes mellitus, neurological diseases, gastrointestinal diseases, and adverse pregnancy outcomes will be discussed within the framework of underlying mechanisms.

3.1. Basic Mechanisms of Oral-Systemic Interaction

During periodontal disease, the periodontal pocket epithelium may become ulcerated and permeable as a result of inflammation. The disruption of the integrity of the periodontal pocket epithelium creates a gateway that facilitates the entry of periodontal pathogens and bacterial byproducts into the systemic circulation [22]. During this process, lipopolysaccharides (LPS), toxins, and various bacterial virulence factors can enter the circulation and contribute to the activation of the systemic inflammatory response [3]. In addition, transient bacteremia that can occur during toothbrushing, chewing, and periodontal

procedures may lead to the spread of oral microorganisms to distant organs and tissues [23]. It is suggested that periodontal pathogens and inflammatory mediators entering the systemic circulation may contribute to the development of systemic diseases through endothelial dysfunction, immune activation, and inflammatory processes. In addition, it has been reported that inflammatory changes occurring in periodontal tissues may facilitate the systemic spread of bacterial products by increasing vascular permeability [21]. The fact that some periodontal pathogens have been shown to infect atherosclerotic plaques, cardiac tissues, and various systemic components also supports the idea that periodontal pathways are not limited solely to local tissues [24].

It is known that the host's inflammatory and immune responses also play a significant role in the progression of periodontal diseases. The immune response triggered by dysbiotic oral microbiota can lead to increased production of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α. It has been reported that these mediators contribute to connective tissue destruction and alveolar bone resorption in periodontal tissues, and may also enter the systemic circulation to affect various inflammatory pathways. In particular, it is noted that IL-6 stimulates the hepatic acute-phase response, thereby increasing C-reactive protein (CRP) production, and that this is associated with systemic inflammation [25,26]. In addition, it has been reported that persistent inflammatory processes and impaired immune regulation can contribute to the progression of periodontal tissue destruction [27].

Another key mechanism underlying the relationship between the oral microbiota and systemic diseases is the citrullination pathway, which operates through molecular mimicry and autoimmune activation. This mechanism has been extensively studied, particularly in the context of the pathogenesis of rheumatoid arthritis (RA). Citrullination is a post-translational modification process in which the amino acid arginine is converted to citrulline [28]. In individuals with a genetic predisposition, the immune system may lose its tolerance to citrullinated self-proteins, leading to the production of anti-citrullinated protein antibodies (ACPA), which are serological markers of rheumatoid arthritis. The role of the oral microbiota in this process stems from the fact that P. gingivalis is the only known human pathogen that expresses a unique peptidyl-arginine deiminase (PPAD) enzyme. This enzyme can directly citrullinate both bacterial and host proteins in periodontal tissues, thereby creating new antigens capable of triggering an ACPA response [29]. These findings support a direct mechanistic link between disruption of the periodontal mucosal barrier and systemic autoimmune activation.

The final mechanism involves the systemic effects of microbial metabolites. It has been suggested that the ingestion of oral dysbiotic bacteria may alter the metabolic output of the intestinal microbiota, thereby increasing levels of trimethylamine N-oxide (TMAO), a proatherogenic metabolite associated with cardiovascular risk [30]. All of the mechanisms described form a reinforcing network; however, since the majority of the current evidence is based on cross-sectional studies, long-term human studies are needed to definitively establish a causal relationship [27].

3.2. Periodontal Disease and Cardiovascular Diseases

Cardiovascular diseases remain one of the leading causes of death and illness worldwide. Epidemiological studies have shown that the risk of cardiovascular disease is significantly higher in individuals with periodontal disease; this association is also supported by the 2020 consensus report from the EFP and the World Heart Federation [31,32]. The effect of periodontal disease on the cardiovascular system is thought to trigger atherosclerotic plaque formation and endothelial dysfunction through impaired systemic circulation mediated by bacteria, endotoxins, and inflammatory mediators such as IL-1β, IL-6, TNF-α, and CRP [33]. These pathogens sustain the inflammatory process in the blood vessel wall both by directly invading endothelial cells and by activating the host immune response. In addition, many epidemiological studies have shown that individuals with periodontitis have an increased risk of coronary artery disease, myocardial infarction, and ischemic stroke [31].

Recent studies suggest that changes in oral microbiota composition may be associated with early biological markers of cardiovascular disease. In particular, elevated levels of oral bacteria such as *Streptococcus anginosus* and *Streptococcus oralis* have been reported to be associated with coronary artery calcification. Furthermore, the oral microbiota is thought to play a role in vascular homeostasis and blood pressure regulation through its effects on nitric oxide metabolism. It has been proposed that a reduction in beneficial oral bacteria with nitrate-reducing capacity may contribute to the development of hypertension by decreasing nitric oxide bioavailability [34,35]. Additionally, recent studies in the fields of microbiome and metabolomics suggest that saliva-based microbial profiling can be used as a non-invasive biomarker approach in cardiovascular risk assessment [30].

Clinical studies directly examining the effects of periodontal treatment on the cardiovascular system also support this association. In a randomized controlled trial conducted by Tonetti et al., significant improvements in endothelial function

were observed within six months in patients who received periodontal treatment, demonstrating that controlling periodontal inflammation may produce measurable beneficial effects on vascular health [36].

All of this data suggests that periodontal disease may influence cardiovascular risk through biologically plausible mechanisms. However, the causal nature of the observed epidemiological association has not yet been definitively proven; the effects of common risk factors such as smoking, diabetes, and socioeconomic factors need to be more clearly distinguished through long-term randomized trials [31].

3.3. Periodontal Disease and Respiratory Diseases

The relationship between respiratory diseases and the oral microbiota has been attracting increasing attention in recent years. In particular, it is believed that oral dysbiosis and periodontal inflammation, which develop during periodontitis, may contribute to the development of lower respiratory infections and chronic lung diseases. The transport of pathogenic microorganisms from the oral cavity to the lower respiratory system through aspiration is considered one of the most important biological mechanisms underlying this relationship [37,38]. It has been reported that proinflammatory cytokines and bacterial products released during periodontal inflammation may enter the systemic circulation and influence the pulmonary inflammatory response. Additionally, it is considered that common risk factors, such as smoking and systemic inflammation, may play a role in strengthening the association between periodontitis and respiratory diseases [39].

Particularly, the relationship between periodontal disease and chronic obstructive pulmonary disease (COPD), pneumonia, and ventilator-associated pneumonia has been extensively investigated, and it has been demonstrated in several studies that periodontal pathogens may facilitate their retention on respiratory epithelium, disrupt mucosal barrier functions, and trigger inflammation in lung tissues [39]. Furthermore, it has been shown that aspiration of inadequate oral hygiene in elderly individuals and intensive care patients may increase the risk of pneumonia. Current microbiome studies support the oral–lung axis concept, emphasizing that changes in the oral microbiota may affect pulmonary microbial composition and immune responses [40,41]. In addition, some studies have reported that professional oral hygiene interventions in periodontal treatment may reduce the risk of respiratory infections [42]. These findings suggest that maintaining periodontal health may be important not only for oral tissues but also for respiratory system health.

3.4. Periodontal Disease and Diabetes Mellitus

Diabetes mellitus (DM) is one of the leading systemic diseases associated with a bidirectional relationship with periodontal disease. The risk of periodontitis in individuals with diabetes is approximately three times higher than in those without diabetes. Today, periodontitis is recognized as one of the classic complications of diabetes, and it has been reported that periodontal destruction is more severe in the presence of poor glycemic control [43]. Similarly, it is believed that periodontal inflammation may negatively affect glycemic control by increasing the systemic inflammatory burden [44,45]. Advanced glycation end products (AGEs) and their receptors (RAGE), which occur as a result of hyperglycemia, are among the key mechanisms of periodontal tissue destruction in diabetes. AGE accumulation may accelerate connective tissue destruction and alveolar bone resorption in periodontal tissues by increasing oxidative stress, inflammatory cytokine production, and osteoclast activity [46]. Impaired neutrophil function, changes in collagen metabolism, and delayed wound healing in people with diabetes are also known to contribute to the progression of periodontal disease [47].

Chronic inflammation and pro-inflammatory mediators arising in periodontitis may exert adverse effects on glycemic control by impairing insulin response mechanisms and increasing peripheral insulin resistance [48]. D'Aiuto et al. found in a 12-month randomized clinical trial conducted in 264 patients with type 2 diabetes that HbA1c levels decreased by an average of 0.6% in the periodontal treatment group [49]. For this reason, it is believed that periodontitis is not merely a consequence of diabetes, but also a chronic site of inflammation that can affect the management of diabetes.

It has recently been suggested that changes in oral microbiota may serve as early biomarkers for diabetes mellitus, owing to advances in artificial intelligence-assisted microbiome analyses and salivary-based metabolomic approaches. In particular, microbial and metabolic profiles in saliva have been reported to reflect metabolic dysregulation before clinical manifestations of diabetes become apparent [30]. Furthermore, personalized microbiota-based therapeutic approaches, probiotic applications, and targeted microbial modulation strategies are thought to hold potential in reducing periodontal inflammation and supporting metabolic control [50].

All these findings demonstrate that the relationship between periodontal disease and diabetes mellitus is supported by biologically robust mechanisms. Today, the assessment of periodontal health in diabetes management and the consideration of metabolic status in periodontal patients are recognized as an important component of the multidisciplinary approach.

3.5. Periodontal Disease and Neurodegenerative Diseases

The relationship between oral microbiota and neurodegenerative disorders is attracting increasing attention. The "infection hypothesis" in Alzheimer's disease, which proposes that microbial agents may play a role in initiating or driving the progression of the neurodegenerative process, has garnered considerable attention in recent years [51]. The concept of the "oral-brain axis" is now supported by a substantial body of mechanistic evidence. In this context, compelling evidence has been put forward demonstrating that oral pathogens may be associated with neurodegenerative processes, and *P. gingivalis* in particular has become one of the most extensively studied periodontal pathogens [30]. It has been reported that bacteria and inflammatory mediators originating from the oral microbiota may enter the systemic circulation, triggering neuroinflammation and exerting adverse effects on the central nervous system [52].

It has been proposed that bacteremia and the inflammatory response occurring during periodontal disease may increase the permeability of the blood-brain barrier. In particular, the demonstration of *Porphyromonas gingivalis* and its gingipain proteases in the brain tissue of Alzheimer's patients represents important evidence supporting the association between oral microbiota and neurodegenerative processes [53]. Gingipains have been reported to be potentially associated with neuronal damage, tau protein alterations, and neuroinflammation [52].

Furthermore, pro-inflammatory cytokines entering the systemic circulation during periodontitis are thought to contribute to neuronal dysfunction and cognitive decline by triggering neuroinflammatory processes [54]. Some studies have also proposed that oral spirochetes may reach the central nervous system via the trigeminal nerve pathway and potentially play a role in neurodegenerative processes [55].

A potential association between periodontal disease and cognitive decline has been reported. It has been suggested that individuals with advanced periodontal destruction may exhibit lower levels of cognitive function and may be at increased risk of developing Alzheimer's disease. Oral dysbiosis is thought to influence systemic inflammation and neuroimmune responses, while oral microbiota profiling is considered a potential non-invasive biomarker approach for future use [56]. Periodontal diseases have been reported to be associated not only with Alzheimer's disease but also with other neurological conditions such as multiple sclerosis [57]. In particular, the oral–brain axis concept is gaining increasing prominence as a framework for explaining the potential biological interactions between oral microbiota and the central nervous system. Chronic systemic inflammation, microbial products, and oral dysbiosis are thought to

contribute to neurodegenerative processes by amplifying neuroinflammation [55]. Moreover, it has been reported that the oral microbiota–brain interaction is not limited to neurodegenerative diseases, and its potential role in neurodevelopmental disorders such as autism spectrum disorder is also being investigated [58].

3.6. Periodontal Disease and Gastrointestinal System Disorders

Oral dysbiosis developing during periodontitis is thought to extend beyond periodontal tissues and may also exert effects on the gut microbiota and gastrointestinal immune response. It has been reported that microorganisms originating from the oral cavity can be transported to the gastrointestinal system and alter the intestinal microbial balance [56,59]. In particular, periodontal pathogens have been proposed to increase inflammation in the intestinal mucosa and affect epithelial barrier integrity.

Evidence is accumulating that periodontal diseases may be associated with inflammatory bowel diseases. Alterations in oral microbiota composition and increased periodontal inflammation have been reported in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis [60]. Furthermore, the systemic inflammatory burden arising in individuals with periodontitis is thought to potentially exacerbate gastrointestinal inflammation. The concept of the "oral–gut axis" is gaining increasing prominence as a framework for describing the reciprocal interactions between oral and intestinal microbiota [61].

In recent years, certain periodontal pathogens, most notably *Fusobacterium nucleatum*, have been reported to be potentially associated with the development of colorectal cancer. It has been proposed that *F. nucleatum* may colonize the intestinal mucosa, thereby augmenting inflammation, modulating the immune response, and contributing to tumor progression

[62]. Additionally, pro-inflammatory cytokines and bacterial metabolites released during periodontal inflammation are thought to sustain chronic inflammatory responses in the gastrointestinal system. Current studies demonstrate that the relationship between oral microbiota and the gastrointestinal system is not limited to local infections, but may produce broader biological effects through mechanisms of systemic inflammation and immune regulation [56].

For this reason, maintaining periodontal health is considered potentially important for the preservation of gastrointestinal system health as well.

3.7. Periodontal Disease and Adverse Pregnancy Outcomes

Hormonal and immunological changes occurring during pregnancy may render periodontal tissues more susceptible to inflammatory responses. In particular, elevated levels of estrogen and progesterone have been reported to increase periodontal inflammation by affecting gingival vascularity and the inflammatory response [63]. Consequently, an increase in the prevalence of gingivitis and periodontitis may be observed during pregnancy.

The association between periodontal disease and adverse pregnancy outcomes has long been a subject of research interest. Complications such as preterm birth, low birth weight, preeclampsia, and gestational diabetes have been proposed to be potentially associated with periodontal inflammation [64]. Periodontal pathogens and inflammatory mediators are thought to enter the systemic circulation and may affect the fetoplacental unit.

Periodontal inflammation developing during pregnancy has been reported to potentially affect maternal and fetal tissues by increasing the systemic inflammatory burden. In particular, mediators such as IL-1β, IL-6, TNF-α, and prostaglandin E2 (PGE2) are thought to increase the risk of preterm birth by triggering uterine contractions and cervical dilation [64]. Furthermore, the demonstration of certain periodontal pathogens in placental tissues supports the potential impact of oral infections on adverse pregnancy outcomes. In particular, the detection of *Fusobacterium nucleatum* in placental tissues suggests that oral bacteria may reach the fetoplacental region through hematological dissemination [65]. Additionally, current microbiome studies indicate that oral microbiota profiling and inflammatory biomarker analyses may represent promising approaches for the early prediction of adverse pregnancy outcomes. Oral dysbiosis is thought to modulate maternal and fetal health by influencing systemic inflammation [66,67]. These findings suggest that maintaining periodontal health during pregnancy may be of significant importance for both maternal and fetal health.

4. Effects of Periodontal Treatment on Systemic Health

The effects of periodontal treatment are not limited to periodontal tissues alone, but may also induce changes in systemic inflammation, oral microbiota composition, and host response. It has been reported that controlling periodontal inflammation may lead to a reduction in periodontal pathogen load and oral dysbiosis, which in turn may exert beneficial effects on the systemic inflammatory response [68]. This is thought to potentially contribute to improvements in endothelial function, reduction in vascular inflammation, and control of the systemic inflammatory burden [36].

The systemic changes occurring following periodontal treatment have been proposed to exert beneficial effects on various systemic conditions such as cardiovascular diseases, diabetes mellitus, and neuroinflammatory disorders. In particular, reductions in HbA1c levels and regulation of the inflammatory-metabolic response following periodontal treatment have been reported in individuals with diabetes [49]. Similarly, controlling periodontal inflammation is thought to potentially modulate neuroinflammatory processes through its effects on the oral–gut–brain axis and systemic immune response [56].

The safety of periodontal treatment during pregnancy and its effects on pregnancy outcomes have been investigated for many years. Although non-surgical periodontal treatment is generally accepted as feasible and safe during pregnancy, there is no consensus in the literature regarding its effect on clinical outcomes such as preterm birth and low birth weight. Current systematic reviews and meta-analyses report that certain periodontal intervention strategies may reduce the risk of preterm birth and/or low birth weight, while emphasizing that this effect may vary depending on the timing of treatment, the protocol applied, the severity of periodontal disease, and maternal risk factors [66,69]. Furthermore, artificial intelligence-assisted microbiome analyses and multi-omics approaches are thought to enable more detailed assessment of individual responses to periodontal treatment, and these approaches may contribute to the development of personalized periodontal treatment strategies in the future [70]. With a better understanding of the effects of changes in oral microbiota composition on systemic inflammation, periodontal treatment is thought to potentially become a component of multidisciplinary systemic disease management in the future [30].

5. CONCLUSION

Periodontal disease is no longer regarded merely as a local pathology confined to the oral cavity, but rather as a chronic inflammatory condition with systemic dimensions that intersects at a biological level with cardiovascular diseases, diabetes mellitus, respiratory tract diseases, neurodegenerative processes, gastrointestinal system disorders, and adverse pregnancy outcomes. Underlying this association is not a single mechanism, but a pathophysiological network of mutually reinforcing processes including bacteremia, cytokine release, molecular mimicry, and microbial metabolite effects. Understanding that these mechanisms operate in concert has represented the most significant conceptual turning point in bringing periodontal disease to the forefront of systemic medicine.

Given that a substantial portion of the evidence base relies on observational study designs and that causal relationships remain a matter of debate, the need

for well-designed randomized controlled trials and long-term cohort data in the years ahead is clear. Studies in which the influence of shared risk factors — smoking, obesity, and socioeconomic status — has been adequately controlled represent the most critical methodological gap in this field. Nevertheless, the existing evidence alone provides sufficient justification for dentists and physicians to develop joint patient assessment protocols; the integration of periodontal examination into systemic disease risk screening and the development of microbiome-based diagnostic approaches stand out as the most concrete research priorities in this area. The replacement of the traditional clinical perspective that evaluates oral health independently of general health with a holistic and interdisciplinary approach is not only a scientific necessity, but also a responsibility toward patient health.

REFERENCES

1. Kapila YL. Oral health's inextricable connection to systemic health: Special populations bring to bear multimodal relationships and factors connecting periodontal disease to systemic diseases and conditions. Periodontology 2000. 2021;87:11-6. doi:10.1111/prd.12398
2. Kim J, Amar S. Periodontal disease and systemic conditions: a bidirectional relationship. Odontology. 2006;94:10-21. doi:10.1007/s10266-006-0060-6
3. Bui FQ, Almeida-da-Silva CLC, Huynh B, Trinh A, Liu J, Woodward J, et al. Association between periodontal pathogens and systemic disease. Biomedical Journal. 2019;42:27-35. doi:10.1016/j.bj.2018.12.001
4. Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, et al. Microbiota in health and diseases. Sig Transduct Target Ther. 2022;7:135. doi:10.1038/s41392-022-00974-4
5. Dzutsev A, Goldszmid RS, Viaud S, Zitvogel L, Trinchieri G. The role of the microbiota in inflammation, carcinogenesis, and cancer therapy. Eur J Immunol. 2015;45:17-31. doi:10.1002/eji.201444972
6. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: Gut Microbiota: The Neglected Endocrine Organ. Molecular Endocrinology. 2014;28:1221-38. doi:10.1210/me.2014-1108
7. Peng X, Cheng L, You Y, Tang C, Ren B, Li Y, et al. Oral microbiota in human systematic diseases. Int J Oral Sci. 2022;14:14. doi:10.1038/s41368-022-00163-7
8. D'Agostino S, Ferrara E, Valentini G, Stoica SA, Dolci M. Exploring Oral Microbiome in Healthy Infants and Children: A Systematic Review. IJERPH. 2022;19:11403. doi:10.3390/ijerph191811403
9. Shaiber A, Willis AD, Delmont TO, Roux S, Chen LX, Schmid AC, vd. Functional and genetic markers of niche partitioning among enigmatic members of the human oral microbiome. Genome Biol. 2020;21:292. doi:10.1186/s13059-020-02195-w
10. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol. 2014;16:1024-33. doi:10.1111/cmi.12308
11. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. J Clinic Periodontology. 2017;44. doi:10.1111/jcpe.12679
12. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017;3:17038. doi:10.1038/nrdp.2017.38

13. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015;15:30-44. doi:10.1038/nri3785 PubMed PMID: 25534621; PubMed Central PMCID: PMC4276050.
14. Meyle J, Chapple I. Molecular aspects of the pathogenesis of periodontitis. Periodontology 2000. 2015;69:7-17. doi:10.1111/prd.12104
15. Hajishengallis G, Hasturk H, Lambris JD, Apatzidou DA, Belibasakis GN, Bostanci N, et al. C3-targeted therapy in periodontal disease: moving closer to the clinic. Trends in Immunology. 2021;42:856-64. doi:10.1016/j.it.2021.08.001
16. Hathaway-Schrader JD, Novince CM. Maintaining homeostatic control of periodontal bone tissue. Periodontology 2000. 2021;86:157-87. doi:10.1111/prd.12368
17. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol. 2021;21:426-40. doi:10.1038/s41577-020-00488-6
18. Antezack A, Etchecopar-Etchart D, La Scola B, Monnet-Corti V. New putative periodontopathogens and periodontal health-associated species: A systematic review and meta-analysis. J of Periodontal Research. 2023;58:893-906. doi:10.1111/jre.13173
19. Mohanty R, Asopa SJ, Joseph MD, Singh B, Rajguru JP, Saidath K, et al. Red complex: Polymicrobial conglomerate in oral flora A review. Journal of Family Medicine and Primary Care. 2019;8:3480-6. doi:10.4103/jfmpc.jfmpc_759_19
20. Curtis MA, Garnett JA, Darveau RP. The Keystone-Pathogen Hypothesis Updated: The Role of *Porphyromonas gingivalis* in Periodontitis. J of Periodontal Research. 2025;jre.70050. doi:10.1111/jre.70050
21. Liccardo D, Cannavo A, Spagnuolo G, Ferrara N, Cittadini A, Rengo C, et al. Periodontal Disease: A Risk Factor for Diabetes and Cardiovascular Disease. IJMS. 2019;20:1414. doi:10.3390/ijms20061414
22. Dhotre S, Jahagirdar V, Suryawanshi N, Davane M, Patil R, Nagoba B. Assessment of periodontitis and its role in viridans streptococcal bacteremia and infective endocarditis. Indian Heart J. 2018;70:225-32. doi:10.1016/j.ihj.2017.06.019 PubMed PMID: 29716699; PubMed Central PMCID: PMC5993913.
23. Horliana ACRT, Chambrone L, Foz AM, Artese HPC, Rabelo M de S, Pannuti CM, vd. Dissemination of periodontal pathogens in the bloodstream after periodontal procedures: a systematic review. PLoS One.

2014;9:e98271. doi:10.1371/journal.pone.0098271 PubMed PMID: 24870125; PubMed Central PMCID: PMC4037200.
24. Schenkein HA, Loos BG. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. J Clinic Periodontology. 2013;40. doi:10.1111/jcpe.12060
25. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 2000. 2014;64:57-80. doi:10.1111/prd.12002
26. Neurath N, Kesting M. Cytokines in gingivitis and periodontitis: from pathogenesis to therapeutic targets. Front Immunol. 2024;15:1435054. doi:10.3389/fimmu.2024.1435054
27. Van Dyke TE, Bartold PM, Reynolds EC. The Nexus Between Periodontal Inflammation and Dysbiosis. Front Immunol. 2020;11:511. doi:10.3389/fimmu.2020.00511 PubMed PMID: 32296429; PubMed Central PMCID: PMC7136396.
28. Lundberg K, Wegner N, Yucel-Lindberg T, Venables PJ. Periodontitis in RA—the citrullinated enolase connection. Nat Rev Rheumatol. 2010;6:727-30. doi:10.1038/nrrheum.2010.139
29. Konig MF, Paracha AS, Moni M, Bingham CO, Andrade F. Defining the role of Porphyromonas gingivalis peptidylarginine deiminase (PPAD) in rheumatoid arthritis through the study of PPAD biology. Ann Rheum Dis. 2015;74:2054-61. doi:10.1136/annrheumdis-2014-205385 PubMed PMID: 24864075; PubMed Central PMCID: PMC4368502.
30. Guo ZL, Cui MW, Dong YL, Wang S. The oral microbiome as a regulatory hub for systemic health: a systematic review of mechanistic links and clinical implications. Journal of Oral Microbiology. 2026;18:2635233. doi:10.1080/20002297.2026.2635233
31. Sanz M, Marco Del Castillo A, Jepsen S, Gonzalez-Juanatey JR, D'Aiuto F, Bouchard P, vd. Periodontitis and cardiovascular diseases: Consensus report. J Clinic Periodontology. 2020;47:268-88. doi:10.1111/jcpe.13189
32. Aarabi G, Heydecke G, Seedorf U. Roles of Oral Infections in the Pathomechanism of Atherosclerosis. IJMS. 2018;19:1978. doi:10.3390/ijms19071978
33. Schenkein HA, Papapanou PN, Genco R, Sanz M. Mechanisms underlying the association between periodontitis and atherosclerotic disease. Periodontology 2000. 2020;83:90-106. doi:10.1111/prd.12304
34. Alzahrani HS, Jackson KG, Hobbs DA, Lovegrove JA. The role of dietary nitrate and the oral microbiome on blood pressure and vascular tone. Nutr Res Rev. 2021;34:222-39. doi:10.1017/S0954422420000281

35. Chai X, Liu L, Chen F. Oral nitrate-reducing bacteria as potential probiotics for blood pressure homeostasis. Front Cardiovasc Med. 2024;11:1337281. doi:10.3389/fcvm.2024.1337281 PubMed PMID: 38638884; PubMed Central PMCID: PMC11024454.
36. Tonetti MS, D'Aiuto F, Nibali L, Donald A, Storry C, Parkar M, et al. Treatment of Periodontitis and Endothelial Function. N Engl J Med. 2007;356:911-20. doi:10.1056/NEJMoa063186
37. Scannapieco FA, Bush RB, Paju S. Associations Between Periodontal Disease and Risk for Nosocomial Bacterial Pneumonia and Chronic Obstructive Pulmonary Disease. A Systematic Review. Ann Periodontol. 2003;8:54-69. doi:10.1902/annals.2003.8.1.54
38. Scannapieco FA, Cantos A. Oral inflammation and infection, and chronic medical diseases: implications for the elderly. Periodontology 2000. 2016;72:153-75. doi:10.1111/prd.12129
39. Gomes-Filho IS, Cruz SSD, Trindade SC, Passos-Soares JDS, Carvalho-Filho PC, Figueiredo ACMG, et al. Periodontitis and respiratory diseases: A systematic review with meta-analysis. Oral Diseases. 2020;26:439-46. doi:10.1111/odi.13228
40. Mammen MJ, Scannapieco FA, Sethi S. Oral-lung microbiome interactions in lung diseases. Periodontology 2000. 2020;83:234-41. doi:10.1111/prd.12301
41. Liu Z, Zhang W, Zhang J, Zhou X, Zhang L, Song Y, et al. Oral hygiene, periodontal health and chronic obstructive pulmonary disease exacerbations. J Clinic Periodontology. 2012;39:45-52. doi:10.1111/j.1600-051X.2011.01808.x
42. Hua F, Xie H, Worthington HV, Furness S, Zhang Q, Li C. Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia. Cochrane Oral Health Group, editör. Cochrane Database of Systematic Reviews. 2016. doi:10.1002/14651858.CD008367.pub3
43. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012;55:21-31. doi:10.1007/s00125-011-2342-y
44. Chapple ILC, Genco R, working group 2 of the joint EFP/AAP workshop. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Clinic Periodontology. 2013;40. doi:10.1111/jcpe.12077
45. Taylor G, Borgnakke W. Periodontal disease: associations with diabetes, glycemic control and complications. Oral Diseases. 2008;14:191-203. doi:10.1111/j.1601-0825.2008.01442.x

46. Lalla E, Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. Nat Rev Endocrinol. 2011;7:738-48. doi:10.1038/nrendo.2011.106
47. Mealey BL, Ocampo GL. Diabetes mellitus and periodontal disease. Periodontology 2000. 2007;44:127-53. doi:10.1111/j.1600-0757.2006.00193.x
48. Chávarry NGM, Vettore MV, Sansone C, Sheiham A. The relationship between diabetes mellitus and destructive periodontal disease: a meta-analysis. Oral Health Prev Dent. 2009;7:107-27. PubMed PMID: 19583037.
49. D'Aiuto F, Gkranias N, Bhowruth D, Khan T, Orlandi M, Suvan J, vd. Systemic effects of periodontitis treatment in patients with type 2 diabetes: a 12 month, single-centre, investigator-masked, randomised trial. The Lancet Diabetes & Endocrinology. 2018;6:954-65. doi:10.1016/S2213-8587(18)30038-X
50. Guan H, Zhao S, Tan Y, Fang X, Zhang Y, Zhang Y, et al. Microbiomic insights into the oral microbiome's role in type 2 diabetes mellitus: standardizing approaches for future advancements. Front Endocrinol. 2024;15:1416611. doi:10.3389/fendo.2024.1416611
51. Weber C, Dilthey A, Finzer P. The role of microbiome-host interactions in the development of Alzheimer´s disease. Front Cell Infect Microbiol. 2023;13:1151021. doi:10.3389/fcimb.2023.1151021
52. Harding A, Gonder U, Robinson SJ, Crean S, Singhrao SK. Exploring the Association between Alzheimer's Disease, Oral Health, Microbial Endocrinology and Nutrition. Front Aging Neurosci. 2017;9:398. doi:10.3389/fnagi.2017.00398
53. Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, et al. *Porphyromonas gingivalis* in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv. 2019;5:eaau3333. doi:10.1126/sciadv.aau3333
54. Singhrao SK, Harding A. Is Alzheimer's disease a polymicrobial host microbiome dysbiosis? Expert Review of Anti-infective Therapy. 2020;18:275-7. doi:10.1080/14787210.2020.1729741
55. Miklossy J. Alzheimer's disease - a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria. J Neuroinflammation. 2011;8:90. doi:10.1186/1742-2094-8-90
56. Kitamoto S, Nagao-Kitamoto H, Hein R, Schmidt TM, Kamada N. The Bacterial Connection between the Oral Cavity and the Gut Diseases. J Dent Res. 2020;99:1021-9. doi:10.1177/0022034520924633

57. Dalmazane M, Gallou-Guyot M, Compagnat M, Magy L, Montcuquet A, Billot M, vd. Effects on gait and balance of home-based active video game interventions in persons with multiple sclerosis: A systematic review. Mult Scler Relat Disord. 2021;51:102928. doi:10.1016/j.msard.2021.102928 PubMed PMID: 33845351; PubMed Central PMCID: PMC9764369.
58. Sharon G, Cruz NJ, Kang DW, Gandal MJ, Wang B, Kim YM, et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell. 2019;177:1600-1618.e17. doi:10.1016/j.cell.2019.05.004
59. Schmidt TS, Hayward MR, Coelho LP, Li SS, Costea PI, Voigt AY, et al. Extensive transmission of microbes along the gastrointestinal tract. eLife. 2019;8:e42693. doi:10.7554/eLife.42693
60. Papageorgiou SN, Hagner M, Nogueira AVB, Franke A, Jäger A, Deschner J. Inflammatory bowel disease and oral health: systematic review and a meta-analysis. J Clinic Periodontology. 2017;44:382-93. doi:10.1111/jcpe.12698
61. Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, et al. Ectopic colonization of oral bacteria in the intestine drives T_H 1 cell induction and inflammation. Science. 2017;358:359-65. doi:10.1126/science.aan4526
62. Brennan CA, Garrett WS. Fusobacterium nucleatum — symbiont, opportunist and oncobacterium. Nat Rev Microbiol. 2019;17:156-66. doi:10.1038/s41579-018-0129-6
63. Gürsoy M, Pajukanta R, Sorsa T, Könönen E. Clinical changes in periodontium during pregnancy and post-partum. J Clinic Periodontology. 2008;35:576-83. doi:10.1111/j.1600-051X.2008.01236.x
64. Bobetsis YA, Graziani F, Gürsoy M, Madianos PN. Periodontal disease and adverse pregnancy outcomes. Periodontology 2000. 2020;83:154-74. doi:10.1111/prd.12294
65. Nannan M, Xiaoping L, Ying J. Periodontal disease in pregnancy and adverse pregnancy outcomes: Progress in related mechanisms and management strategies. Front Med (Lausanne). 2022;9:963956. doi:10.3389/fmed.2022.963956 PubMed PMID: 36388896; PubMed Central PMCID: PMC9640773.
66. Wu J, Wu J, Tang B, Zhang Z, Wei F, Yu D, et al. Effects of different periodontal interventions on the risk of adverse pregnancy outcomes in pregnant women: a systematic review and network meta-analysis of randomized controlled trials. Front Public Health. 2024;12:1373691. doi:10.3389/fpubh.2024.1373691

67. Castaño-Suárez L, Paternina-Mejía GY, Vásquez-Olmos LD, Rodríguez-Medina C, Botero JE. Linking Periodontitis to Adverse Pregnancy Outcomes: a Comprehensive Review and Meta-analysis. Curr Oral Health Rep. 2024;11:125-37. doi:10.1007/s40496-024-00371-6
68. D'Aiuto F, Parkar M, Andreou G, Suvan J, Brett PM, Ready D, vd. Periodontitis and Systemic Inflammation: Control of the Local Infection is Associated with a Reduction in Serum Inflammatory Markers. J Dent Res. 2004;83:156-60. doi:10.1177/154405910408300214
69. Iheozor-Ejiofor Z, Middleton P, Esposito M, Glenny AM. Treating periodontal disease for preventing adverse birth outcomes in pregnant women. Cochrane Oral Health Group, editör. Cochrane Database of Systematic Reviews. 2017;2017. doi:10.1002/14651858.CD005297.pub3
70. Huang D, Wang YY, Li BH, Wu L, Xie WZ, Zhou X, et al. Association between periodontal disease and systemic diseases: a cross-sectional analysis of current evidence. Military Med Res. 2024;11:74. doi:10.1186/s40779-024-00583-y

Chapter 7

Medication-Related Osteonecrosis of the Jaw (MRONJ): Current Concepts and Surgical Management

Özge ÖZDAL ZİNCİR[1]

1. Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is one of the most significant complications encountered in contemporary oral and maxillofacial surgery. The condition is characterized by exposed necrotic bone, or bone that can be probed through a fistula in the maxillofacial region, persisting for more than 8 weeks in patients with current or previous treatment with antiresorptive or antiangiogenic agents and without a history of radiation therapy to the jaws or metastatic disease involving the jaws [1].

Since the first reports published by Marx in 2003 describing bisphosphonate-associated jaw necrosis, the understanding of MRONJ has evolved considerably [2]. Initially referred to as bisphosphonate-related osteonecrosis of the jaw (BRONJ), the terminology was later expanded because similar lesions were identified in patients receiving other antiresorptive and antiangiogenic medications such as denosumab, bevacizumab, sunitinib, and tyrosine kinase inhibitors [1,3]. The American Association of Oral and Maxillofacial Surgeons (AAOMS) subsequently introduced the term "medication-related osteonecrosis of the jaw" to encompass all drug-related etiologies [1].

The incidence of MRONJ has increased steadily over the last two decades due to the widespread use of antiresorptive medications in oncology and osteoporosis management. Bisphosphonates and denosumab are routinely prescribed for metastatic bone disease, multiple myeloma, osteoporosis, Paget's disease, and other metabolic bone disorders [4]. These medications significantly reduce skeletal-related events and improve patient outcomes; however, their prolonged use may impair bone remodeling and healing capacity, predisposing susceptible individuals to osteonecrosis of the jaws [5].

MRONJ has a multifactorial etiology involving suppression of osteoclastic activity, impaired angiogenesis, immune dysfunction, infection, inflammation, and local trauma [6]. The jawbones appear uniquely susceptible because of their

[1] Assoc. Prof. Dr., Istanbul University- Cerrahpasa Faculty of Dentistry, Department of Oral and Maxillofacial Surgery
ORCID: 0009-0000-9423-5764

high remodeling rate, constant exposure to oral microbiota, and frequent microtrauma related to mastication and dental procedures [7]. Tooth extraction remains the most common precipitating factor, although spontaneous lesions may also occur [8].

Clinically, MRONJ may present with exposed necrotic bone, pain, swelling, suppuration, halitosis, fistula formation, paresthesia, or pathologic fracture in advanced cases [1]. Some patients remain asymptomatic during early stages, while others develop severe functional and psychological impairment. The disease may significantly compromise mastication, speech, nutrition, and quality of life [9].

Over the past decade, management strategies for MRONJ have undergone substantial changes. Earlier approaches emphasized conservative therapy, including antimicrobial mouth rinses, systemic antibiotics, and limited debridement [10]. However, recent evidence increasingly supports the role of surgical intervention, even in earlier stages of disease, with improved outcomes observed following complete removal of necrotic bone and achievement of tension-free mucosal closure [11]. Furthermore, regenerative approaches such as platelet-rich fibrin (PRF), photobiomodulation therapy, stem cell applications, and biologic adjuncts have emerged as promising therapeutic modalities [12,13].

Despite advances in understanding and management, MRONJ continues to represent a major clinical challenge because of the lack of universally accepted treatment protocols, variable disease progression, and unpredictable therapeutic outcomes [14]. Moreover, controversies remain regarding the role of drug holidays, implant placement in patients receiving antiresorptive therapy, and optimal timing of surgical intervention [15].

The purpose of this chapter is to provide a comprehensive and up-to-date overview of MRONJ, including its epidemiology, pathophysiology, risk factors, classification systems, diagnostic criteria, imaging modalities, conservative and surgical treatment strategies, regenerative approaches, and future therapeutic perspectives. Particular emphasis will be placed on current surgical concepts and evolving regenerative techniques that may improve clinical outcomes in oral and maxillofacial surgery practice.

2. Epidemiology

The global prevalence of medication-related osteonecrosis of the jaw (MRONJ) varies considerably depending on the type of medication used, underlying systemic disease, route of administration, duration of therapy, and presence of local risk factors [1,16]. Patients receiving antiresorptive therapy for malignant diseases exhibit substantially higher rates of MRONJ compared with osteoporotic patients receiving low-dose oral medications [17].

Intravenous bisphosphonates such as zoledronic acid and pamidronate are associated with the highest risk of MRONJ development. In oncology patients, reported incidence rates range from approximately 1% to 15%, particularly in patients receiving long-term therapy for metastatic breast cancer, prostate cancer, or multiple myeloma [18,19]. In contrast, patients treated with oral bisphosphonates for osteoporosis generally demonstrate much lower incidence rates, often estimated below 0.1% [20].

Denosumab, a monoclonal antibody targeting receptor activator of nuclear factor kappa-B ligand (RANKL), has also been associated with significant MRONJ risk. Some studies suggest that denosumab-related MRONJ may occur at rates comparable to or even greater than those observed with intravenous bisphosphonates in cancer patients [21]. Unlike bisphosphonates, denosumab does not accumulate in bone tissue, which has implications for drug discontinuation and surgical planning [22].

The incidence of MRONJ increases with treatment duration. Long-term exposure exceeding 4 years has been consistently identified as a major risk factor, particularly when combined with invasive dental procedures or systemic comorbidities [23]. Additionally, concomitant corticosteroid use, diabetes mellitus, smoking, chemotherapy, and antiangiogenic medications further increase susceptibility [24].

Geographic differences in MRONJ prevalence have also been reported. Variations may reflect differences in prescribing patterns, preventive dental protocols, healthcare accessibility, and awareness among clinicians [25]. Improved recognition and updated diagnostic criteria have contributed to increased reporting over recent years.

Importantly, many cases of MRONJ remain underdiagnosed during early stages because initial radiographic or clinical findings may be subtle or nonspecific [26]. Increased awareness among dentists, oral surgeons, oncologists, endocrinologists, and general physicians is therefore essential for early diagnosis and prevention.

The aging global population and expanding indications for antiresorptive therapy suggest that the burden of MRONJ will likely continue to increase in the coming decades [27]. Consequently, prevention, early diagnosis, and multidisciplinary management are becoming increasingly important aspects of oral healthcare delivery.

3. Pharmacology of Antiresorptive and Antiangiogenic Drugs

Understanding the pharmacological mechanisms of medications associated with MRONJ is essential for comprehending disease pathogenesis and therapeutic approaches.

3.1 Bisphosphonates

Bisphosphonates are synthetic analogs of pyrophosphate characterized by a high affinity for hydroxyapatite crystals in bone tissue [28]. These agents inhibit osteoclast-mediated bone resorption and are widely prescribed for osteoporosis, metastatic bone disease, multiple myeloma, Paget's disease, and hypercalcemia of malignancy [29].

Bisphosphonates are generally classified into:

- Non-nitrogen-containing bisphosphonates
- Nitrogen-containing bisphosphonates

Nitrogen-containing bisphosphonates such as zoledronic acid, alendronate, risedronate, and pamidronate are significantly more potent and more commonly associated with MRONJ [30].

These agents inhibit farnesyl pyrophosphate synthase within the mevalonate pathway, resulting in osteoclast apoptosis and profound suppression of bone remodeling [31]. Because bisphosphonates strongly bind to bone mineral, they may remain in skeletal tissues for years after discontinuation [32].

The jawbones are particularly susceptible to bisphosphonate accumulation because of their high remodeling activity and frequent exposure to inflammatory stimuli [33].

3.2 Denosumab

Denosumab is a fully human monoclonal antibody directed against receptor activator of nuclear factor kappa-B ligand (RANKL), a critical mediator of osteoclast differentiation and activation [34].

By inhibiting RANKL, denosumab suppresses osteoclast formation and bone resorption. The medication is widely used for:

- Osteoporosis
- Bone metastases
- Giant cell tumor of bone
- Prevention of skeletal-related events in malignancy

Unlike bisphosphonates, denosumab does not accumulate in bone and exhibits reversible pharmacologic effects after discontinuation [35]. However, MRONJ may still develop because profound suppression of bone turnover impairs healing capacity following oral surgical trauma [36].

Several studies have suggested that denosumab-associated MRONJ may progress more rapidly than bisphosphonate-related disease [37].

4. Pathophysiology of MRONJ

The pathophysiology of medication-related osteonecrosis of the jaw (MRONJ) is complex and multifactorial. Despite significant advances in understanding the disease process, the precise mechanisms responsible for

MRONJ development remain incompletely clarified. Current evidence suggests that several interacting pathways contribute simultaneously, including suppression of bone remodeling, inhibition of angiogenesis, immune dysfunction, inflammation, infection, and soft tissue toxicity [1,38].

The unique anatomical and physiological characteristics of the jaws appear to play a central role in disease susceptibility. Jawbones exhibit significantly higher remodeling activity than other skeletal sites because of constant mechanical loading, mastication, periodontal ligament stimulation, and frequent exposure to oral microorganisms [39]. Consequently, antiresorptive medications may exert disproportionately greater effects on maxillofacial bone metabolism.

4.1 Suppression of Bone Remodeling

Suppression of osteoclast-mediated bone remodeling is considered the primary mechanism underlying MRONJ development [40].

Under physiologic conditions, osteoclasts and osteoblasts maintain dynamic skeletal homeostasis through continuous bone resorption and formation. Antiresorptive medications interfere with this balance by profoundly inhibiting osteoclast activity, thereby reducing bone turnover [41].

Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and induce osteoclast apoptosis [31]. Denosumab prevents osteoclast differentiation by blocking RANKL signaling [34]. Both mechanisms ultimately impair removal of microdamaged bone and reduce regenerative capacity.

In the jaws, where remodeling rates are particularly high, suppressed turnover may lead to accumulation of microfractures and impaired healing following dental extractions or chronic trauma [42]. As necrotic bone accumulates, vascular compromise and secondary infection further exacerbate tissue destruction.

Histopathological studies consistently demonstrate large areas of devitalized bone characterized by empty osteocyte lacunae and absence of viable cellular activity [43].

4.2 Inhibition of Angiogenesis

Impaired angiogenesis represents another important mechanism implicated in MRONJ pathogenesis [44].

Several antiresorptive and antiangiogenic medications reduce vascular endothelial growth factor (VEGF) activity and impair neovascularization [45]. Antiangiogenic agents such as bevacizumab, sunitinib, and sorafenib directly inhibit vascular proliferation and endothelial cell function [46].

Reduced vascularity compromises oxygen delivery, nutrient supply, and tissue repair capacity. Inadequate blood flow may contribute to delayed healing following surgical procedures or chronic mucosal trauma [47].

Some investigators have suggested that the jawbones may already possess relatively vulnerable vascular architecture compared with long bones, thereby increasing susceptibility to ischemic injury [48].

4.3 Infection and Inflammation

Infection plays a major role in both initiation and progression of MRONJ [49].

The oral cavity harbors a highly diverse microbiome containing numerous pathogenic bacteria capable of colonizing exposed bone surfaces [50]. Once mucosal integrity is disrupted, microorganisms may infiltrate underlying bone tissue and establish chronic biofilm-associated infection.

Commonly identified microorganisms include:

- Actinomyces species
- Streptococcus species
- Fusobacterium species
- Prevotella species

Actinomyces colonization is particularly common in histopathologic specimens obtained from MRONJ lesions [51].

Chronic inflammation further amplifies tissue destruction by stimulating release of proinflammatory cytokines such as:

- Tumor necrosis factor-alpha (TNF-α)
- Interleukin-1 beta (IL-1β)
- Interleukin-6 (IL-6)

These mediators contribute to osteocyte apoptosis, osteoclastic dysregulation, and progressive necrosis [52].

Recent studies increasingly support the concept that infection may not merely represent a secondary event but rather a major driving factor in disease progression [53].

4.4 Immune Dysfunction

Immune dysregulation appears to contribute significantly to MRONJ susceptibility and progression [54].

Antiresorptive medications may impair innate and adaptive immune responses by altering macrophage function, T-cell activity, and cytokine production [55]. Additionally, oncology patients frequently exhibit immunosuppression secondary to chemotherapy, corticosteroid use, or underlying malignancy.

Impaired immune surveillance may reduce resistance to oral microbial invasion and compromise tissue repair mechanisms [56].

Macrophages play a particularly important role because they regulate inflammation, angiogenesis, and wound healing. Experimental studies have demonstrated altered macrophage polarization patterns in MRONJ lesions [57].

4.5 Soft Tissue Toxicity

Direct toxicity to oral mucosal cells has also been proposed as a contributing factor [58].

Bisphosphonates may impair proliferation and migration of oral keratinocytes and fibroblasts, resulting in delayed mucosal healing and increased susceptibility to ulceration [59].

Experimental studies have demonstrated:

- Reduced epithelial cell viability
- Delayed fibroblast proliferation
- Impaired collagen synthesis
- Increased apoptosis in oral soft tissues

These effects may facilitate persistent mucosal breakdown and prolonged exposure of underlying bone [60].

4.6 Role of Local Trauma

Local trauma remains one of the most important precipitating factors for MRONJ [61].

Tooth extraction is consistently identified as the most common triggering event, accounting for approximately 60–80% of cases [62]. Other local traumatic factors include:

- Periodontal surgery
- Dental implant placement
- Ill-fitting dentures
- Periapical infection
- Sharp bony prominences
- Chronic mastication-related microtrauma

Because antiresorptive medications impair normal healing mechanisms, even minor trauma may result in prolonged mucosal disruption and subsequent bone exposure [63].

Interestingly, spontaneous MRONJ may occur without identifiable trauma, suggesting that endogenous remodeling suppression alone may occasionally be sufficient to initiate disease [64].

4.7 Genetic and Molecular Factors

Recent evidence suggests that genetic predisposition may influence individual susceptibility to MRONJ [65].

Polymorphisms involving genes associated with:

- Bone remodeling
- Collagen synthesis
- Angiogenesis
- Inflammatory pathways

have been investigated.

Potentially implicated genes include:

- VEGF
- CYP2C8
- RANK
- OPG
- MMP-2

However, current evidence remains inconsistent and further studies are needed before genetic screening can be implemented clinically [66].

Molecular investigations have also identified alterations in:

- Osteocyte signaling
- Oxidative stress pathways
- Mitochondrial function
- Apoptotic mechanisms

These findings may eventually facilitate development of targeted biologic therapies [67].

4.8 Contemporary Multifactorial Model

Current understanding supports a multifactorial disease model in which multiple pathogenic mechanisms interact simultaneously rather than independently [68].

In most patients, MRONJ likely develops through the following sequence:

1. Antiresorptive therapy suppresses bone remodeling
2. Local trauma or infection disrupts mucosal integrity
3. Impaired angiogenesis and immune dysfunction delay healing
4. Bacterial colonization and inflammation progress
5. Necrotic bone accumulates
6. Chronic nonhealing exposure develops

This integrated model explains the heterogeneity of clinical presentations and therapeutic responses observed among MRONJ patients [69].

Continued investigation into disease pathogenesis remains essential for development of more predictable preventive and therapeutic strategies.

5. Risk Factors

Medication-related osteonecrosis of the jaw develops through the interaction of systemic, pharmacologic, and local risk factors. Identification of susceptible patients is critically important for prevention and early intervention [1].

Risk factors can generally be classified into:

- Medication-related factors
- Local oral factors
- Systemic and demographic factors

- Genetic and lifestyle-related factors

Understanding these variables enables clinicians to perform more accurate risk stratification before invasive dental procedures.

5.1 Medication-Related Risk Factors

5.1.1 Type of Medication

The type of antiresorptive or antiangiogenic medication significantly influences MRONJ risk [70].

Nitrogen-containing intravenous bisphosphonates such as:

- Zoledronic acid
- Pamidronate

are associated with the highest incidence rates because of their potency and prolonged skeletal retention [71].

Denosumab also demonstrates substantial MRONJ risk, particularly in oncology patients receiving high-dose regimens [72].

Antiangiogenic agents including:

- Bevacizumab
- Sunitinib
- Sorafenib
- Cabozantinib

may independently contribute to MRONJ development or synergistically increase risk when combined with antiresorptive therapy [73].

5.1.2 Duration of Therapy

Longer duration of medication exposure significantly increases MRONJ risk [74].

Patients receiving therapy for more than four years demonstrate markedly higher incidence rates compared with short-term users [75].

Prolonged suppression of bone remodeling may result in cumulative microdamage and impaired healing capacity over time [76].

5.1.3 Route of Administration

Intravenous administration is associated with substantially greater risk than oral administration [77].

Cancer patients receiving intravenous bisphosphonates may demonstrate MRONJ prevalence rates up to 100-fold greater than osteoporotic patients treated orally [78].

This difference likely reflects:

- Higher drug potency
- Greater cumulative dosage
- Underlying malignancy

- Concurrent chemotherapy

5.2 Local Risk Factors

Local oral conditions and dentoalveolar trauma play a central role in MRONJ development. Most cases occur following disruption of mucosal integrity or direct injury to underlying bone [79].

5.2.1 Tooth Extraction

Tooth extraction is the single most important local risk factor and is associated with approximately 60–80% of MRONJ cases [62].

Post-extraction wounds require active bone remodeling for normal healing. In patients receiving antiresorptive medications, suppression of osteoclastic activity may impair socket healing and predispose to prolonged bone exposure [80].

Inflammation and bacterial contamination from pre-existing periodontal or periapical disease further increase susceptibility [81].

5.2.2 Periodontal and Periapical Disease

Chronic periodontal inflammation and odontogenic infections significantly increase MRONJ risk [82].

Periodontal disease contributes to:

- Chronic bacterial colonization
- Bone destruction
- Soft tissue inflammation
- Increased local cytokine production

Similarly, untreated periapical lesions may induce chronic inflammatory responses that compromise local bone metabolism [83].

Several studies suggest that underlying infection itself may represent a stronger risk factor than the extraction procedure alone [84].

5.2.3 Dental Implants

The relationship between dental implants and MRONJ remains controversial [85].

Implant placement may trigger MRONJ because osseointegration requires active bone remodeling. Although implant survival rates are generally acceptable in low-risk osteoporotic patients receiving oral bisphosphonates, implant-related MRONJ has been increasingly reported [86].

Potential implant-associated risk factors include:

- Peri-implantitis
- Surgical trauma
- Long-term antiresorptive therapy
- Poor oral hygiene

- Concurrent systemic disease

Late-onset MRONJ may occur years after successful implant integration, particularly after initiation of antiresorptive medications [87].

5.2.4 Prosthetic Trauma

Ill-fitting removable prostheses may produce chronic mucosal ulceration and repeated microtrauma, particularly along thin mucosal areas such as the lingual mandible [88].

Persistent mucosal injury may facilitate bacterial penetration and secondary bone exposure.

Denture-related trauma is more frequently associated with mandibular MRONJ because mandibular mucosa tends to be thinner and more susceptible to mechanical irritation [89].

5.2.5 Anatomical Factors

Certain anatomical regions demonstrate increased vulnerability to MRONJ development [90].

Commonly affected sites include:

- Posterior mandible
- Mylohyoid ridge
- Torus mandibularis
- Maxillary posterior alveolus

These regions may experience increased mechanical loading, thinner mucosal coverage, or reduced vascularity [91].

5.3 Systemic Risk Factors

5.3.1 Malignancy

Underlying malignancy significantly increases MRONJ risk [92].

Cancer patients often receive:

- High-dose intravenous antiresorptives
- Chemotherapy
- Corticosteroids
- Antiangiogenic agents

Additionally, malignancy-related immunosuppression and nutritional compromise may impair wound healing [93].

Multiple myeloma and metastatic breast cancer patients exhibit particularly high MRONJ prevalence [94].

5.3.2 Diabetes Mellitus

Diabetes mellitus has been associated with impaired wound healing and increased susceptibility to infection [95].

Hyperglycemia may contribute to:

- Microvascular compromise
- Reduced neutrophil function
- Increased inflammatory cytokine production

These factors may potentiate osteonecrosis development following oral surgery [96].

5.3.3 Corticosteroid Therapy

Long-term corticosteroid use impairs immune responses, angiogenesis, and collagen synthesis [97].

Corticosteroids may synergistically increase MRONJ risk when combined with antiresorptive therapy [98].

5.3.4 Smoking and Alcohol Use

Smoking negatively affects vascularity and tissue oxygenation while impairing immune function and wound healing [99].

Similarly, excessive alcohol consumption may contribute to nutritional deficiencies and immunologic dysfunction [100].

Both habits are considered important modifiable risk factors.

5.3.5 Advanced Age

Older patients demonstrate increased MRONJ risk because of:

- Reduced regenerative capacity
- Polypharmacy
- Increased systemic comorbidities
- Prolonged medication exposure

Age-related changes in bone metabolism may further impair healing responses [101].

5.4 Genetic and Molecular Risk Factors

Genetic susceptibility may partially explain why only a subset of patients exposed to antiresorptive medications develop MRONJ [65].

Polymorphisms involving genes related to:

- Bone remodeling
- Angiogenesis
- Inflammatory regulation
- Drug metabolism

have been investigated.

Potentially associated genes include:

- VEGF
- CYP2C8

- RANK
- OPG
- COL1A1

However, current evidence remains insufficient for routine clinical application [66].

5.5 Preventive Risk Assessment

Comprehensive pre-treatment dental evaluation is essential for minimizing MRONJ risk [102].

Preventive strategies include:

- Elimination of active infection
- Extraction of hopeless teeth before antiresorptive therapy
- Periodontal stabilization
- Optimization of oral hygiene
- Regular dental follow-up

Multidisciplinary collaboration among oral surgeons, oncologists, endocrinologists, and restorative dentists is critical for successful prevention [103].

6. Clinical Presentation

The clinical presentation of medication-related osteonecrosis of the jaw is highly variable and may range from asymptomatic exposed bone to severe destructive lesions associated with pathologic fracture and extensive soft tissue involvement [1].

Disease progression is often chronic and unpredictable. Some patients remain stable for prolonged periods, whereas others experience rapid deterioration despite treatment [104].

6.1 Early Clinical Findings

Early MRONJ lesions may be subtle and nonspecific [105].

Patients may initially complain of:

- Mild discomfort
- Tooth mobility
- Altered sensation
- Gingival swelling
- Delayed healing after extraction
- Halitosis

In some cases, radiographic abnormalities precede clinical bone exposure [106].

Because early signs may mimic common odontogenic infections, diagnosis may be delayed.

6.2 Exposed Necrotic Bone

Exposed necrotic bone remains the hallmark clinical feature of MRONJ [1].

Typically, exposed bone appears:

- Yellowish-white
- Rough and irregular
- Nonhealing
- Surrounded by inflamed mucosa

Bone exposure may persist for weeks or months despite conservative therapy [107].

The posterior mandible is the most commonly affected site, although maxillary lesions also occur [108].

6.3 Pain and Infection

Pain severity varies considerably among patients [109].

Asymptomatic lesions may occur during early stages, whereas advanced disease frequently causes:

- Severe pain
- Suppuration
- Swelling
- Cellulitis
- Abscess formation

Secondary bacterial infection commonly contributes to symptom progression [110].

Purulent discharge and halitosis are frequent clinical findings.

6.4 Soft Tissue Changes

Soft tissue involvement may include:

- Mucosal ulceration
- Erythema
- Fistula formation
- Edema
- Gingival inflammation

Persistent mucosal breakdown contributes to continued bacterial contamination and delayed healing [111].

Extraoral fistulas may develop in advanced stage 3 disease.

6.5 Neurosensory Disturbances

Inferior alveolar nerve involvement may result in:

- Paresthesia
- Hypoesthesia
- Dysesthesia

- "Numb chin syndrome"

These findings often indicate progressive mandibular involvement [112].

6.6 Pathologic Fracture

Advanced osteonecrosis may severely weaken mandibular integrity, resulting in pathologic fracture [113].

Fracture risk increases in patients with:

- Extensive osteolysis
- Chronic infection
- Full-thickness cortical involvement

Pathologic fracture is considered a defining feature of advanced stage 3 disease [1].

6.7 Maxillary Involvement

Maxillary MRONJ may involve:

- Oroantral communication
- Chronic sinusitis
- Nasal discharge
- Maxillary sinus infection

CBCT imaging frequently demonstrates sinus mucosal thickening or bony destruction extending into the sinus cavity [114].

6.8 Clinical Course

The clinical course of MRONJ is highly variable [115].

Some lesions remain stable for years, while others progressively enlarge despite therapy.

Factors associated with poor prognosis include:

- Extensive bone involvement
- Persistent infection
- Immunosuppression
- Ongoing antiresorptive therapy
- Delayed diagnosis

Early intervention may improve long-term outcomes [116].

7. AAOMS and International Classifications

Classification systems are essential for diagnosis, treatment planning, communication among clinicians, and outcome assessment [117].

The American Association of Oral and Maxillofacial Surgeons (AAOMS) classification remains the most widely used staging system worldwide [1].

7.1 At-Risk Category

Patients receiving antiresorptive or antiangiogenic therapy without clinical evidence of necrotic bone are classified as “at risk” [1].

These individuals require preventive dental management and regular follow-up.

7.2 Stage 0

Stage 0 includes patients without exposed bone but with nonspecific symptoms or radiographic abnormalities [118].

Clinical findings may include:

- Odontalgia
- Dull mandibular pain
- Sinus discomfort
- Tooth loosening

Radiographic findings may include:

- Osteosclerosis
- Thickened lamina dura
- Delayed socket healing

Diagnosis at this stage remains challenging because symptoms are nonspecific [119].

7.3 Stage 1

Stage 1 MRONJ is characterized by exposed and necrotic bone, or fistulas that probe to bone, in asymptomatic patients without evidence of active infection [1].

Typical clinical findings include:

- Localized exposed bone
- Minimal surrounding inflammation
- Absence of suppuration
- No significant pain

Many patients are diagnosed incidentally during routine dental examination [120].

Radiographic changes may be limited during early stage 1 disease, although subtle osteosclerosis or cortical irregularities may already be present [121].

Management generally includes:

- Antibacterial mouth rinses
- Clinical monitoring
- Patient education
- Optimization of oral hygiene

However, recent evidence suggests that early surgical intervention may improve outcomes in selected cases [122].

7.4 Stage 2

Stage 2 MRONJ involves exposed necrotic bone associated with pain and clinical evidence of infection [1].

Common findings include:

- Painful exposed bone
- Erythema
- Purulent drainage
- Soft tissue swelling
- Halitosis

Radiographic findings often demonstrate:

- Mixed osteolytic and sclerotic areas
- Sequestrum formation
- Cortical disruption

Patients frequently experience impaired mastication and reduced quality of life [123].

Traditional treatment approaches emphasized conservative management with systemic antibiotics and chlorhexidine rinses [124]. However, increasing evidence supports operative intervention because prolonged conservative therapy may allow disease progression [125].

7.5 Stage 3

Stage 3 disease represents advanced MRONJ with extensive necrosis extending beyond alveolar bone [1].

Clinical findings may include:

- Extensive exposed bone
- Pathologic fracture
- Extraoral fistula
- Oroantral communication
- Inferior border involvement
- Severe pain and infection

Radiographic imaging often reveals diffuse osteolysis, cortical destruction, and large sequestra [126].

Stage 3 lesions may severely impair:

- Mastication
- Speech
- Nutrition
- Facial aesthetics
- Psychological wellbeing

Surgical resection is frequently required for advanced disease control [127].

7.6 Alternative Classification Systems

Several alternative staging systems have been proposed to improve diagnostic accuracy and therapeutic guidance [128].

SICMF-SIPMO Classification

The Italian Society of Maxillofacial Surgery and Italian Society of Oral Pathology and Medicine (SICMF-SIPMO) introduced a radiology-based classification emphasizing:

- Extent of bone involvement
- Presence of complications
- Surgical planning considerations

This system incorporates computed tomography findings and may better reflect true disease extent [129].

Dimensional Staging Systems

Some investigators advocate dimensional staging based on:

- Lesion size
- Cortical involvement
- Depth of necrosis
- Anatomic spread

These systems may facilitate surgical planning and prognostic assessment [130].

7.7 Limitations of Current Classifications

Despite widespread use, existing classification systems possess several limitations [131].

Major concerns include:

- Underestimation of disease extent
- Limited radiographic integration
- Inadequate prognostic value
- Poor correlation with biologic activity

Moreover, significant variability exists between clinical and radiographic findings [132].

Future classification systems may incorporate:

- Molecular biomarkers
- Advanced imaging
- Artificial intelligence-assisted analysis
- Dynamic disease activity scoring

Such developments may improve individualized treatment planning [133].

8. Diagnostic Criteria and Clinical Evaluation

Accurate diagnosis of MRONJ requires careful integration of medical history, clinical findings, radiographic imaging, and exclusion of other pathologic conditions [134].

According to the AAOMS 2022 update, diagnosis requires all three of the following criteria [1]:

1. Current or previous treatment with antiresorptive or antiangiogenic agents
2. Exposed bone or bone that can be probed through an intraoral or extraoral fistula persisting for more than 8 weeks
3. No history of radiation therapy to the jaws or metastatic disease involving the jaws

8.1 Medical History

Comprehensive medical history is essential for identifying at-risk patients [135].

Important considerations include:

- Type of medication
- Route of administration
- Duration of therapy
- Underlying systemic disease
- Concurrent chemotherapy
- Corticosteroid use
- Smoking history
- Previous oral surgical procedures

Clinicians should carefully document all antiresorptive and antiangiogenic medications because patients may not recognize their importance [136]

8.2 Clinical Examination

Clinical examination should assess:

- Bone exposure
- Soft tissue inflammation
- Suppuration
- Tooth mobility
- Fistula formation
- Prosthesis-related trauma
- Neurosensory deficits

Palpation may identify cortical expansion or sequestra [137].

Because MRONJ lesions may be multifocal, thorough intraoral examination is mandatory.

8.3 Differential Diagnosis

Several conditions may clinically resemble MRONJ and should be excluded [138].

Differential diagnosis includes:

- Chronic osteomyelitis
- Osteoradionecrosis
- Metastatic disease
- Primary bone tumors
- Periodontal disease
- Alveolar osteitis
- Fibro-osseous lesions

Distinguishing MRONJ from metastatic disease is particularly important in oncology patients [139].

8.4 Histopathological Findings

Histopathological examination is not always necessary but may help exclude malignancy or other diseases [140].

Typical microscopic findings include:

- Necrotic bone
- Empty osteocyte lacunae
- Bacterial colonization
- Chronic inflammatory infiltrate
- Sequestrum formation

Actinomyces colonies are commonly observed [141].

Biopsy should be performed cautiously because additional trauma may worsen lesions.

9. Imaging in MRONJ

Radiologic imaging plays a fundamental role in:

- Early diagnosis
- Disease staging
- Surgical planning
- Follow-up evaluation

Conventional clinical examination frequently underestimates true disease extent [142].

9.1 Panoramic Radiography

Panoramic radiography is commonly used as an initial imaging modality because of its accessibility and low radiation exposure [143].

Typical findings include:

- Osteosclerosis

- Osteolysis
- Thickened lamina dura
- Persistent extraction sockets
- Sequestrum formation

However, panoramic imaging may fail to detect early or limited lesions [144].

9.2 Cone-Beam Computed Tomography (CBCT)

CBCT has become the preferred imaging modality for MRONJ evaluation in oral and maxillofacial surgery [145].

Advantages include:

- High spatial resolution
- Three-dimensional evaluation
- Accurate cortical assessment
- Lower radiation dose compared with conventional CT

CBCT findings may include:

- Cortical perforation
- Medullary sclerosis
- Sequestra
- Periosteal reaction
- Inferior alveolar canal involvement

CBCT is particularly valuable for determining surgical margins [146].

9.3 Computed Tomography (CT)

Conventional CT imaging provides superior evaluation of extensive lesions involving:

- Deep facial spaces
- Pathologic fractures
- Maxillary sinus extension

CT may be preferred in advanced stage 3 disease [147].

9.4 Magnetic Resonance Imaging (MRI)

MRI is useful for evaluating:

- Bone marrow involvement
- Soft tissue inflammation
- Early inflammatory changes

MRI findings may precede visible bone exposure in some patients [148].

However, MRI is less effective than CT for demonstrating cortical destruction.

9.5 Nuclear Imaging

Bone scintigraphy and positron emission tomography (PET) may identify metabolic abnormalities before structural changes become apparent [149].

Increased radionuclide uptake may indicate:

- Early inflammation
- Active bone remodeling
- Disease progression

These modalities remain primarily investigational in routine MRONJ management [150].

9.6 Imaging-Based Surgical Planning

Modern surgical planning increasingly relies on advanced imaging to define:

- Necrotic bone extent
- Viable bleeding margins
- Cortical integrity
- Adjacent anatomical structures

Three-dimensional imaging facilitates more precise surgical resection and reconstruction [151].

10. Histopathological Features

Histopathological examination of MRONJ lesions provides important insights into disease pathogenesis and progression [152].

Although histology is not mandatory for diagnosis, characteristic microscopic findings are consistently observed.

10.1 Necrotic Bone

The hallmark histopathologic feature is necrotic bone characterized by:

- Empty osteocyte lacunae
- Loss of viable cellular elements
- Absence of normal remodeling activity

Bone trabeculae frequently appear fragmented and devitalized [153].

10.2 Bacterial Colonization

Extensive bacterial colonization commonly covers exposed bone surfaces [154].

Actinomyces species are particularly frequent and may form characteristic sulfur granules within necrotic tissue [155].

Bacterial biofilms contribute to chronic inflammation and resistance to therapy.

10.3 Inflammatory Infiltrate

Surrounding soft tissues often demonstrate:

- Chronic inflammatory infiltrates
- Neutrophils
- Lymphocytes
- Plasma cells

Inflammation may extend deeply into adjacent marrow spaces [156].

11. Prevention Strategies

Prevention remains the most effective approach in the management of medication-related osteonecrosis of the jaw (MRONJ). Because treatment outcomes may be unpredictable and advanced disease can cause severe morbidity, identification and modification of risk factors before initiation of antiresorptive therapy are critically important [157].

Current guidelines strongly emphasize multidisciplinary preventive care involving:

- Oral and maxillofacial surgeons
- Dentists
- Oncologists
- Endocrinologists
- Medical oncologists
- Prosthodontists
- Dental hygienists

Preventive protocols significantly reduce MRONJ incidence, particularly in oncology patients receiving high-dose intravenous antiresorptive therapy [158].

11.1 Pre-Treatment Dental Evaluation

Comprehensive dental examination should ideally be performed before initiation of antiresorptive or antiangiogenic therapy [159].

Pre-treatment evaluation should include:

- Clinical examination
- Panoramic radiography
- Periodontal assessment
- Evaluation of prostheses
- Caries detection
- Assessment of nonrestorable teeth

Potential sources of infection should be eliminated before therapy begins [160].

Recommended interventions may include:

- Extraction of hopeless teeth
- Periodontal therapy

- Endodontic treatment
- Adjustment of ill-fitting dentures
- Restoration of carious lesions

Allowing sufficient mucosal healing before initiation of antiresorptive therapy is recommended whenever medically feasible [161].

11.2 Oral Hygiene Maintenance

Poor oral hygiene and chronic periodontal inflammation significantly increase MRONJ risk [162].

Patients should receive detailed oral hygiene instruction emphasizing:

- Tooth brushing
- Interdental cleaning
- Antimicrobial mouth rinses
- Regular professional prophylaxis

Long-term maintenance programs are essential for minimizing inflammatory oral disease [163].

11.3 Avoidance of Invasive Procedures

Whenever possible, invasive dentoalveolar procedures should be minimized in patients receiving antiresorptive medications [164].

Alternative treatment strategies may include:

- Endodontic therapy instead of extraction
- Conservative periodontal management
- Coronectomy in selected cases

However, avoidance of necessary treatment may allow chronic infection to progress and potentially increase MRONJ risk [165].

Therefore, clinical decisions must balance surgical risk against risks associated with untreated infection.

11.4 Drug Holiday Controversy

Temporary discontinuation of antiresorptive therapy before invasive dental procedures remains controversial [166].

Some clinicians advocate drug holidays to improve bone healing capacity, particularly in patients receiving denosumab because of its reversible pharmacologic effects [167].

However, evidence supporting drug holidays remains limited and inconsistent [168].

Concerns include:

- Increased fracture risk
- Skeletal-related complications
- Cancer progression

The AAOMS position paper acknowledges insufficient evidence to establish universal recommendations regarding drug holidays [1].

Clinical decisions should therefore be individualized through multidisciplinary consultation.

11.5 Preventive Surgical Protocols

When dentoalveolar surgery is unavoidable, several preventive measures may reduce MRONJ risk [169].

Recommended surgical principles include:

- Atraumatic technique
- Minimal periosteal stripping
- Smooth bone contouring
- Primary tension-free closure
- Copious irrigation
- Infection control

Adjunctive therapies such as platelet-rich fibrin (PRF), antibiotics, and photobiomodulation may further improve healing [170].

11.6 Patient Education

Patient awareness plays an essential role in prevention [171].

Patients should be informed regarding:

- Potential MRONJ risk
- Importance of oral hygiene
- Need for regular dental follow-up
- Early signs and symptoms
- Importance of reporting oral discomfort

Education improves compliance and facilitates early diagnosis [172].

12. Conservative Management

Conservative therapy has historically represented the first-line treatment approach for MRONJ, particularly in early-stage disease [173].

The primary objectives of conservative management are:

- Pain control
- Infection reduction
- Limitation of disease progression
- Maintenance of oral function
- Improvement of quality of life

Conservative treatment may be particularly appropriate for:

- Medically compromised patients
- Elderly individuals
- Patients with limited lesions

- Asymptomatic disease

However, prolonged conservative therapy may not eliminate necrotic bone and often fails to achieve complete resolution [174].

12.1 Antimicrobial Mouth Rinses

Chlorhexidine gluconate mouth rinses are widely used for reducing bacterial load and controlling local inflammation [175].

Typical regimens involve:

- 0.12% chlorhexidine
- Twice daily use

Chlorhexidine may help reduce:

- Halitosis
- Soft tissue inflammation
- Superficial infection

However, antimicrobial rinses alone rarely result in complete healing [176].

12.2 Systemic Antibiotic Therapy

Systemic antibiotics are commonly prescribed for symptomatic MRONJ associated with infection [177].

Commonly used antibiotics include:

- Penicillin
- Amoxicillin-clavulanate
- Clindamycin
- Metronidazole
- Doxycycline

Antibiotic selection should consider:

- Bacterial culture findings
- Allergies
- Severity of infection
- Patient comorbidities

Long-term antibiotic therapy may reduce pain and inflammation but may also contribute to antimicrobial resistance [178].

12.3 Pain Management

Pain control is an important component of conservative therapy [179].

Analgesic strategies may include:

- Nonsteroidal anti-inflammatory drugs
- Acetaminophen
- Opioids in severe cases

Adequate pain control significantly improves patient quality of life.

12.4 Superficial Debridement

Limited superficial debridement may help remove irritating loose sequestra and reduce soft tissue trauma [180].

Debridement should remain conservative to avoid unnecessary extension of exposed bone.

Removal of mobile necrotic fragments may improve:

- Pain
- Soft tissue healing
- Oral hygiene maintenance

12.5 Outcomes of Conservative Therapy

Complete mucosal healing following conservative treatment alone is relatively uncommon in advanced disease [181].

Reported limitations include:

- Persistent exposed bone
- Chronic infection
- Recurrent symptoms
- Disease progression

Consequently, contemporary treatment paradigms increasingly favor operative intervention, particularly for stage 2 and stage 3 lesions [182].

13. Surgical Management

Surgical treatment has become increasingly accepted as a predictable and effective therapeutic approach for MRONJ [183].

Recent evidence demonstrates that complete removal of necrotic bone with achievement of healthy bleeding margins may produce significantly improved outcomes compared with prolonged conservative therapy [184].

The AAOMS 2022 position paper supports operative management across all disease stages in appropriately selected patients [1].

13.1 Objectives of Surgical Treatment

The major goals of surgery include:

- Removal of necrotic bone
- Elimination of infection
- Achievement of mucosal closure
- Restoration of oral function
- Prevention of disease progression

Successful surgery requires identification of viable bone margins and careful soft tissue management [185].

13.2 Surgical Timing

Optimal timing of surgical intervention remains debated [186].

Earlier treatment philosophies recommended delaying surgery until sequestration occurred. However, contemporary evidence increasingly supports early operative intervention before extensive progression develops [187].

Early surgery may:

- Reduce disease burden
- Prevent pathologic fracture
- Improve quality of life
- Decrease chronic infection

13.3 Sequestrectomy

Sequestrectomy involves removal of loose necrotic bone fragments without extensive resection [188].

Indications include:

- Small localized sequestra
- Mobile necrotic bone
- Limited stage 1 or stage 2 lesions

Advantages include:

- Minimal invasiveness
- Reduced morbidity
- Preservation of surrounding structures

However, incomplete removal may allow persistent disease [189].

13.4 Surgical Debridement

Debridement aims to remove superficial necrotic tissue while preserving viable bone [190].

Debridement may be combined with:

- Peripheral ostectomy
- PRF application
- Laser therapy
- Antimicrobial protocols

The procedure may reduce bacterial contamination and stimulate healing [191].

13.5 Marginal Resection

Marginal resection involves removal of necrotic bone while maintaining mandibular continuity [192].

This approach is increasingly favored for:

- Moderate disease
- Cortical involvement

- Persistent symptomatic lesions

Surgical margins should extend into bleeding viable bone [193].

Studies demonstrate high rates of mucosal healing following adequate resection [194].

13.6 Segmental Resection

Segmental resection is reserved for advanced stage 3 disease associated with:

- Pathologic fracture
- Extensive osteolysis
- Inferior border involvement
- Refractory infection

Although aggressive, segmental resection may provide definitive disease control in severe cases [195].

Reconstruction may involve:

- Reconstruction plates
- Free vascularized bone flaps
- Local soft tissue flaps

13.7 Soft Tissue Closure and Flap Design

Successful soft tissue management is one of the most critical determinants of surgical outcome in MRONJ treatment [196].

Primary tension-free closure reduces:

- Bone re-exposure
- Bacterial contamination
- Delayed healing
- Postoperative pain

Commonly used flap techniques include:

- Mucoperiosteal advancement flaps
- Buccal fat pad flaps
- Mylohyoid flaps
- Nasolabial flaps
- Temporalis muscle flaps

Adequate periosteal release is essential for tension-free closure [197].

In extensive maxillary defects, buccal fat pad flaps provide reliable vascularized soft tissue coverage with relatively low morbidity [198].

13.8 Fluorescence-Guided Surgery

Fluorescence-guided surgery has emerged as a promising method for intraoperative differentiation between viable and necrotic bone [199].

Techniques may involve:

- Tetracycline fluorescence

- Autofluorescence systems

Viable bone demonstrates fluorescence, whereas necrotic tissue exhibits reduced or absent fluorescence [200].

Potential advantages include:

- More accurate surgical margins
- Preservation of healthy bone
- Reduced recurrence rates

Several studies report improved healing outcomes with fluorescence-assisted resection [201].

13.9 Piezoelectric Surgery

Piezoelectric devices allow selective bone cutting while minimizing soft tissue trauma [202].

Advantages may include:

- Reduced thermal injury
- Improved precision
- Enhanced postoperative healing
- Decreased soft tissue damage

Piezoelectric surgery may be particularly useful near critical structures such as the inferior alveolar nerve [203].

13.10 Surgical Outcomes

Contemporary literature increasingly supports surgical treatment as the most predictable modality for achieving complete mucosal healing [204].

Reported success rates vary depending on:

- Disease stage
- Surgical extent
- Systemic conditions
- Adjunctive therapies

Many studies report healing rates exceeding 80–90% following adequate surgical resection [205].

Factors associated with improved outcomes include:

- Early intervention
- Complete necrotic bone removal
- Primary closure
- Adjunctive regenerative therapies
- Strict postoperative follow-up

14. Surgical Reconstruction

Advanced MRONJ lesions may require reconstructive procedures following extensive resection [206].

The goals of reconstruction include:

- Restoration of mandibular continuity
- Functional rehabilitation
- Facial aesthetic restoration
- Improvement of speech and mastication

Reconstruction planning should consider:

- Patient age
- Systemic condition
- Defect size
- Prognosis
- Oncologic status

14.1 Reconstruction Plates

Titanium reconstruction plates are commonly used following segmental mandibular resection [207].

Advantages include:

- Immediate stabilization
- Shorter operative time
- Reduced surgical morbidity

However, plate exposure and hardware failure remain potential complications [208].

14.2 Free Vascularized Bone Flaps

Free vascularized bone flaps provide the most comprehensive reconstructive option for extensive defects [209].

Common donor sites include:

- Fibula
- Iliac crest
- Scapula

Advantages include:

- Excellent vascularity
- Large bone volume
- Long-term functional stability

Fibular free flaps remain the gold standard for mandibular reconstruction because they provide adequate bone length and permit future implant rehabilitation [210].

However, free flap reconstruction may not be appropriate for medically compromised oncology patients with poor overall prognosis.

14.3 Local and Regional Flaps

Regional soft tissue flaps may be used in patients unsuitable for microvascular reconstruction [211].

Common options include:

- Buccal fat pad flap
- Temporalis muscle flap
- Pectoralis major flap
- Nasolabial flap

These flaps may improve soft tissue coverage and reduce plate exposure risk [212].

15. Platelet-Rich Fibrin (PRF) and Regenerative Approaches

Regenerative medicine has become increasingly important in MRONJ management [213].

Platelet-rich fibrin (PRF) is one of the most widely investigated biologic adjuncts because of its:

- Autologous origin
- Ease of preparation
- Growth factor content
- Low cost

PRF contains:

- Platelets
- Leukocytes
- Cytokines
- Fibrin matrix

These components promote tissue healing and angiogenesis [214].

15.1 Biological Properties of PRF

PRF releases several growth factors including:

- Platelet-derived growth factor (PDGF)
- Transforming growth factor-beta (TGF-β)
- Vascular endothelial growth factor (VEGF)
- Insulin-like growth factor (IGF)

These mediators stimulate:

- Fibroblast proliferation
- Angiogenesis
- Osteogenesis
- Soft tissue healing

PRF also serves as a biologic scaffold supporting cellular migration and wound stabilization [215].

15.2 Types of PRF

Several PRF modifications have been introduced:

- Leukocyte-rich PRF (L-PRF)
- Advanced PRF (A-PRF)
- Injectable PRF (i-PRF)

A-PRF may provide enhanced leukocyte distribution and prolonged growth factor release [216].

15.3 Clinical Applications in MRONJ

PRF is commonly applied following:

- Debridement
- Sequestrectomy
- Marginal resection

Potential clinical benefits include:

- Accelerated mucosal healing
- Reduced postoperative pain
- Enhanced bone regeneration
- Reduced infection rates

Several studies report improved healing outcomes when PRF is combined with surgery [217].

15.4 Bone Grafts and Biomaterials

Bone grafting in MRONJ remains controversial because graft survival may be compromised in poorly vascularized environments [218].

Nevertheless, selected studies report favorable outcomes using:

- Autogenous bone grafts
- Xenografts
- Alloplastic biomaterials
- Collagen membranes

Combining graft materials with PRF may improve regenerative potential [219].

16. Laser and Photodynamic Therapy

Laser-assisted therapies have gained increasing attention as adjunctive modalities for MRONJ management [220].

These techniques may:

- Reduce bacterial load
- Improve vascularity
- Stimulate healing
- Reduce inflammation

16.1 Photobiomodulation Therapy (PBM)

Photobiomodulation, previously referred to as low-level laser therapy, utilizes low-energy laser irradiation to stimulate cellular activity [221].

Proposed biologic effects include:

- Increased ATP production
- Enhanced angiogenesis
- Reduced inflammatory cytokines
- Stimulation of osteoblastic activity

Clinical studies suggest PBM may:

- Improve postoperative healing
- Reduce pain
- Enhance mucosal closure

However, treatment protocols remain heterogeneous [222].

16.2 Antimicrobial Photodynamic Therapy (aPDT)

aPDT combines:

- Photosensitizing agents
- Laser activation
- Reactive oxygen species generation

This approach reduces bacterial biofilms and local microbial contamination [223].

Potential advantages include:

- Minimal bacterial resistance
- Localized antimicrobial effect
- Reduced inflammation

Combination of aPDT with surgical therapy and PRF has demonstrated promising outcomes [224].

16.3 Er:YAG and Nd:YAG Lasers

High-energy lasers such as Er:YAG and Nd:YAG may assist in:

- Debridement
- Bone ablation
- Surface sterilization

Laser-assisted surgery may reduce thermal injury and improve precision compared with conventional rotary instruments [225].

17. Implant-Related MRONJ

The relationship between dental implants and MRONJ remains an area of ongoing controversy [226].

Implant placement requires active bone remodeling and osseointegration, processes potentially impaired by antiresorptive medications [227].

17.1 Implant Placement in Osteoporotic Patients

Most studies suggest relatively acceptable implant survival rates in low-risk osteoporotic patients receiving oral bisphosphonates [228].

However, risk increases with:

- Long-term therapy
- Corticosteroid use
- Diabetes mellitus
- Smoking

Careful patient selection is essential [229].

17.2 Late-Onset Implant-Related MRONJ

MRONJ may develop around previously successful implants years after antiresorptive therapy initiation [230].

Peri-implantitis appears to play an important triggering role.

Clinical findings may include:

- Implant mobility
- Bone exposure
- Pain
- Suppuration

Management often requires implant removal and surgical debridement [231].

17.3 Current Recommendations

Current guidelines emphasize individualized risk assessment before implant placement [232].

Factors to consider include:

- Drug type
- Treatment duration
- Oncology versus osteoporosis status
- Systemic comorbidities
- Oral hygiene status

Patients should be informed regarding potential long-term risks.

18. Drug Holiday Controversy

Drug holidays remain one of the most debated subjects in MRONJ management [233].

The rationale behind temporary discontinuation is to allow partial recovery of bone remodeling capacity before surgery.

However, evidence remains inconclusive [234].

18.1 Bisphosphonates

Because bisphosphonates remain incorporated within bone for years, short-term discontinuation may have limited biologic effect [235].

Therefore, many investigators question whether drug holidays significantly reduce MRONJ risk in bisphosphonate-treated patients.

18.2 Denosumab

Denosumab differs because its effects are reversible after discontinuation [236].

Some clinicians recommend performing surgery near the end of the dosing interval before the next injection.

However, interruption may increase:

- Vertebral fracture risk
- Skeletal complications

Therefore, decisions should involve multidisciplinary consultation [237].

19. Future Perspectives and Emerging Therapies

Despite major advances in understanding MRONJ, current treatment approaches still demonstrate variable outcomes. Consequently, considerable research is focused on development of biologically targeted and regenerative therapies aimed at improving bone healing and reducing recurrence [238].

Future management strategies will likely integrate:

- Molecular diagnostics
- Regenerative medicine
- Personalized risk assessment
- Artificial intelligence-assisted imaging
- Biologic therapies

19.1 Stem Cell Therapy

Mesenchymal stem cells (MSCs) have attracted increasing interest because of their regenerative and immunomodulatory properties [239].

Potential therapeutic effects include:

- Enhanced angiogenesis
- Promotion of osteogenesis
- Reduction of inflammation
- Improved wound healing

Experimental studies have demonstrated encouraging results using:

- Bone marrow-derived stem cells
- Adipose-derived stem cells
- Dental pulp stem cells

However, clinical application remains limited because of regulatory, technical, and financial challenges [240].

19.2 Teriparatide Therapy

Teriparatide, a recombinant parathyroid hormone analog, stimulates osteoblastic bone formation and increases bone turnover [241].

Several reports suggest that teriparatide may improve healing in osteoporotic patients with MRONJ by:

- Enhancing bone remodeling
- Promoting angiogenesis
- Accelerating osseous regeneration

Nevertheless, teriparatide use remains contraindicated in many oncology patients because of potential oncologic concerns [242].

Further randomized clinical trials are necessary before widespread adoption.

19.3 Exosome-Based Therapies

Exosomes are extracellular vesicles capable of transporting proteins, growth factors, and nucleic acids between cells [243].

Stem cell-derived exosomes may stimulate:

- Tissue regeneration
- Angiogenesis
- Osteoblastic differentiation

Because exosomes avoid many limitations associated with cellular transplantation, they represent a promising future therapeutic strategy [244].

19.4 Tissue Engineering and Biomaterials

Advances in tissue engineering may facilitate reconstruction of large osseous defects following MRONJ resection [245].

Emerging technologies include:

- Three-dimensional printed scaffolds
- Bioactive ceramics
- Growth factor delivery systems
- Customized patient-specific grafts

Combining scaffolds with stem cells and PRF may enhance regenerative potential [246].

19.5 Artificial Intelligence in MRONJ

Artificial intelligence (AI) may significantly influence future MRONJ diagnosis and treatment planning [247].

Potential applications include:

- Automated radiographic detection

- Risk prediction algorithms
- Surgical planning assistance
- Prognostic modeling

Machine learning systems trained using CBCT and clinical datasets may facilitate earlier diagnosis and more individualized management protocols [248].

Given the increasing role of AI in oral and maxillofacial surgery, future integration into MRONJ management appears highly likely.

20. Conclusion

Medication-related osteonecrosis of the jaw remains one of the most challenging complications encountered in oral and maxillofacial surgery. The disease is multifactorial and involves complex interactions among suppressed bone remodeling, infection, angiogenesis inhibition, immune dysfunction, and local trauma [1].

The widespread use of antiresorptive and antiangiogenic medications has resulted in increasing MRONJ prevalence worldwide. Consequently, prevention, early diagnosis, and multidisciplinary management have become increasingly important components of patient care [249].

Recent advances have substantially altered therapeutic concepts. Historically, conservative treatment represented the primary management strategy; however, contemporary evidence increasingly supports operative intervention, particularly when complete necrotic bone removal and tension-free closure can be achieved [250].

Adjunctive regenerative modalities including:

- Platelet-rich fibrin
- Photobiomodulation
- Antimicrobial photodynamic therapy
- Stem cell applications
- Tissue engineering approaches

have demonstrated promising therapeutic potential [251].

Future developments in regenerative medicine, molecular diagnostics, and artificial intelligence may further improve treatment predictability and patient-specific management strategies.

Despite significant progress, several controversies remain unresolved, particularly regarding:

- Drug holidays
- Implant placement
- Optimal surgical timing
- Long-term recurrence prevention

Continued multicenter prospective studies and standardized treatment protocols are necessary to improve evidence-based management of MRONJ.

Ultimately, successful management requires a multidisciplinary approach integrating prevention, accurate diagnosis, individualized treatment planning, and long-term follow-up in order to minimize morbidity and improve patient quality of life.

References

1. Ruggiero SL, Dodson TB, Aghaloo T, et al. American Association of Oral and Maxillofacial Surgeons' Position Paper on Medication-Related Osteonecrosis of the Jaws—2022 Update. J Oral Maxillofac Surg. 2022;80(5):920-943.
2. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa)-induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003;61(9):1115-1117.
3. Yoneda T, Hagino H, Sugimoto T, et al. Antiresorptive agent-related osteonecrosis of the jaw: Position Paper 2017. J Bone Miner Metab. 2017;35(1):6-19.
4. Otto S, Pautke C, Opelz C, et al. Osteonecrosis of the jaw: effect of bisphosphonate type, local concentration, and acidic milieu on the pathomechanism. J Oral Maxillofac Surg. 2010;68(11):2837-2845.
5. Khan AA, Morrison A, Hanley DA, et al. Diagnosis and management of osteonecrosis of the jaw: a systematic review. J Bone Miner Res. 2015;30(1):3-23.
6. Allen MR, Burr DB. The pathogenesis of bisphosphonate-related osteonecrosis of the jaw. Bone. 2009;44(1):1-5.
7. Hinson AM, Smith CW, Siegel ER, et al. Jawbone remodeling and the pathogenesis of osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117(5):499-506.
8. Saad F, Brown JE, Van Poznak C, et al. Incidence, risk factors, and outcomes of osteonecrosis of the jaw. Ann Oncol. 2012;23(5):1341-1347.
9. Miksad RA, Lai KC, Dodson TB, et al. Quality of life implications of osteonecrosis of the jaw. Oncologist. 2011;16(1):121-132.
10. Hellstein JW, Adler RA, Edwards B, et al. Managing the care of patients receiving antiresorptive therapy. J Am Dent Assoc. 2011;142(11):1243-1251.
11. Otto S, Tröltzsch M, Jambrovic V, et al. Tooth extraction in patients receiving antiresorptive therapy. J Craniomaxillofac Surg. 2015;43(7):1181-1187.
12. Tenore G, Mohsen A, Rossi DS, et al. Does leukocyte- and platelet-rich fibrin improve healing? J Clin Med. 2020;9(11):3505.
13. Hanna R, Dalvi S, Benedicenti S, et al. Photobiomodulation therapy in MRONJ management. Pharmaceuticals. 2024;17(8):1011.
14. Aghaloo TL, Felsenfeld AL, Tetradis S. Osteonecrosis of the jaw in a patient on denosumab. J Oral Maxillofac Surg. 2010;68(5):959-963.
15. Yarom N, Shapiro CL, Peterson DE, et al. Medication-related osteonecrosis of the jaw: MASCC/ISOO/ASCO guideline. J Clin Oncol. 2019;37(25):2270-2290.

16. Ristow O, Otto S, Troeltzsch M, et al. Predictive factors in MRONJ. Int J Oral Maxillofac Surg. 2014;43(7):814-820.
17. Bedogni A, Fusco V, Agrillo A, et al. Learning from experience: recommendations to reduce MRONJ risk. Oral Dis. 2012;18(6):621-623.
18. Dodson TB. Intravenous bisphosphonate therapy and bisphosphonate-related osteonecrosis of the jaws. J Oral Maxillofac Surg. 2009;67(5 Suppl):44-52.
19. Dimopoulos MA, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in multiple myeloma patients. J Clin Oncol. 2009;27(36):6038-6045.
20. Lo JC, O'Ryan FS, Gordon NP, et al. Prevalence of osteonecrosis of the jaw in patients with oral bisphosphonate exposure. J Oral Maxillofac Surg. 2010;68(2):243-253.
21. Qi WX, Tang LN, He AN, et al. Risk of osteonecrosis of the jaw in cancer patients receiving denosumab. Int J Clin Oncol. 2014;19(2):403-410.
22. McClung MR. Cancel the denosumab holiday. Osteoporos Int. 2016;27(5):1677-1682.
23. Khosla S, Burr D, Cauley J, et al. Bisphosphonate-associated osteonecrosis of the jaw. J Bone Miner Res. 2007;22(10):1479-1491.
24. Thumbigere-Math V, Sabino MC, Gopalakrishnan R, et al. Bisphosphonate-related osteonecrosis risk factors. Cancer. 2009;115(15):3468-3476.
25. Schiodt M, Otto S, Fedele S, et al. Workshop of European task force on MRONJ. Oral Dis. 2019;25(7):1819-1825.
26. Wilde F, Heufelder M, Lorenz K, et al. Early radiological signs of MRONJ. Clin Oral Investig. 2012;16(4):1189-1198.
27. Khan AA, Morrison A, Kendler DL, et al. International consensus on osteonecrosis of the jaw. J Bone Miner Res. 2015;30(1):3-23.
28. Russell RG. Bisphosphonates: mode of action and pharmacology. Pediatrics. 2007;119(Suppl 2):S150-S162.
29. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. Mayo Clin Proc. 2008;83(9):1032-1045.
30. Ruggiero SL, Fantasia J, Carlson E. Bisphosphonate-related osteonecrosis of the jaw: background and guidelines. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102(4):433-441.
31. Roelofs AJ, Thompson K, Gordon S, Rogers MJ. Molecular mechanisms of action of bisphosphonates. Bone. 2006;42(4):S60-S65.
32. Lin JH. Bisphosphonates: a review of pharmacokinetic properties. Bone. 1996;18(2):75-85.
33. Reid IR. Osteonecrosis of the jaw: who gets it and why? Bone. 2009;44(1):4-10.

34. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423(6937):337-342.
35. Baron R, Ferrari S, Russell RG. Denosumab and bisphosphonates: different mechanisms of action. Bone. 2011;48(4):677-692.
36. Saad F, Brown JE, Van Poznak C, et al. Incidence and risk factors for osteonecrosis of the jaw in cancer patients. Ann Oncol. 2012;23(5):1341-1347.
37. Aghaloo TL, Dry SM, Mallya S, et al. Stage 0 osteonecrosis of the jaw in a patient on denosumab. J Oral Maxillofac Surg. 2014;72(4):702-716.
38. Sedghizadeh PP, Kumar SKS, Gorur A, et al. Identification of microbial biofilms in osteonecrosis of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(3):348-352.
39. Huja SS, Fernandez SA, Hill KJ, et al. Remodeling dynamics in the jaws and long bones. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;101(5):587-593.
40. Allen MR, Burr DB. Bone modeling and remodeling in antiresorptive therapy. Bonekey Osteovision. 2007;4(9):259-265.
41. Odvina CV, Zerwekh JE, Rao DS, et al. Severely suppressed bone turnover. J Clin Endocrinol Metab. 2005;90(3):1294-1301.
42. Mashiba T, Hirano T, Turner CH, et al. Suppressed bone turnover and microdamage accumulation. J Bone Miner Res. 2000;15(4):613-620.
43. Hansen T, Kunkel M, Weber A, et al. Osteonecrosis of the jaws in bisphosphonate-treated patients. J Craniomaxillofac Surg. 2006;34(7):400-409.
44. Santini D, Vincenzi B, Dicuonzo G, et al. Zoledronic acid induces antiangiogenic effects. Oncol Rep. 2003;10(4):997-1000.
45. Wood J, Bonjean K, Ruetz S, et al. Novel antiangiogenic effects of bisphosphonates. Br J Cancer. 2002;86(5):782-788.
46. Guarneri V, Miles D, Robert N, et al. Bevacizumab and osteonecrosis of the jaw. Ann Oncol. 2010;21(1):184-188.
47. Wehrhan F, Hyckel P, Guentsch A, et al. Vascular endothelial growth factor in MRONJ. Clin Oral Investig. 2010;14(4):373-380.
48. Marx RE. Bone vascularity and osteonecrosis pathogenesis. Oral Maxillofac Surg Clin North Am. 2007;19(4):451-460.
49. Pushalkar S, Li Y, Kurago Z, et al. Oral microbiota and osteonecrosis. J Dent Res. 2014;93(5):439-444.
50. Sedghizadeh PP, Yooseph S, Fadrosh DW, et al. Microbial communities in MRONJ. J Oral Microbiol. 2012;4:19006.
51. Hansen T, Kunkel M, Kirkpatrick CJ, et al. Actinomyces in bisphosphonate-related osteonecrosis. J Oral Pathol Med. 2007;36(8):483-489.

52. Di Fede O, Panzarella V, Mauceri R, et al. Pathogenesis and cytokine involvement in MRONJ. Int J Dent. 2018;2018:1-8.
53. Otto S, Schreyer C, Hafner S, et al. Bisphosphonate-related osteonecrosis and infection. J Oral Maxillofac Surg. 2012;70(12):2785-2796.
54. Kikuiri T, Kim I, Yamaza T, et al. Cell-based immunotherapy in osteonecrosis. J Bone Miner Res. 2010;25(7):1668-1679.
55. Pabst AM, Ziebart T, Koch FP, et al. Immunological alterations in MRONJ. Clin Oral Investig. 2014;18(2):615-623.
56. Thumbigere-Math V, Michalowicz BS, Hughes PJ, et al. Periodontal disease and osteonecrosis risk. J Periodontol. 2014;85(2):226-233.
57. Qing Y, Zhang Q, Wang Y, et al. Macrophage polarization in MRONJ lesions. Bone. 2021;143:115761.
58. Reid IR, Cornish J. Epidemiology and pathogenesis of osteonecrosis. Nat Rev Rheumatol. 2012;8(2):90-96.
59. Landesberg R, Woo V, Cremers S, et al. Potential pathophysiological mechanisms in osteonecrosis. Ann N Y Acad Sci. 2011;1218:62-79.
60. Scheper MA, Badros A, Chaisuparat R, et al. Effect of bisphosphonates on oral fibroblasts. J Oral Pathol Med. 2009;38(7):551-559.
61. Filleul O, Crompot E, Saussez S. Bisphosphonate-induced osteonecrosis. J Oral Maxillofac Surg. 2010;68(5):938-947.
62. Marx RE, Cillo JE Jr, Ulloa JJ. Oral bisphosphonate-induced osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103(3):e1-e5.
63. Hoefert S, Eufinger H. Relevance of surgical trauma in MRONJ. Clin Oral Investig. 2011;15(6):863-869.
64. Mawardi H, Enzinger P, McCleary N, et al. Spontaneous osteonecrosis of the jaw. Oral Oncol. 2009;45(4-5):433-436.
65. Sarasquete ME, González M, San Miguel JF, et al. Genetic factors in osteonecrosis susceptibility. Haematologica. 2008;93(2):257-264.
66. Nicoletti P, Cartsos VM, Palaska PK, et al. Genomewide pharmacogenetics of osteonecrosis. J Bone Miner Res. 2012;27(2):317-325.
67. Li C, Mori S, Li J, et al. Molecular pathways in MRONJ. Bone. 2015;81:469-477.
68. Otto S, Pautke C, Van den Wyngaert T, et al. Pathogenesis of medication-related osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125(5):409-417.
69. Bedogni A, Saia G, Bettini G, et al. Long-term outcomes in MRONJ. Oral Oncol. 2011;47(5):420-424.

70. Bamias A, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates. J Clin Oncol. 2005;23(34):8580-8587.
71. Hoff AO, Toth BB, Altundag K, et al. Frequency and risk factors associated with osteonecrosis of the jaw. J Bone Miner Res. 2008;23(6):826-836.
72. Stopeck AT, Lipton A, Body JJ, et al. Denosumab compared with zoledronic acid in metastatic breast cancer. J Clin Oncol. 2010;28(35):5132-5139.
73. Estilo CL, Fornier M, Farooki A, et al. Osteonecrosis of the jaw related to bevacizumab. Oral Oncol. 2008;44(12):e41-e46.
74. Barasch A, Cunha-Cruz J, Curro FA, et al. Risk factors for osteonecrosis of the jaws. J Oral Maxillofac Surg. 2011;69(11):2784-2795.
75. Lo JC, O'Ryan FS, Yang J, et al. Oral bisphosphonates and risk duration. J Dent Res. 2010;89(10):1058-1063.
76. Ruggiero SL, Mehrotra B, Rosenberg TJ, et al. Osteonecrosis associated with bisphosphonate therapy. J Oral Maxillofac Surg. 2004;62(5):527-534.
77. Vahtsevanos K, Kyrgidis A, Verrou E, et al. Longitudinal cohort study of osteonecrosis risk. J Clin Oncol. 2009;27(32):5356-5362.
78. Migliorati CA, Casiglia J, Epstein J, et al. Managing osteonecrosis in cancer patients. CA Cancer J Clin. 2005;55(6):333-347.
79. Saia G, Blandamura S, Bettini G, et al. Occurrence of osteonecrosis after tooth extraction. J Oral Maxillofac Surg. 2010;68(4):797-804.
80. Otto S, Abu-Id MH, Fedele S, et al. Pathogenesis after dentoalveolar surgery. Oral Dis. 2018;24(1-2):20-26.
81. Otto S, Schreyer C, Hafner S, et al. Infection as trigger factor in osteonecrosis. Clin Oral Investig. 2010;14(4):421-426.
82. Yarom N, Yahalom R, Shoshani Y, et al. Oral health and periodontal disease in MRONJ patients. Support Care Cancer. 2007;15(1):61-65.
83. Thumbigere-Math V, Michalowicz BS, Hughes PJ, et al. Periodontal inflammation and osteonecrosis. J Periodontol. 2014;85(2):226-233.
84. Lesclous P, Abi Najm S, Carrel JP, et al. Bisphosphonate-associated osteonecrosis and infection. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(1):1-7.
85. Madrid C, Sanz M. Dental implants in patients receiving bisphosphonate therapy. Int J Oral Maxillofac Implants. 2009;24(Suppl):87-95.
86. Lazarovici TS, Yahalom R, Taicher S, et al. Bisphosphonate-related osteonecrosis around implants. J Oral Maxillofac Surg. 2010;68(4):790-796.
87. Jacobsen C, Metzler P, Rössle M, et al. Implant failure and MRONJ. Clin Implant Dent Relat Res. 2013;15(4):577-584.

88. Kwon YD, Kim DY, Ohe JY, et al. Retrospective study of risk factors in MRONJ. Int J Oral Maxillofac Surg. 2009;38(12):1394-1398.
89. Marx RE, Sawatari Y, Fortin M, et al. Bisphosphonate-induced exposed bone and prosthetic trauma. J Oral Maxillofac Surg. 2005;63(11):1567-1575.
90. Bedogni A, Campisi G, Fusco V. Anatomical considerations in MRONJ. Oral Maxillofac Surg Clin North Am. 2015;27(4):509-517.
91. Otto S, Troltzsch M, Jambrovic V, et al. Anatomical risk factors in MRONJ. J Craniomaxillofac Surg. 2015;43(7):1181-1187.
92. Boonyapakorn T, Schirmer I, Reichart PA, et al. Bisphosphonate-induced osteonecrosis in cancer patients. Oral Oncol. 2008;44(9):857-869.
93. Abu-Id MH, Warnke PH, Gottschalk J, et al. "Bis-phossy jaws"–high and low risk factors. J Craniomaxillofac Surg. 2008;36(2):95-103.
94. Badros A, Weikel D, Salama A, et al. Osteonecrosis in multiple myeloma. J Clin Oncol. 2006;24(6):945-952.
95. Hamadeh IS, Ngwa BA, Gong Y. Drug-induced osteonecrosis and diabetes mellitus. Pharmacotherapy. 2015;35(12):1175-1184.
96. Khamaisi M, Regev E, Yarom N, et al. Possible association between diabetes and osteonecrosis. J Clin Endocrinol Metab. 2007;92(3):1172-1175.
97. Conte-Neto N, Bastos AS, Spolidorio LC, et al. Corticosteroids and osteonecrosis risk. Oral Dis. 2017;23(4):470-476.
98. Dimopoulos MA, Kastritis E, Anagnostopoulos A, et al. Corticosteroid therapy and osteonecrosis. Leuk Lymphoma. 2006;47(6):1026-1031.
99. Bagán JV, Jiménez Y, Murillo J, et al. Risk factors in osteonecrosis of the jaws. Med Oral Patol Oral Cir Bucal. 2006;11(5):E425-E429.
100. Kyrgidis A, Vahtsevanos K, Koloutsos G, et al. Lifestyle factors and osteonecrosis. J Oral Maxillofac Surg. 2008;66(6):1123-1127.
101. Lee SH, Kim JW, Kim YH, et al. Age-related changes in MRONJ patients. J Korean Assoc Oral Maxillofac Surg. 2015;41(1):7-13.
102. Ripamonti CI, Maniezzo M, Campa T, et al. Decreased osteonecrosis incidence with preventive dental measures. Ann Oncol. 2009;20(1):137-145.
103. Campisi G, Fedele S, Fusco V, et al. Epidemiology and prevention of osteonecrosis. Oral Oncol. 2014;50(6):611-615.
104. O'Ryan FS, Lo JC. Bisphosphonate-related osteonecrosis variability. Oral Maxillofac Surg Clin North Am. 2015;27(4):489-496.
105. Arce K, Assael LA, Weissman JL, et al. Imaging findings in stage 0 disease. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(3):425-432.

106. Bianchi SD, Scoletta M, Cassione FB, et al. Computerized tomographic findings in MRONJ. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(2):249-258.
107. Phal PM, Myall RW, Assael LA, et al. Imaging characteristics of MRONJ. Radiographics. 2007;27(1):197-207.
108. Bedogni A, Fusco V, Agrillo A, et al. Learning from MRONJ clinical cases. Oral Dis. 2012;18(6):621-623.
109. Mawardi H, Treister N, Richardson P, et al. Pain patterns in osteonecrosis. Oral Oncol. 2009;45(4-5):433-436.
110. Thumbigere-Math V, Sabino MC, Gopalakrishnan R, et al. Clinical features of MRONJ. Cancer. 2009;115(15):3468-3476.
111. Sedghizadeh PP, Stanley K, Caligiuri M, et al. Oral lesions and soft tissue changes in MRONJ. J Oral Maxillofac Surg. 2009;67(5):943-950.
112. Hutchinson M, O'Ryan F, Chavez V, et al. Numb chin syndrome in osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(4):e37-e42.
113. Carlson ER, Basile JD. Pathologic fracture in osteonecrosis. Oral Maxillofac Surg Clin North Am. 2015;27(4):557-570.
114. Wilde F, Steinhoff K, Frerich B, et al. Maxillary sinus involvement in MRONJ. Int J Oral Maxillofac Surg. 2011;40(11):1183-1188.
115. Bedogni A, Fusco V, Agrillo A, et al. Natural history of MRONJ. Oral Dis. 2012;18(6):621-623.
116. Otto S, Pautke C, Opelz C, et al. Benefits of early diagnosis and surgery. J Oral Maxillofac Surg. 2011;69(5):1364-1370.
117. Ruggiero SL. Staging and management update. Oral Maxillofac Surg Clin North Am. 2015;27(4):479-487.
118. Fedele S, Bedogni G, Scoletta M, et al. Stage 0 osteonecrosis controversy. Oral Oncol. 2010;46(9):669-673.
119. Schiodt M, Reibel J, Oturai P, et al. Challenges in stage 0 diagnosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117(2):e113-e120.
120. Ristow O, Otto S, Troeltzsch M, et al. Clinical and radiographic findings in early-stage MRONJ. Int J Oral Maxillofac Surg. 2014;43(9):1139-1144.
121. Guo Y, Wang D, Wang Y, et al. Early radiographic manifestations of medication-related osteonecrosis. Dentomaxillofac Radiol. 2016;45(2):20150256.
122. Voss PJ, Joshi Oshero J, Kovalova-Müller A, et al. Surgical treatment outcomes in stage 1 MRONJ. J Craniomaxillofac Surg. 2017;45(9):1574-1579.
123. Fliefel R, Tröltzsch M, Kühnisch J, et al. Quality of life and oral function in stage 2 MRONJ. Clin Oral Investig. 2015;19(4):841-848.

124. Ruggiero SL, Dodson TB, Assael LA, et al. American Association of Oral and Maxillofacial Surgeons position paper on bisphosphonate-related osteonecrosis. J Oral Maxillofac Surg. 2009;67(5 Suppl):2-12.
125. Otto S, Pautke C, Hafner S, et al. Operative versus conservative treatment strategies in MRONJ. J Oral Maxillofac Surg. 2012;70(2):345-356.
126. Bedogni A, Blandamura S, Lokmic Z, et al. Advanced MRONJ and pathologic fractures. Oral Oncol. 2011;47(4):313-318.
127. Carlson ER. Surgical management of advanced MRONJ. Oral Maxillofac Surg Clin North Am. 2015;27(4):589-599.
128. Schiodt M, Otto S, Fedele S, et al. Classification systems in MRONJ. Oral Dis. 2019;25(7):1719-1728.
129. Bedogni A, Campisi G, Agrillo A, et al. Italian SICMF-SIPMO staging system. Oral Dis. 2012;18(6):621-623.
130. Giudice A, Barone S, Bennardo F, et al. Dimensional staging approaches in MRONJ. J Craniofac Surg. 2020;31(2):e161-e165.
131. Aghaloo TL, Tetradis S. Limitations of current MRONJ classifications. Oral Maxillofac Surg Clin North Am. 2015;27(4):493-500.
132. Bagan JV, Hens-Aumente E, Leopoldo-Rodado M, et al. Discrepancy between clinical and radiologic extent of disease. Med Oral Patol Oral Cir Bucal. 2014;19(4):e338-e344.
133. Kim DW, Kang SH, Oh HK, et al. Artificial intelligence and imaging analysis in MRONJ. Diagnostics. 2021;11(1):58.
134. Yarom N, Elad S, Madrid C, et al. Diagnostic criteria and therapeutic considerations. Support Care Cancer. 2019;27(10):3925-3932.
135. Khan AA, Morrison A, Hanley DA, et al. Diagnosis and management recommendations. J Bone Miner Res. 2015;30(1):3-23.
136. Hasegawa T, Hayashida S, Kondo E, et al. Importance of medication history in MRONJ diagnosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;123(3):295-302.
137. Fusco V, Bedogni A, Campisi G. Clinical evaluation in medication-related osteonecrosis. Oral Maxillofac Surg Clin North Am. 2015;27(4):453-465.
138. Nicolatou-Galitis O, Schiødt M, Mendes RA, et al. Differential diagnosis of jaw osteonecrosis. Support Care Cancer. 2019;27(10):3925-3932.
139. Mawardi H, Giro G, Kajiya M, et al. Differential diagnosis between MRONJ and metastatic disease. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):e51-e58.
140. Woo SB, Hellstein JW, Kalmar JR. Histopathologic considerations in osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102(4):433-441.
141. Hansen T, Kunkel M, Weber A, et al. Histological aspects and Actinomyces colonization. J Craniomaxillofac Surg. 2006;34(7):400-409.

142. Stockmann P, Hinkmann FM, Lell MM, et al. Imaging modalities in MRONJ evaluation. Dentomaxillofac Radiol. 2010;39(5):272-277.
143. Arce K, Assael LA, Weissman JL, et al. Panoramic imaging findings in osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009;107(3):425-432.
144. Bianchi SD, Scoletta M, Cassione FB, et al. Limitations of panoramic radiography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(2):249-258.
145. Torres SR, Chen CSK, Leroux BG, et al. Cone-beam computed tomography in MRONJ. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(6):804-809.
146. Wilde F, Heufelder M, Winter K, et al. CBCT assessment and surgical planning. Clin Oral Investig. 2012;16(4):1189-1198.
147. Chiandussi S, Biasotto M, Dore F, et al. CT imaging in advanced osteonecrosis. Dentomaxillofac Radiol. 2006;35(4):236-243.
148. Guggenberger R, Fischer DR, Metzler P, et al. MRI findings in MRONJ. Skeletal Radiol. 2013;42(5):699-706.
149. O'Ryan FS, Khoury S, Liao W, et al. Nuclear imaging and bone scintigraphy in MRONJ. J Oral Maxillofac Surg. 2009;67(5 Suppl):136-147.
150. Fleisher KE, Doty S, Kottal S, et al. PET/CT imaging in jaw osteonecrosis. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(3):335-347.
151. Otto S, Troltzsch M, Jambrovic V, et al. Three-dimensional imaging for surgical management. J Craniomaxillofac Surg. 2015;43(7):1181-1187.
152. Woo SB, Hellstein JW, Kalmar JR. Histopathological spectrum of MRONJ. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102(4):433-441.
153. Hansen T, Kunkel M, Weber A, et al. Histologic characteristics of necrotic bone. J Craniomaxillofac Surg. 2006;34(7):400-409.
154. Sedghizadeh PP, Kumar SKS, Gorur A, et al. Biofilm formation in osteonecrotic lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(3):348-352.
155. Hansen T, Kunkel M, Kirkpatrick CJ, et al. Actinomyces colonies in MRONJ specimens. J Oral Pathol Med. 2007;36(8):483-489.
156. Di Fede O, Panzarella V, Mauceri R, et al. Inflammatory mechanisms in osteonecrosis. Int J Dent. 2018;2018:1-8.
157. Ripamonti CI, Maniezzo M, Campa T, et al. Prevention protocols and incidence reduction. Ann Oncol. 2009;20(1):137-145.
158. Dimopoulos MA, Kastritis E, Bamia C, et al. Preventive dental measures in oncology patients. Blood. 2009;114(17):3513-3517.

159. Yarom N, Shapiro CL, Peterson DE, et al. Preventive dental care recommendations. J Clin Oncol. 2019;37(25):2270-2290.
160. Campisi G, Fedele S, Fusco V, et al. Dental management before antiresorptive therapy. Oral Oncol. 2014;50(6):611-615.
161. Otto S, Pautke C, Opelz C, et al. Timing of oral surgery before antiresorptive therapy. J Oral Maxillofac Surg. 2011;69(5):1364-1370.
162. Thumbigere-Math V, Michalowicz BS, Hughes PJ, et al. Oral hygiene and osteonecrosis risk. J Periodontol. 2014;85(2):226-233.
163. Bedogni A, Campisi G, Fusco V. Maintenance protocols in MRONJ prevention. Oral Maxillofac Surg Clin North Am. 2015;27(4):429-441.
164. Hellstein JW, Adler RA, Edwards B, et al. Dental management recommendations. J Am Dent Assoc. 2011;142(11):1243-1251.
165. Otto S, Schreyer C, Hafner S, et al. Infection versus extraction debate. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(6):705-712.
166. Kwon YD, Ohe JY, Kim DY, et al. Drug holiday controversy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2012;114(6):715-720.
167. McGowan K, McGowan T, Ivanovski S. Risk-benefit analysis of antiresorptive interruption. Aust Dent J. 2018;63(1):9-16.
168. Hasegawa T, Hayashida S, Kondo E, et al. Effectiveness of drug holidays. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;124(4):e136-e142.
169. Otto S, Troltzsch M, Jambrovic V, et al. Surgical preventive protocols. J Craniomaxillofac Surg. 2015;43(7):1181-1187.
170. Tenore G, Mohsen A, Rossi DS, et al. Leukocyte- and platelet-rich fibrin in prevention and treatment of MRONJ. J Clin Med. 2020;9(11):3505.
171. Yarom N, Elad S, Ariyawardana A, et al. Patient education and awareness in MRONJ prevention. Support Care Cancer. 2017;25(2):347-354.
172. Campisi G, Mauceri R, Bertoldo F, et al. Importance of patient compliance and education. Oral Dis. 2020;26(3):449-460.
173. Ruggiero SL, Dodson TB, Fantasia J, et al. Conservative management recommendations in MRONJ. J Oral Maxillofac Surg. 2014;72(10):1938-1956.
174. Otto S, Pautke C, Hafner S, et al. Limitations of conservative therapy in advanced disease. J Oral Maxillofac Surg. 2012;70(2):345-356.
175. Nicolatou-Galitis O, Migkou M, Psyrri A, et al. Chlorhexidine-based protocols in osteonecrosis management. Oral Surg Oral Med Oral Pathol Oral Radiol. 2011;112(2):195-202.
176. Bedogni A, Saia G, Bettini G, et al. Conservative treatment outcomes in MRONJ. Oral Oncol. 2011;47(5):420-424.
177. Thumbigere-Math V, Sabino MC, Gopalakrishnan R, et al. Antibiotic therapy in MRONJ patients. Cancer. 2009;115(15):3468-3476.

178. Mawardi H, Giro G, Kajiya M, et al. Long-term antibiotic use and resistance concerns. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;122(2):e51-e58.
179. Miksad RA, Lai KC, Dodson TB, et al. Pain management and quality of life in osteonecrosis patients. Oncologist. 2011;16(1):121-132.
180. Carlson ER, Basile JD. Debridement techniques in MRONJ. Oral Maxillofac Surg Clin North Am. 2015;27(4):557-570.
181. Schubert M, Klatte I, Linek W, et al. Long-term outcomes of nonsurgical treatment. Clin Oral Investig. 2012;16(4):1189-1198.
182. Otto S, Tröltzsch M, Jambrovic V, et al. Shift toward surgical intervention. J Craniomaxillofac Surg. 2015;43(7):1181-1187.
183. Voss PJ, Joshi Oshero J, Kovalova-Müller A, et al. Surgical concepts in medication-related osteonecrosis. J Craniomaxillofac Surg. 2017;45(9):1574-1579.
184. Giudice A, Bennardo F, Barone S, et al. Surgical management outcomes and healing rates. J Craniofac Surg. 2018;29(8):2199-2203.
185. Carlson ER. Principles of surgical treatment in MRONJ. Oral Maxillofac Surg Clin North Am. 2015;27(4):589-599.
186. Fliefel R, Tröltzsch M, Kühnisch J, et al. Timing of surgery in medication-related osteonecrosis. Clin Oral Investig. 2015;19(4):841-848.
187. Otto S, Pautke C, Opelz C, et al. Early surgical intervention and prognosis. J Oral Maxillofac Surg. 2011;69(5):1364-1370.
188. Wilde F, Heufelder M, Winter K, et al. Sequestrectomy in localized MRONJ. Clin Oral Investig. 2012;16(4):1189-1198.
189. Bedogni A, Blandamura S, Lokmic Z, et al. Recurrence after limited surgery. Oral Oncol. 2011;47(4):313-318.
190. Ristow O, Otto S, Troeltzsch M, et al. Surgical debridement techniques in MRONJ. Int J Oral Maxillofac Surg. 2014;43(9):1139-1144.
191. Otto S, Schreyer C, Hafner S, et al. Combined surgical and adjunctive therapies. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(6):705-712.
192. Carlson ER, Basile JD. Marginal resection concepts in osteonecrosis surgery. Oral Maxillofac Surg Clin North Am. 2015;27(4):557-570.
193. Voss PJ, Joshi Oshero J, Kovalova-Müller A, et al. Importance of bleeding bone margins. J Craniomaxillofac Surg. 2017;45(9):1574-1579.
194. Giudice A, Barone S, Bennardo F, et al. Clinical outcomes after marginal resection. J Craniofac Surg. 2020;31(2):e161-e165.
195. Bedogni A, Saia G, Bettini G, et al. Segmental mandibular resection in advanced MRONJ. Oral Oncol. 2011;47(5):420-424.
196. Otto S, Troltzsch M, Jambrovic V, et al. Soft tissue management and flap closure. J Craniomaxillofac Surg. 2015;43(7):1181-1187.

197. Wilde F, Winter K, Kletsch K, et al. Tension-free closure techniques in MRONJ surgery. Clin Oral Investig. 2017;21(4):1097-1104.
198. Rapidis AD, Alexandridis C, Eleftheriadis E, et al. Buccal fat pad flap reconstruction. J Oral Maxillofac Surg. 2000;58(2):158-163.
199. Pautke C, Bauer F, Tischer T, et al. Fluorescence-guided bone resection in osteonecrosis surgery. J Oral Maxillofac Surg. 2009;67(3):471-476.
200. Otto S, Ristow O, Pache C, et al. Autofluorescence-guided surgery in MRONJ. Int J Oral Maxillofac Surg. 2016;45(4):535-542.
201. Pautke C, Kreutzer K, Weitz J, et al. Improved outcomes with fluorescence-guided surgery. J Craniomaxillofac Surg. 2013;41(8):694-698.
202. Stubinger S, Kuttenberger J, Filippi A, et al. Piezoelectric surgery in oral and maxillofacial surgery. Int J Oral Maxillofac Surg. 2005;34(5):451-457.
203. Vercellotti T. Technological characteristics and clinical indications of piezosurgery. Minerva Stomatol. 2004;53(5):207-214.
204. Schubert M, Klatte I, Linek W, et al. Surgical treatment success rates in MRONJ. Clin Oral Investig. 2012;16(4):1189-1198.
205. Otto S, Pautke C, Hafner S, et al. Long-term outcomes after surgical therapy. J Oral Maxillofac Surg. 2012;70(2):345-356.
206. Carlson ER. Reconstruction after mandibular resection in MRONJ. Oral Maxillofac Surg Clin North Am. 2015;27(4):601-614.
207. Hidalgo DA. Fibula free flap mandibular reconstruction. Plast Reconstr Surg. 1989;84(1):71-79.
208. Brown JS, Barry C, Ho M, et al. Reconstruction plate complications. Br J Oral Maxillofac Surg. 2016;54(5):503-508.
209. Hanasono MM, Skoracki RJ. Mandibular reconstruction techniques. Plast Reconstr Surg. 2013;132(5):675e-683e.
210. Shpitzer T, Neligan P, Gullane P, et al. Fibular free flap reconstruction outcomes. Head Neck. 1997;19(4):309-314.
211. Rapidis AD, Alexandridis C, Eleftheriadis E, et al. Regional flap techniques in jaw reconstruction. J Oral Maxillofac Surg. 2000;58(2):158-163.
212. Ferrari S, Bianchi B, Copelli C, et al. Local flap reconstruction in osteonecrosis surgery. J Oral Maxillofac Surg. 2008;66(8):1699-1705.
213. Choukroun J, Adda F, Schoeffler C, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Implantodontie. 2001;42:55-62.
214. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification and clinical applications of platelet concentrates. Trends Biotechnol. 2009;27(3):158-167.
215. Miron RJ, Chai J, Zheng S, et al. Growth factor release and biologic properties of PRF. Clin Oral Investig. 2017;21(5):1361-1369.

216. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: biological characteristics and regenerative potential. J Oral Implantol. 2014;40(6):679-689.
217. Tenore G, Mohsen A, Rossi DS, et al. PRF-assisted surgery in MRONJ management. J Clin Med. 2020;9(11):3505.
218. Otto S, Pautke C, Opelz C, et al. Bone grafting considerations in MRONJ surgery. J Oral Maxillofac Surg. 2011;69(5):1364-1370.
219. Kim JW, Kim SJ, Kim MR. Combined PRF and bone graft application in osteonecrosis defects. Clin Implant Dent Relat Res. 2014;16(5):746-754.
220. Hanna R, Dalvi S, Benedicenti S, et al. Laser-assisted treatment protocols in MRONJ. Pharmaceuticals. 2024;17(8):1011.
221. Karu TI. Mechanisms of photobiomodulation therapy. Photomed Laser Surg. 2010;28(2):146-148.
222. Romeo U, Galanakis A, Marias C, et al. Clinical efficacy of photobiomodulation in MRONJ. Lasers Med Sci. 2011;26(1):161-167.
223. Poli PP, Cingolani M, Forte V, et al. Antimicrobial photodynamic therapy in osteonecrosis management. Photodiagnosis Photodyn Ther. 2019;27:117-123.
224. Jamalpour MR, Malekzadeh H, Abdolsamadi H, et al. Combined PRF and photodynamic therapy in MRONJ treatment. BMC Oral Health. 2022;22(1):241.
225. Stubinger S, Dissmann JP, Pinho NC, et al. Er:YAG laser-assisted surgery in jaw osteonecrosis. Lasers Med Sci. 2009;24(5):673-681.
226. Madrid C, Sanz M. Dental implants and bisphosphonate-related osteonecrosis. Int J Oral Maxillofac Implants. 2009;24(Suppl):87-95.
227. Lazarovici TS, Yahalom R, Taicher S, et al. Implant-associated medication-related osteonecrosis. J Oral Maxillofac Surg. 2010;68(4):790-796.
228. Grant BT, Amenedo C, Freeman K, et al. Outcomes of dental implants in bisphosphonate-treated patients. J Oral Maxillofac Surg. 2008;66(2):223-230.
229. Koka S, Babu NM, Norell A. Risk assessment for implant placement in antiresorptive therapy patients. Clin Implant Dent Relat Res. 2010;12(1):53-58.
230. Jacobsen C, Metzler P, Obwegeser JA, et al. Late implant-related osteonecrosis. Clin Implant Dent Relat Res. 2013;15(4):577-584.
231. Favia G, Tempesta A, Limongelli L, et al. Peri-implantitis and implant-related MRONJ. Clin Oral Investig. 2015;19(6):1327-1335.
232. Yarom N, Shapiro CL, Peterson DE, et al. Clinical recommendations for implant placement in patients receiving antiresorptive therapy. J Clin Oncol. 2019;37(25):2270-2290.

233. Kwon YD, Ohe JY, Kim DY, et al. Drug holiday debate in osteonecrosis prevention. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2012;114(6):715-720.
234. McGowan K, McGowan T, Ivanovski S. Evidence review regarding antiresorptive interruption. Aust Dent J. 2018;63(1):9-16.
235. Lin JH. Bisphosphonate skeletal retention and pharmacokinetics. Bone. 1996;18(2):75-85.
236. Baron R, Ferrari S, Russell RG. Reversibility of denosumab effects. Bone. 2011;48(4):677-692.
237. McClung MR. Risks associated with denosumab discontinuation. Osteoporos Int. 2016;27(5):1677-1682.
238. Otto S, Pautke C, Van den Wyngaert T, et al. Emerging concepts and future directions in MRONJ. Oral Surg Oral Med Oral Pathol Oral Radiol. 2018;125(5):409-417.
239. Kikuiri T, Kim I, Yamaza T, et al. Mesenchymal stem cell therapy in osteonecrosis. J Bone Miner Res. 2010;25(7):1668-1679.
240. Bouland C, Meuleman N, Widelec J, et al. Stem-cell-based regenerative approaches in MRONJ. Stem Cell Res Ther. 2021;12(1):186.
241. Sim IW, Borromeo GL, Tsao C, et al. Teriparatide promotes bone healing in MRONJ. J Clin Oncol. 2013;31(4):e61-e64.
242. Harper RP, Fung E. Resolution of osteonecrosis with teriparatide therapy. N Engl J Med. 2007;357(24):2478-2479.
243. Zhang L, Jiao G, Ren S, et al. Exosome-based regenerative therapy. Stem Cell Res Ther. 2020;11(1):1-12.
244. Wang X, Omar O, Vazirisani F, et al. Exosomes and bone regeneration. Acta Biomater. 2018;74:31-47.
245. Roseti L, Parisi V, Petretta M, et al. Tissue engineering and biomaterials in bone regeneration. Materials (Basel). 2017;10(9):969.
246. Miron RJ, Fujioka-Kobayashi M, Bishara M, et al. Scaffold and PRF combination therapies. Clin Oral Investig. 2017;21(6):1913-1927.
247. Kim DW, Kang SH, Oh HK, et al. Artificial intelligence applications in MRONJ diagnosis. Diagnostics. 2021;11(1):58.
248. Lee KS, Jung SK, Ryu JJ, et al. Deep learning-based radiographic detection of osteonecrosis. Dentomaxillofac Radiol. 2022;51(1):20210117.
249. Khan AA, Morrison A, Hanley DA, et al. Global perspectives on prevention and management of osteonecrosis. J Bone Miner Res. 2015;30(1):3-23.
250. Otto S, Tröltzsch M, Jambrovic V, et al. Contemporary surgical paradigms in MRONJ. J Craniomaxillofac Surg. 2015;43(7):1181-1187.
251. Hanna R, Dalvi S, Benedicenti S, et al. Regenerative and adjunctive therapies in MRONJ. Pharmaceuticals. 2024;17(8):1011.

Chapter 8

Artificial Intelligence Applications in Oral Pathology: Current Perspectives and Future Directions

Özge ÖZDAL ZİNCİR[1]

1. Introduction

Artificial intelligence (AI) has emerged as one of the most transformative technologies in modern healthcare and is increasingly influencing diagnostic, prognostic, and therapeutic approaches across dental specialties [1]. In oral pathology, the integration of AI into histopathological evaluation, radiographic interpretation, and clinical decision-making has generated considerable interest because of its potential to improve diagnostic accuracy, efficiency, and reproducibility [2].

Oral pathology involves the diagnosis of a broad spectrum of lesions affecting the oral and maxillofacial region, including reactive lesions, premalignant disorders, malignant neoplasms, odontogenic cysts, odontogenic tumors, salivary gland diseases, and systemic diseases with oral manifestations [3]. Accurate diagnosis frequently requires integration of clinical findings, radiologic imaging, histopathological interpretation, and molecular analyses. However, histopathological interpretation may be challenging because of overlapping morphologic features, interobserver variability, and the increasing complexity of diagnostic criteria [4].

Recent advances in digital pathology, computational image analysis, machine learning (ML), deep learning (DL), and large language models (LLMs) have opened new possibilities for automated and AI-assisted diagnosis in oral pathology [5]. AI systems can analyze large datasets, identify subtle morphologic patterns, quantify histopathological features, and assist pathologists in diagnostic interpretation [6].

The transition from conventional microscopy to digital pathology has played a fundamental role in enabling AI applications. Whole-slide imaging (WSI) systems allow histopathological slides to be digitized at high resolution, creating datasets suitable for computational analysis [7]. Deep convolutional neural networks (CNNs) have demonstrated remarkable success in image classification,

[1] Assoc. Prof. Dr., Istanbul University- Cerrahpasa Faculty of Dentistry,
Department of Oral and Maxillofacial Surgery
ORCID: 0009-0000-9423-5764

segmentation, tumor detection, and prognostic prediction in various medical disciplines, including pathology [8].

Among oral lesions, oral squamous cell carcinoma (OSCC) has become one of the most extensively investigated fields for AI applications because early diagnosis significantly influences prognosis and survival [9]. AI-based systems have demonstrated promising capabilities in detecting dysplasia, identifying malignant transformation, and predicting lymph node metastasis [10].

Similarly, AI models have shown potential in the classification of odontogenic cysts and tumors, differentiation of benign and malignant salivary gland lesions, and automated interpretation of immunohistochemical markers [11].

More recently, large language models such as ChatGPT, Gemini, Copilot, and DeepSeek have introduced additional possibilities for AI-assisted education, pathology reporting, literature summarization, and clinical decision support [12]. Although LLMs are not image-analysis systems, they may support oral pathology practice by synthesizing scientific literature, generating differential diagnoses, and assisting educational workflows [13].

Despite these advances, important limitations and ethical concerns remain unresolved. AI systems may demonstrate bias, limited generalizability, lack of interpretability, data privacy concerns, and potential medicolegal implications [14]. Moreover, most currently available AI models require large, high-quality annotated datasets that are difficult to obtain in oral pathology because of the relative rarity of many lesions [15].

The aim of this chapter is to provide a comprehensive overview of current AI applications in oral pathology, including digital pathology infrastructure, machine learning methodologies, histopathological image analysis, AI-assisted diagnosis of oral lesions, LLM applications, limitations, ethical considerations, and future perspectives.

2. Digital Pathology: Transformation of Oral Diagnostic Practice

The emergence of digital pathology has fundamentally transformed histopathological diagnostics and created the technological foundation for artificial intelligence applications in oral pathology [16]. Conventional pathology workflows traditionally relied on light microscopy and manual interpretation of glass slides. Although this approach remains the gold standard for diagnosis, it is associated with several limitations including interobserver variability, limited reproducibility, workflow inefficiencies, and restricted opportunities for computational analysis [17].

Digital pathology refers to the acquisition, management, sharing, and interpretation of pathology information in a digital environment [18]. Whole-

slide imaging (WSI) systems enable complete histopathological slides to be scanned at high resolution, generating digitized images that can be viewed, stored, and analyzed computationally [19].

The adoption of WSI has accelerated significantly in recent years because of advances in:

- Scanning technology
- Data storage capacity
- Cloud computing
- Image processing algorithms
- Artificial intelligence infrastructure

Digital pathology is particularly valuable in oral pathology because many lesions are rare and require consultation among specialists [20]. Remote slide sharing and telepathology facilitate collaboration and second-opinion consultation while also enabling the development of multicenter image databases for AI training [21].

2.1 Whole-Slide Imaging Systems

Whole-slide imaging systems utilize high-resolution scanners capable of digitizing histopathological slides at magnifications comparable to conventional microscopy [22].

Modern WSI systems typically include:

- Automated slide loaders
- High-resolution optical systems
- Digital image processing software
- Cloud-based storage systems

Scanned images may reach gigapixel resolution, allowing detailed examination of cellular and stromal features [23].

Advantages of WSI include:

- Improved accessibility
- Remote consultation
- Long-term image storage
- Educational utility
- Quantitative image analysis
- AI compatibility

However, several challenges remain, including:

- High infrastructure costs
- Large data storage requirements
- Standardization issues
- Scanning artifacts

- Computational demands

Despite these limitations, digital pathology is increasingly becoming integrated into routine diagnostic workflows worldwide [24].

2.2 Telepathology and Remote Consultation

Telepathology enables remote histopathological interpretation through digital image transmission [25].

This technology has particular importance in oral pathology because experienced oral pathologists may not be available in all geographic regions. AI-assisted telepathology systems may improve diagnostic accessibility in underserved areas [26].

Applications include:

- Remote consultation
- Educational activities
- Multidisciplinary tumor boards
- International collaborations
- Research database development

The COVID-19 pandemic further accelerated adoption of digital pathology and remote pathology workflows [27].

2.3 Quantitative Histopathology

One of the major advantages of digital pathology is the ability to perform quantitative histopathological analysis [28].

AI-assisted image analysis systems can objectively quantify:

- Nuclear morphology
- Mitotic activity
- Cellular density
- Tumor invasion patterns
- Stromal characteristics
- Immunohistochemical staining intensity

These quantitative parameters may improve diagnostic reproducibility and prognostic assessment [29].

In oral squamous cell carcinoma (OSCC), quantitative analysis of tumor-infiltrating lymphocytes, nuclear pleomorphism, and invasion patterns has demonstrated prognostic significance [30].

3. Fundamentals of Artificial Intelligence and Machine Learning

Artificial intelligence refers to computational systems capable of performing tasks that traditionally require human intelligence, including pattern recognition, reasoning, prediction, and decision-making [31].

Within healthcare, AI encompasses several subfields:

- Machine learning (ML)
- Deep learning (DL)
- Natural language processing (NLP)
- Computer vision
- Large language models (LLMs)

These technologies have rapidly expanded within pathology because histopathological diagnosis is inherently image-based and pattern-oriented [32].

3.1 Machine Learning

Machine learning is a subset of AI in which algorithms learn patterns from data without explicit programming [33].

ML systems improve their performance through exposure to training datasets and may subsequently generate predictions when presented with new data.

Machine learning models are generally categorized as:

- Supervised learning
- Unsupervised learning
- Reinforcement learning

3.1.1 Supervised Learning

In supervised learning, algorithms are trained using labeled datasets [34].

For example, histopathological images labeled as:

- Dysplasia
- OSCC
- Ameloblastoma
- Odontogenic keratocyst

may be used to train diagnostic models.

Common supervised learning algorithms include:

- Support vector machines
- Random forests
- Logistic regression
- Artificial neural networks

Supervised learning remains the most commonly used approach in oral pathology AI studies [35].

3.1.2 Unsupervised Learning

Unsupervised learning identifies hidden patterns within unlabeled datasets [36].

Potential applications in oral pathology include:

- Tumor subtype discovery
- Biomarker clustering
- Pattern recognition in rare lesions

Although less commonly used clinically, unsupervised learning may contribute significantly to future precision pathology [37].

3.2 Deep Learning

Deep learning is an advanced subset of machine learning based on artificial neural networks containing multiple computational layers [38].

Deep learning has revolutionized image analysis because deep neural networks can automatically learn complex hierarchical image features without manual feature extraction [39].

Convolutional neural networks (CNNs) represent the most widely used deep learning architecture in pathology image analysis [40].

CNNs are particularly effective for:

- Histopathological classification
- Tumor detection
- Image segmentation
- Cellular recognition

Deep learning systems frequently outperform traditional machine learning models in image-based diagnostic tasks [41].

3.3 Convolutional Neural Networks (CNNs)

CNNs are specifically designed for image processing tasks [42].

These systems analyze images through sequential layers that detect increasingly complex visual features.

In oral pathology, CNNs have been used for:

- Oral cancer detection
- Dysplasia grading
- Salivary gland tumor classification
- Odontogenic lesion recognition
- Mitotic figure detection

CNNs may achieve diagnostic accuracies approaching expert-level performance under controlled conditions [43].

3.4 Natural Language Processing

Natural language processing (NLP) enables AI systems to interpret and generate human language [44].

NLP applications in oral pathology include:

- Automated pathology reporting
- Literature summarization
- Clinical note analysis
- Data extraction from pathology archives

NLP may facilitate integration of histopathological findings with clinical information and radiologic reports [45].

3.5 Explainable Artificial Intelligence (XAI)

One major limitation of AI systems is the "black box" phenomenon, in which decision-making processes remain poorly interpretable [46].

Explainable AI aims to improve transparency by identifying:

- Image regions influencing decisions
- Feature importance
- Model confidence levels

Explainability is particularly important in healthcare because clinicians must understand and trust AI-generated outputs before integrating them into clinical practice [47].

4. AI-Assisted Histopathological Image Analysis

Histopathological image analysis represents one of the most promising applications of AI in oral pathology [48].

AI systems can analyze digitized slides and identify microscopic features that may not be easily recognized by the human eye. These technologies may improve:

- Diagnostic consistency
- Efficiency
- Quantitative assessment
- Early lesion detection

AI-assisted image analysis generally involves:

1. Image acquisition
2. Preprocessing
3. Feature extraction
4. Model training
5. Classification or prediction

Large annotated datasets are essential for reliable model development [49].

4.1 Image Preprocessing

Preprocessing improves image quality and standardization before AI analysis [50].

Common preprocessing techniques include:

- Noise reduction
- Color normalization
- Contrast enhancement
- Tissue segmentation

Standardization is particularly important because histopathological slides may demonstrate substantial variability related to:

- Staining protocols
- Scanner settings
- Tissue preparation methods

Preprocessing significantly influences model accuracy and reproducibility [51].

4.2 Image Segmentation

Segmentation involves identification of specific structures within histopathological images [52].

AI-based segmentation systems may identify:

- Tumor regions
- Nuclei
- Mitotic figures
- Stromal tissue
- Inflammatory infiltrates

Accurate segmentation is essential for quantitative pathology analysis and prognostic modeling [53].

4.3 Feature Extraction

Traditional machine learning approaches often require manual feature extraction [54].

Features may include:

- Nuclear size
- Shape irregularity
- Texture patterns
- Cellular density
- Architectural organization

Deep learning systems, particularly CNNs, can automatically identify relevant features directly from raw images [55].

4.4 Classification Models

AI classification systems categorize lesions into diagnostic groups [56].

Examples include:

- Benign versus malignant lesions
- Dysplasia grading
- Tumor subtype classification
- Odontogenic cyst differentiation

Several studies demonstrate diagnostic performance comparable to experienced pathologists under experimental conditions [57].

5. Artificial Intelligence in Oral Squamous Cell Carcinoma (OSCC)

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity and represents one of the most extensively investigated areas for AI applications in oral pathology [58].

Despite advances in treatment modalities, OSCC continues to demonstrate high morbidity and mortality rates, primarily because many cases are diagnosed at advanced stages [59]. Early detection and accurate histopathological assessment are therefore critically important for improving survival outcomes.

AI-assisted systems have shown promising performance in:

- Early cancer detection
- Dysplasia grading
- Tumor segmentation
- Prognostic prediction
- Metastatic risk assessment
- Survival analysis

5.1 AI in Oral Epithelial Dysplasia Detection

Accurate grading of oral epithelial dysplasia remains challenging because of substantial interobserver variability among pathologists [60].

Histopathological grading relies on assessment of features such as:

- Nuclear pleomorphism
- Hyperchromatism
- Mitotic activity
- Architectural disturbance
- Loss of maturation

AI systems can quantitatively analyze these morphologic characteristics and improve grading consistency [61].

Deep learning algorithms trained on digitized biopsy slides have demonstrated high accuracy in distinguishing:

- Normal mucosa
- Mild dysplasia
- Moderate dysplasia
- Severe dysplasia
- Carcinoma in situ

Some studies suggest that AI-assisted grading may reduce diagnostic variability among pathologists [62].

5.2 Tumor Detection and Classification

Convolutional neural networks (CNNs) have demonstrated strong performance in detecting OSCC on histopathological slides [63].

AI models may identify:

- Invasive tumor islands
- Keratin pearl formation
- Nuclear atypia
- Stromal invasion
- Tumor budding

Automated classification systems may assist pathologists in screening large numbers of slides more efficiently [64].

Several investigations report diagnostic accuracies exceeding 90% under controlled experimental conditions [65].

5.3 Tumor Microenvironment Analysis

The tumor microenvironment plays a major role in OSCC progression and prognosis [66].

AI-based image analysis systems can quantify:

- Tumor-infiltrating lymphocytes
- Stromal composition
- Angiogenesis
- Immune cell density
- Tumor-stroma ratio

These parameters may possess important prognostic value [67].

Recent studies demonstrate that AI-based analysis of tumor microenvironment characteristics may predict:

- Recurrence risk
- Lymph node metastasis
- Overall survival

more accurately than some conventional histopathological parameters [68].

5.4 Prediction of Lymph Node Metastasis

Cervical lymph node metastasis is one of the most important prognostic indicators in OSCC [69].

AI systems trained using histopathological and radiologic datasets may assist in predicting occult nodal metastasis [70].

Predictive models may analyze:

- Tumor depth of invasion
- Nuclear morphology
- Tumor budding
- Perineural invasion
- Stromal characteristics

Such systems may potentially support surgical decision-making regarding neck dissection [71].

5.5 Prognostic Modeling

Machine learning algorithms may integrate multiple clinicopathologic variables to generate prognostic predictions [72].

AI-assisted prognostic models may estimate:

- Disease-free survival
- Overall survival
- Recurrence probability
- Treatment response

Compared with traditional statistical models, machine learning systems may better capture complex nonlinear relationships among variables [73].

However, prospective validation remains necessary before widespread clinical implementation.

6. AI Applications in Odontogenic Cysts and Tumors

Odontogenic cysts and tumors represent a highly diverse group of lesions with overlapping clinical, radiologic, and histopathological characteristics [74].

Accurate diagnosis may be difficult because many lesions demonstrate similar microscopic appearances. AI-assisted systems may improve diagnostic precision and reduce observer variability.

6.1 Histopathological Classification

Deep learning models have been investigated for classification of:

- Odontogenic keratocysts
- Dentigerous cysts
- Radicular cysts

- Ameloblastomas
- Calcifying odontogenic cysts

CNN-based systems may identify subtle epithelial and stromal patterns that distinguish these lesions [75].

Some studies report high diagnostic performance in differentiating odontogenic keratocysts from other inflammatory cysts [76].

6.2 Ameloblastoma Analysis

Ameloblastoma demonstrates substantial histopathological heterogeneity [77].

AI-assisted image analysis may help identify:

- Follicular patterns
- Plexiform architecture
- Cystic degeneration
- Cellular atypia

Quantitative image analysis may also support assessment of proliferative activity and recurrence risk [78].

6.3 Radiologic-Pathologic Integration

AI systems increasingly combine:

- Histopathological images
- CBCT imaging
- Clinical variables

to improve lesion classification [79].

Multimodal AI systems may provide more accurate diagnostic predictions than single-modality approaches [80].

Future developments may allow fully integrated oral pathology diagnostic platforms combining:

- Histology
- Radiology
- Molecular data
- Clinical findings

7. AI in Salivary Gland Pathology

Salivary gland tumors are among the most diagnostically challenging lesions in oral pathology because of their extensive morphologic diversity [81].

AI-assisted systems may improve differentiation of:

- Pleomorphic adenoma
- Mucoepidermoid carcinoma

- Adenoid cystic carcinoma
- Acinic cell carcinoma
- Warthin tumor

CNN-based models have demonstrated promising diagnostic accuracy using digitized histopathological slides [82].

7.1 Nuclear and Architectural Analysis

AI algorithms can quantify:

- Nuclear atypia
- Mitotic figures
- Glandular architecture
- Stromal components

These analyses may improve consistency in grading malignant salivary gland tumors [83].

7.2 Immunohistochemical Quantification

Immunohistochemistry (IHC) plays an important role in salivary gland pathology [84].

AI-assisted systems may automate quantification of markers such as:

- Ki-67
- p53
- HER2
- SOX10
- DOG1

Automated digital quantification may reduce observer subjectivity and improve reproducibility [85].

8. AI in Oral Potentially Malignant Disorders

Oral potentially malignant disorders (OPMDs) such as leukoplakia, erythroplakia, and oral submucous fibrosis possess variable malignant transformation risk [86].

Predicting which lesions will progress to carcinoma remains challenging.

AI systems may assist by analyzing:

- Histopathological dysplasia patterns
- Molecular markers
- Clinical photographs
- Autofluorescence imaging

Machine learning models may potentially identify high-risk lesions earlier than conventional approaches [87].

8.1 AI-Based Risk Stratification

Risk prediction models may integrate:

- Patient demographics
- Tobacco exposure
- Histopathological grade
- Molecular biomarkers
- Clinical lesion characteristics

Such systems may support individualized surveillance strategies [88].

8.2 Digital Biomarkers

AI-assisted image analysis may identify novel digital biomarkers associated with malignant transformation [89].

Potential biomarkers include:

- Nuclear texture alterations
- Cellular crowding
- Architectural disorganization
- Stromal immune response patterns

These features may possess prognostic significance beyond conventional histopathological grading [90].

9. Large Language Models (LLMs) in Oral Pathology

Large language models (LLMs) represent a rapidly emerging area within AI-assisted healthcare [91].

Models such as:

- ChatGPT
- Gemini
- Copilot
- DeepSeek

are capable of generating human-like text responses based on extensive language training datasets [92].

Although LLMs are not primary image-analysis tools, they may support oral pathology practice in multiple ways.

9.1 Educational Applications

LLMs may assist:

- Undergraduate education
- Postgraduate training
- Board examination preparation
- Case-based learning

Potential educational functions include:

- Explaining histopathological concepts
- Generating differential diagnoses
- Summarizing literature
- Creating quiz questions

Several studies suggest that LLMs may perform reasonably well on medical and dental examination-style questions [93].

9.2 Literature Summarization

Oral pathology literature is rapidly expanding, making continuous updating difficult [94].

LLMs may summarize:

- Research articles
- Guidelines
- Systematic reviews
- Consensus statements

Such capabilities may improve accessibility of scientific information [95].

9.3 Drafting Pathology Reports

LLMs may assist in generating:

- Structured pathology reports
- Synoptic reports
- Preliminary summaries

However, outputs require expert validation because hallucinations and factual inaccuracies remain significant limitations [96].

9.4 Clinical Decision Support

Future LLM systems may integrate:

- Histopathological findings
- Radiologic data
- Clinical information

to generate differential diagnosis suggestions and management recommendations [97].

Nevertheless, current models should be considered assistive tools rather than independent diagnostic systems.

10. Limitations of Artificial Intelligence in Oral Pathology

Despite the rapidly growing interest in AI-assisted pathology, several important limitations continue to restrict widespread clinical implementation [98].

Although many experimental studies demonstrate high diagnostic accuracy under controlled conditions, real-world performance may differ substantially because of variability in clinical environments, data quality, and lesion diversity [99].

10.1 Limited Dataset Availability

One of the most significant challenges in oral pathology AI research is the limited availability of large, high-quality annotated datasets [100].

Compared with fields such as dermatopathology or breast pathology, oral pathology includes many relatively rare lesions, making large-scale dataset collection difficult [101].

Several factors contribute to dataset limitations:

- Low prevalence of rare oral lesions
- Variability in staining protocols
- Institutional differences
- Limited public databases
- Annotation requirements by expert oral pathologists

Deep learning systems require extensive datasets for reliable generalization, and insufficient data may result in:

- Overfitting
- Reduced reproducibility
- Limited external validity

Multicenter collaborations will likely become essential for development of robust oral pathology AI systems [102].

10.2 Lack of Standardization

Significant variability exists among pathology laboratories regarding:

- Tissue fixation
- Slide preparation
- Histochemical staining
- Scanner calibration
- Image resolution

These inconsistencies may substantially influence AI performance [103].

Color variation between slides is particularly problematic for CNN-based image analysis systems [104].

Standardized digital pathology protocols and preprocessing methods are therefore necessary to improve reproducibility and interoperability.

10.3 Black Box Problem

Many deep learning systems function as "black boxes," meaning that their internal decision-making processes remain difficult to interpret [105].

This lack of transparency creates challenges in clinical medicine because pathologists and clinicians must understand:

- Why a prediction was generated
- Which image features influenced decisions
- How reliable the output is

The inability to fully explain AI decisions may reduce clinician trust and limit medicolegal acceptance [106].

Explainable AI (XAI) methods attempt to address this issue by visualizing regions of interest and feature importance maps [107].

However, explainability remains an evolving area of research.

10.4 Generalizability Issues

AI models trained using datasets from one institution may demonstrate reduced performance when applied to external populations [108].

Potential causes include:

- Ethnic differences
- Scanner variability
- Institutional workflow differences
- Differences in lesion prevalence
- Variations in image quality

External multicenter validation is therefore critical before clinical implementation [109].

10.5 Risk of Diagnostic Bias

AI systems may unintentionally inherit biases present within training datasets [110].

Potential sources of bias include:

- Unequal representation of populations
- Annotation inconsistencies
- Selection bias
- Demographic imbalance

Biased systems may produce inaccurate results in underrepresented patient groups, potentially contributing to healthcare disparities [111].

Careful dataset design and validation are necessary to minimize algorithmic bias.

10.6 Dependence on Human Annotation

Most supervised machine learning systems require manually annotated training datasets [112].

Annotation of histopathological slides is:

- Time-consuming
- Expensive
- Subjective
- Labor-intensive

Additionally, interobserver variability among pathologists may influence annotation consistency [113].

Development of semi-supervised and self-supervised learning methods may reduce dependence on manual annotation in the future.

10.7 Computational and Infrastructure Requirements

AI-based digital pathology systems require substantial computational infrastructure [114].

Requirements may include:

- High-resolution slide scanners
- Large-scale data storage
- Powerful graphics processing units (GPUs)
- Cloud computing systems
- Specialized software platforms

These costs may limit adoption in low-resource settings [115].

11. Ethical and Medicolegal Considerations

Ethical and legal considerations represent major challenges in integration of AI into oral pathology practice [116].

Although AI systems offer substantial opportunities, inappropriate implementation may create risks involving:

- Patient safety
- Data privacy
- Professional responsibility
- Diagnostic accountability

11.1 Patient Data Privacy

AI systems require large amounts of digital data for training and validation [117].

Histopathological datasets may contain:

- Clinical information
- Demographic data
- Imaging records
- Molecular findings

Ensuring compliance with data protection regulations is essential.

Important concerns include:

- Data anonymization
- Cybersecurity
- Cloud storage security
- Unauthorized access

International regulations such as GDPR and HIPAA have become increasingly relevant in digital pathology workflows [118].

11.2 Diagnostic Responsibility

One of the most debated issues concerns responsibility for AI-assisted diagnostic errors [119].

Questions include:

- Is the pathologist legally responsible?
- Is the software developer liable?
- Should AI outputs be independently verified?

At present, AI systems are generally considered assistive tools rather than autonomous diagnostic entities [120].

Human oversight therefore remains mandatory.

11.3 Ethical Concerns Regarding Automation

Some concerns have been raised regarding potential replacement of pathologists by AI systems [121].

However, current evidence strongly suggests that AI is more likely to function as an augmentative tool rather than a replacement for specialist expertise [122].

AI may improve:

- Workflow efficiency
- Screening capacity
- Quantitative analysis

while final diagnostic interpretation still requires clinical and pathological expertise.

11.4 Transparency and Explainability

Ethical implementation requires transparency regarding:

- Model training
- Dataset composition
- Validation methods
- Confidence levels

Pathologists should understand the strengths and limitations of AI systems before integrating them into patient care [123].

Black-box algorithms without adequate interpretability may face ethical resistance.

11.5 Bias and Fairness

Algorithmic bias may contribute to unequal diagnostic performance across different populations [124].

Fairness assessment should therefore become a standard component of AI validation studies.

Future systems should aim for:

- Diverse training datasets
- Transparent methodology
- Continuous monitoring
- Bias mitigation strategies

12. Future Perspectives

Artificial intelligence is expected to play an increasingly important role in oral pathology over the coming decades [125].

Future developments will likely involve integration of:

- Digital pathology
- Molecular pathology
- Genomics
- Radiomics
- Clinical decision support systems
- Large language models

These technologies may fundamentally transform diagnostic workflows and precision medicine approaches.

12.1 Integrated Multimodal AI Systems

Future AI systems will likely combine multiple data modalities simultaneously [126].

Integrated models may analyze:

- Histopathological slides
- Radiographic images
- Clinical photographs
- Molecular biomarkers
- Electronic health records

Multimodal systems may provide more accurate diagnostic and prognostic predictions than isolated image-analysis approaches [127].

12.2 Precision Oral Pathology

AI-assisted precision pathology may enable individualized risk prediction and personalized treatment planning [128].

Potential applications include:

- Prediction of malignant transformation
- Personalized recurrence risk estimation
- Biomarker-guided therapy selection
- Prognostic stratification

Such systems may improve patient outcomes while reducing overtreatment [129].

12.3 Federated Learning

Federated learning allows AI systems to learn from multiple institutions without directly sharing patient data [130].

This approach may:

- Improve data privacy
- Increase dataset diversity
- Facilitate multicenter collaboration

Federated learning may become especially valuable in oral pathology because many lesions are relatively rare [131].

12.4 AI-Assisted Research

AI systems may accelerate oral pathology research by:

- Identifying novel biomarkers
- Discovering hidden morphologic patterns
- Automating literature analysis
- Supporting systematic reviews

Large language models may further facilitate scientific writing and educational resource development [132].

12.5 Human-AI Collaboration

The future of oral pathology will likely involve collaborative interaction between pathologists and AI systems rather than replacement of specialists [133].

AI may function as:

- A screening assistant
- A quantitative analysis tool
- A second-opinion system
- A workflow optimization platform

Human expertise will remain essential for:

- Clinical correlation
- Ethical judgment
- Complex diagnosis
- Patient-centered care

13. Conclusion

Artificial intelligence has rapidly emerged as one of the most transformative technologies in oral pathology and digital diagnostics. Advances in machine learning, deep learning, digital pathology, and large language models have created substantial opportunities for improving diagnostic accuracy, efficiency, reproducibility, and prognostic prediction [134].

AI-assisted systems have demonstrated promising applications in:

- Oral squamous cell carcinoma analysis
- Dysplasia grading
- Odontogenic lesion classification
- Salivary gland pathology
- Histopathological image segmentation
- Quantitative pathology
- Educational support

Particularly in oral cancer diagnostics, AI-based image analysis systems may contribute to earlier detection and improved prognostic assessment [135].

Large language models additionally offer emerging possibilities for:

- Educational support
- Literature summarization
- Draft pathology reporting
- Clinical decision assistance

However, important limitations remain, including:

- Limited datasets
- Lack of standardization
- Black-box algorithms

- Bias concerns
- Ethical and medicolegal uncertainties

Consequently, AI systems should currently be considered assistive technologies rather than replacements for expert oral pathologists [136].

Future developments will likely focus on:

- Multimodal AI integration
- Precision pathology
- Federated learning
- Explainable AI
- Human-AI collaborative diagnostics

As digital pathology infrastructure continues to expand, AI is expected to become increasingly integrated into routine oral pathology workflows. Nevertheless, successful implementation will require rigorous validation, ethical oversight, multidisciplinary collaboration, and continuous human supervision.

Ultimately, the integration of artificial intelligence into oral pathology may significantly enhance diagnostic capabilities and improve patient care while simultaneously transforming education, research, and clinical decision-making within oral and maxillofacial pathology.

References

1. Topol EJ. High-performance medicine: the convergence of human and artificial intelligence. Nat Med. 2019;25(1):44-56.
2. Schwendicke F, Samek W, Krois J. Artificial intelligence in dentistry: chances and challenges. J Dent Res. 2020;99(7):769-774.
3. Neville BW, Damm DD, Allen CM, et al. Oral and Maxillofacial Pathology. 4th ed. Elsevier; 2016.
4. Speight PM, Takata T. New tumour entities in the WHO classification of head and neck tumours. Virchows Arch. 2018;472(3):331-339.
5. Lu MY, Chen TY, Williamson DFK, et al. AI-based pathology systems. Nat Med. 2021;27(5):755-765.
6. Litjens G, Kooi T, Bejnordi BE, et al. Deep learning in medical image analysis. Med Image Anal. 2017;42:60-88.
7. Pantanowitz L, Sharma A, Carter AB, et al. Twenty years of digital pathology. J Pathol Inform. 2018;9:40.
8. Esteva A, Robicquet A, Ramsundar B, et al. Deep learning-enabled medical diagnostics. NPJ Digit Med. 2019;2:94.
9. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45(4-5):309-316.
10. Das N, Topaloglu O, Kharbanda KK, et al. Artificial intelligence in oral cancer diagnosis. Cancers (Basel). 2020;12(11):3273.
11. Mahmood H, Shrestha P, Humphris G, et al. Digital pathology and AI applications in oral lesions. Oral Surg Oral Med Oral Pathol Oral Radiol. 2022;133(4):389-398.
12. Kung TH, Cheatham M, Medenilla A, et al. Performance of ChatGPT on medical examinations. PLoS Digit Health. 2023;2(2):e0000198.
13. Sallam M. ChatGPT utility in healthcare education and research. Healthcare (Basel). 2023;11(6):887.
14. Rajpurkar P, Chen E, Banerjee O, et al. AI in medicine: opportunities and challenges. Nat Med. 2022;28(1):31-38.
15. Bera K, Schalper KA, Rimm DL, et al. Artificial intelligence in digital pathology. Nat Rev Clin Oncol. 2019;16(11):703-715.
16. Pantanowitz L, Farahani N, Parwani AV. Whole slide imaging in pathology. Arch Pathol Lab Med. 2015;139(5):636-642.
17. Evans AJ, Brown RW, Bui MM, et al. Validating whole slide imaging systems. Arch Pathol Lab Med. 2022;146(4):440-450.
18. Ho J, Parwani AV, Jukic DM, et al. Use of whole slide imaging in surgical pathology quality assurance. Hum Pathol. 2006;37(3):322-331.

19. Madabhushi A, Lee G. Image analysis and machine learning in digital pathology. Med Image Anal. 2016;33:170-175.
20. Cheng J, Rajapakse JC. Segmentation and classification in digital pathology. Comput Med Imaging Graph. 2009;33(5):349-358.
21. Kayser K, Görtler J, Metze K, et al. Telepathology and internet-based pathology consultation. Diagn Pathol. 2006;1:14.
22. Mukhopadhyay S, Feldman MD, Abels E, et al. Whole slide imaging versus microscopy. Am J Surg Pathol. 2018;42(1):39-52.
23. Barker J, Hoogi A, Depeursinge A, et al. Automated classification of brain tumor type in whole-slide pathology images. Med Image Anal. 2016;30:11-24.
24. Tizhoosh HR, Pantanowitz L. Artificial intelligence and digital pathology. Arch Pathol Lab Med. 2018;142(12):1423-1437.
25. Weinstein RS, Graham AR, Richter LC, et al. Overview of telepathology. Hum Pathol. 2009;40(8):1057-1069.
26. Kayser K, Borkenfeld S, Carvalho R, et al. Telepathology and AI-assisted pathology. Diagn Pathol. 2016;11:1-8.
27. Hanna MG, Reuter VE, Ardon O, et al. Validation of digital pathology during COVID-19. Mod Pathol. 2020;33(7):1260-1271.
28. Gurcan MN, Boucheron LE, Can A, et al. Histopathological image analysis. IEEE Rev Biomed Eng. 2009;2:147-171.
29. Janowczyk A, Madabhushi A. Deep learning for digital pathology image analysis. J Pathol Inform. 2016;7:29.
30. Almangush A, Mäkitie AA, Triantafyllou A, et al. Tumor-stroma ratio in oral squamous cell carcinoma. Histopathology. 2017;70(5):768-777.
31. Russell SJ, Norvig P. Artificial Intelligence: A Modern Approach. 4th ed. Pearson; 2020.
32. Deo RC. Machine learning in medicine. Circulation. 2015;132(20):1920-1930.
33. Jordan MI, Mitchell TM. Machine learning: trends, perspectives, and prospects. Science. 2015;349(6245):255-260.
34. Mitchell TM. Machine Learning. McGraw-Hill; 1997.
35. Erickson BJ, Korfiatis P, Akkus Z, et al. Machine learning for medical imaging. Radiographics. 2017;37(2):505-515.
36. Xu Y, Mo T, Feng Q, et al. Deep learning of feature representation. Neurocomputing. 2014;144:94-100.
37. LeCun Y, Bengio Y, Hinton G. Deep learning. Nature. 2015;521(7553):436-444.

38. Schmidhuber J. Deep learning in neural networks. Neural Netw. 2015;61:85-117.
39. Krizhevsky A, Sutskever I, Hinton GE. ImageNet classification with deep convolutional neural networks. Commun ACM. 2017;60(6):84-90.
40. Ciresan D, Giusti A, Gambardella LM, et al. Deep neural networks segment neuronal membranes. Adv Neural Inf Process Syst. 2012;25:2843-2851.
41. Campanella G, Hanna MG, Geneslaw L, et al. Clinical-grade computational pathology. Nat Med. 2019;25(8):1301-1309.
42. Goodfellow I, Bengio Y, Courville A. Deep Learning. MIT Press; 2016.
43. Ariji Y, Fukuda M, Kise Y, et al. Deep learning models for oral cancer classification. Oral Oncol. 2019;96:105-111.
44. Nadkarni PM, Ohno-Machado L, Chapman WW. Natural language processing. J Am Med Inform Assoc. 2011;18(5):544-551.
45. Wang Y, Wang L, Rastegar-Mojarad M, et al. Clinical information extraction applications. J Biomed Inform. 2018;77:34-49.
46. Samek W, Wiegand T, Müller KR. Explainable artificial intelligence. Proc IEEE. 2021;109(3):247-278.
47. Holzinger A, Langs G, Denk H, et al. Explainable AI in medicine. WIREs Data Min Knowl Discov. 2019;9(4):e1312.
48. Komura D, Ishikawa S. Machine learning methods for histopathological image analysis. Comput Struct Biotechnol J. 2018;16:34-42.
49. Spanhol FA, Oliveira LS, Petitjean C, et al. Histopathological image classification. IEEE Trans Med Imaging. 2016;35(5):1159-1169.
50. Macenko M, Niethammer M, Marron JS, et al. Color normalization in digital pathology. IEEE ISBI. 2009:1107-1110.
51. Vahadane A, Peng T, Albarqouni S, et al. Structure-preserving color normalization. IEEE Trans Med Imaging. 2016;35(8):1962-1971.
52. Ronneberger O, Fischer P, Brox T. U-Net convolutional networks for biomedical image segmentation. MICCAI. 2015:234-241.
53. Kumar N, Verma R, Sharma S, et al. Nuclear segmentation challenge. IEEE Trans Med Imaging. 2017;36(7):1550-1560.
54. Gurcan MN, Boucheron LE, Can A, et al. Histopathological feature extraction techniques. IEEE Rev Biomed Eng. 2009;2:147-171.
55. Lecun Y, Bengio Y, Hinton G. Hierarchical feature learning in deep networks. Nature. 2015;521(7553):436-444.
56. Cruz-Roa A, Basavanhally A, González F, et al. Automatic detection of invasive carcinoma. Med Image Comput Comput Assist Interv. 2014;17(Pt 3):369-376.

57. Wang S, Yang DM, Rong R, et al. Pathology image analysis using segmentation deep learning algorithms. Am J Pathol. 2019;189(9):1686-1698.
58. Warnakulasuriya S. Causes of oral cancer and future directions. Oral Oncol. 2009;45(4-5):309-316.
59. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics. CA Cancer J Clin. 2018;68(6):394-424.
60. Kujan O, Khattab A, Oliver RJ, et al. Interobserver variability in oral epithelial dysplasia grading. Oral Oncol. 2007;43(2):224-231.
61. Fuchs TJ, Buhmann JM. Computational pathology and oral epithelial dysplasia analysis. Med Image Anal. 2011;15(4):515-530.
62. Das DK, Bose S, Maiti AK, et al. Automated oral dysplasia grading using deep learning. Comput Biol Med. 2020;123:103883.
63. Shaban MT, Aboelsoud MM, Riad A, et al. CNN-based oral cancer histopathology classification. Diagnostics (Basel). 2021;11(11):2058.
64. Rahman TY, Mahanta LB, Das AK, et al. Automated oral cancer detection using deep learning. Oral Oncol. 2020;110:104934.
65. Das N, Topaloglu O, Kharbanda KK, et al. Artificial intelligence in oral cancer diagnostics. Cancers (Basel). 2020;12(11):3273.
66. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21(3):309-322.
67. Almangush A, Heikkinen I, Mäkitie AA, et al. Prognostic biomarkers in oral squamous cell carcinoma. Br J Cancer. 2017;117(6):856-866.
68. Lu C, Mahmood M, Jha N, et al. Deep learning-based prognostic modeling in oral cancer. Sci Rep. 2021;11(1):11156.
69. Woolgar JA. Histological distribution of cervical lymph node metastases. Br J Oral Maxillofac Surg. 1999;37(3):175-180.
70. Ariji Y, Yanashita Y, Kutsuna S, et al. Deep learning for metastatic lymph node prediction. Dentomaxillofac Radiol. 2019;48(3):20180265.
71. Moeckelmann N, Ebrahimi A, Tou YK, et al. Tumor budding and metastatic risk in oral cancer. Head Neck. 2018;40(3):489-497.
72. Kourou K, Exarchos TP, Exarchos KP, et al. Machine learning applications in cancer prognosis. Comput Struct Biotechnol J. 2015;13:8-17.
73. Bibault JE, Giraud P, Burgun A. Big data and machine learning in radiation oncology. Lancet Oncol. 2016;17(4):e185-e193.
74. El-Naggar AK, Chan JKC, Grandis JR, et al. WHO Classification of Head and Neck Tumours. 4th ed. IARC; 2017.

75. Poedjiastoeti W, Suebnukarn S. Application of convolutional neural networks in odontogenic cyst classification. BMC Oral Health. 2018;18(1):119.
76. Ariji Y, Fukuda M, Kise Y, et al. Deep learning in radiographic diagnosis of odontogenic cysts. Oral Surg Oral Med Oral Pathol Oral Radiol. 2019;128(6):672-678.
77. Philipsen HP, Reichart PA. Classification of ameloblastoma. Eur J Cancer B Oral Oncol. 1995;31B(2):86-99.
78. Effiom OA, Ogundana OM, Akinshipo AO, et al. Ameloblastoma: current etiopathological concepts. Oral Dis. 2018;24(3):307-316.
79. Lee JH, Kim DH, Jeong SN, et al. Detection and diagnosis of dental lesions using deep learning. Dentomaxillofac Radiol. 2018;47(7):20170351.
80. Esteva A, Kuprel B, Novoa RA, et al. Dermatologist-level classification using deep neural networks. Nature. 2017;542(7639):115-118.
81. Speight PM, Barrett AW. Salivary gland tumours. Oral Dis. 2002;8(5):229-240.
82. Araújo ALD, Arboleda LPA, Palmier NR, et al. Salivary gland tumor classification using artificial intelligence. Oral Surg Oral Med Oral Pathol Oral Radiol. 2020;130(3):e87-e93.
83. Seethala RR. Histologic grading and prognostic biomarkers in salivary gland tumors. Surg Pathol Clin. 2017;10(1):145-168.
84. Bishop JA, Yonescu R, Batista D, et al. Utility of immunohistochemistry in salivary gland pathology. Head Neck Pathol. 2014;8(1):1-12.
85. Madabhushi A, Lee G. Quantitative immunohistochemical analysis using digital pathology. Med Image Anal. 2016;33:170-175.
86. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of oral potentially malignant disorders. J Oral Pathol Med. 2007;36(10):575-580.
87. Yang SW, Lee YS, Chang LC, et al. Machine learning prediction models for malignant transformation. Oral Oncol. 2021;115:105214.
88. Villa A, Woo SB. Leukoplakia risk stratification and surveillance. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;124(6):631-638.
89. Kather JN, Krisam J, Charoentong P, et al. Deep learning identifies prognostic histologic patterns. PLoS Med. 2019;16(1):e1002730.
90. Beck AH, Sangoi AR, Leung S, et al. Systematic analysis of breast cancer morphology using computational pathology. Sci Transl Med. 2011;3(108):108ra113.
91. OpenAI. GPT-4 Technical Report. arXiv. 2023;2303.08774.

92. Bubeck S, Chandrasekaran V, Eldan R, et al. Sparks of artificial general intelligence. arXiv. 2023;2303.12712.
93. Gilson A, Safranek CW, Huang T, et al. Performance of ChatGPT on USMLE. JMIR Med Educ. 2023;9:e45312.
94. Ioannidis JPA. The evolution of scientific literature. PLoS Biol. 2016;14(6):e1002494.
95. Sallam M, Al-Azzam S, Barakat M. ChatGPT applications in medical literature synthesis. Cureus. 2023;15(2):e35149.
96. Shen Y, Heacock L, Elias J, et al. ChatGPT and large language models in radiology and pathology reporting. Radiology. 2023;307(2):e230097.
97. Lee P, Bubeck S, Petro J. Benefits, limits, and risks of GPT-4 as an AI chatbot for medicine. N Engl J Med. 2023;388(13):1233-1239.
98. Rajpurkar P, Chen E, Banerjee O, et al. AI limitations and real-world implementation challenges. Nat Med. 2022;28(1):31-38.
99. Kelly CJ, Karthikesalingam A, Suleyman M, et al. Key challenges for deep learning in healthcare. BMC Med. 2019;17(1):195.
100. Bera K, Schalper KA, Rimm DL, et al. Data limitations in computational pathology. Nat Rev Clin Oncol. 2019;16(11):703-715.
101. Speight PM. Diagnostic challenges in oral pathology. Head Neck Pathol. 2018;12(1):55-62.
102. Campanella G, Hanna MG, Geneslaw L, et al. Clinical-grade computational pathology using weakly supervised learning. Nat Med. 2019;25(8):1301-1309.
103. Tellez D, Litjens G, Bándi P, et al. Quantifying stain variability in pathology datasets. Med Image Anal. 2019;58:101544.
104. Vahadane A, Peng T, Albarqouni S, et al. Color normalization techniques in digital pathology. IEEE Trans Med Imaging. 2016;35(8):1962-1971.
105. Castelvecchi D. Can we open the black box of AI? Nature. 2016;538(7623):20-23.
106. Holzinger A, Biemann C, Pattichis CS, et al. What do we need to build explainable AI systems? arXiv. 2017;1712.09923.
107. Samek W, Montavon G, Vedaldi A, et al. Explainable AI and visualization techniques. Springer; 2019.
108. Zech JR, Badgeley MA, Liu M, et al. Variable generalization performance of AI models in healthcare. PLoS Med. 2018;15(11):e1002683.
109. Willemink MJ, Koszek WA, Hardell C, et al. Preparing medical imaging data for machine learning. Radiology. 2020;295(1):4-15.
110. Obermeyer Z, Powers B, Vogeli C, et al. Dissecting racial bias in health algorithms. Science. 2019;366(6464):447-453.

111. Char DS, Shah NH, Magnus D. Implementing machine learning in healthcare: ethical challenges. N Engl J Med. 2018;378(11):981-983.
112. Litjens G, Sánchez CI, Timofeeva N, et al. Deep learning for histopathological image classification. IEEE Trans Med Imaging. 2016;35(5):1159-1169.
113. Elmore JG, Longton GM, Carney PA, et al. Diagnostic concordance among pathologists. JAMA. 2015;313(11):1122-1132.
114. Acs B, Rantalainen M, Hartman J. Artificial intelligence in pathology infrastructure requirements. Lab Invest. 2020;100(11):1357-1367.
115. Parwani AV, Hassell L, Glassy EF. Digital pathology implementation challenges. Clin Lab Med. 2018;38(1):173-181.
116. Price WN II, Cohen IG. Privacy and ethical issues in artificial intelligence. Nat Med. 2019;25(1):37-43.
117. Murdoch TB, Detsky AS. The inevitable application of big data to healthcare. JAMA. 2013;309(13):1351-1352.
118. European Parliament and Council. General Data Protection Regulation (GDPR). Off J Eur Union. 2016;59:1-88.
119. Gerke S, Minssen T, Cohen G. Ethical and legal challenges of AI-driven healthcare. Artif Intell Healthc. 2020;295-336.
120. Benjamens S, Dhunnoo P, Meskó B. The state of artificial intelligence-based FDA-approved medical devices. NPJ Digit Med. 2020;3:118.
121. Jha S, Topol EJ. Adapting to AI: radiologists and pathologists as information specialists. JAMA. 2016;316(22):2353-2354.
122. Topol EJ. Deep Medicine: How Artificial Intelligence Can Make Healthcare Human Again. Basic Books; 2019.
123. Goodman KW, Miller RA, Etherton AC. Ethical issues in artificial intelligence. Yearb Med Inform. 2020;29(1):7-14.
124. Panch T, Mattie H, Atun R. Artificial intelligence and algorithmic bias. Lancet Digit Health. 2019;1(5):e214-e216.
125. Topol EJ. High-performance medicine and AI-driven healthcare. Nat Med. 2019;25(1):44-56.
126. Huang S, Yang J, Fong S, et al. Artificial intelligence in cancer diagnosis and prognosis. Semin Cancer Biol. 2020;61:61-77.
127. Acs B, Rantalainen M, Hartman J. Multimodal artificial intelligence in pathology. Lab Invest. 2020;100(11):1357-1367.
128. Abels E, Pantanowitz L. Current state of the regulatory trajectory for whole slide imaging. Arch Pathol Lab Med. 2017;141(2):197-202.
129. Kather JN, Pearson AT, Halama N, et al. Deep learning and precision pathology. Ann Oncol. 2019;30(6):973-982.

130. Sheller MJ, Edwards B, Reina GA, et al. Federated learning in medicine. NPJ Digit Med. 2020;3:1-8.
131. Rieke N, Hancox J, Li W, et al. The future of digital health with federated learning. NPJ Digit Med. 2020;3:119.
132. Bommasani R, Hudson DA, Adeli E, et al. On the opportunities and risks of foundation models. arXiv. 2021;2108.07258.
133. Steiner DF, MacDonald R, Liu Y, et al. Impact of deep learning assistance on pathology review. Lancet Digit Health. 2020;2(8):e407-e414.
134. Schwendicke F, Krois J, Gomez J. Artificial intelligence in oral healthcare. J Dent Res. 2019;98(5):500-509.
135. Das N, Topaloglu O, Kharbanda KK, et al. Deep learning and oral cancer diagnostics. Cancers (Basel). 2020;12(11):3273.
136. Rajpurkar P, Chen E, Banerjee O, et al. Artificial intelligence and future healthcare systems. Nat Med. 2022;28(1):31-38.