

INTERNATIONAL STUDIES IN THE FIELD OF

# HEALTH SCIENCES

**MART 2026**

**EDITORS**

**Prof. Dr. Engin ŞAHNA**

**Prof. Dr. Hasan AKGÜL**

**Prof. Dr. Zeliha SELAMOĞLU**

**Doç. Dr. Nevra ALKANLI**

**İmtiyaz Sahibi** / Yaşar Hız  
**Yayına Hazırlayan** / Gece Kitaplığı

**Birinci Basım** / Mart 2026 - Ankara  
**ISBN** / 978-625-8449-25-9

**© copyright**

Bu kitabın tüm yayın hakları Gece Kitaplığı'na aittir.  
Kaynak gösterilmeden alıntı yapılamaz, izin almadan hiçbir yolla çoğaltılamaz.

**Gece Kitaplığı**

Kızılay Mah. Fevzi Çakmak 1. Sokak  
Ümit Apt No: 22/A Çankaya/ANKARA  
0312 384 80 40  
www.gecekitapligi.com / gecekitapligi@gmail.com

**Baskı & Cilt**

Bizim Büro  
**Sertifika No:** 42488

**INTERNATIONAL STUDIES  
IN THE FIELD OF  
HEALTH SCIENCES**

**MARCH 2026**

EDITORS

Prof. Dr. Engin ŞAHNA

Prof. Dr. Hasan AKGÜL

Prof. Dr. Zeliha SELAMOĞLU

Doç. Dr. Nevra ALKANLI

**gece**  
kitaplığı



## CONTENTS

### CHAPTER 1

#### **ELECTROCHEMICAL DETERMINATION OF CETIRIZINE AND LORATADINE: ADVANCES, ELECTRODE STRATEGIES, AND ANALYTICAL PERSPECTIVES**

*Elif ŞİŞMAN, Fatma AĞIN* ..... 7

### CHAPTER 2

#### **TRANSCENDING THE BOUNDARIES OF BIOENERGETICS: MOLECULAR FOUNDATIONS OF PURINERGIC SIGNALING**

*Leyla ATAÇ DOĞAN* ..... 27

### CHAPTER 3

#### **ORAL POTENTIALLY MALIGNANT DISORDERS**

*Hatice BİLGİÇ , Emre KÖSE , Selime CHAUSH* ..... 45

### CHAPTER 4

#### **ACCOUNTABILITY IN THE UNITED KINGDOM PUBLIC HEALTH SYSTEM**

*Kenan YURTSAL* ..... 65

### CHAPTER 5

#### **BIOELECTRICAL SIGNALING IN CANCER: A BIOPHYSICAL PERSPECTIVE**

*Cagatay Han TURKSEVEN* ..... 77

### CHAPTER 6

#### **EVALUATION OF PUBLIC HEALTH SYSTEMS IN SCANDINAVIAN COUNTRIES: SWEDEN, NORWAY AND FINLAND**

*Kenan YURTSAL* ..... 87

### CHAPTER 7

#### **MALE BREAST CANCER: THREE CASE STUDIES**

*Selim YALÇIN* ..... 109

### CHAPTER 8

#### **THE ROLE OF GUT MICROBIOTA IN BRAIN HEALTH AND PSYCHOLOGICAL - NEUROLOGICAL DISEASES**

*Esra TAŞ* ..... 117



# CHAPTER 1

---

## ELECTROCHEMICAL DETERMINATION OF CETIRIZINE AND LORATADINE: ADVANCES, ELECTRODE STRATEGIES, AND ANALYTICAL PERSPECTIVES

*Elif ŞİŞMAN<sup>1</sup>, Fatma AĞIN<sup>2</sup>*

---

<sup>1</sup> Research Assist. Department of Analytical Chemistry, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye, <sup>2</sup>Department of Analytical Chemistry, Graduate School of Health Sciences, Karadeniz Technical University, Trabzon, Türkiye (<https://orcid.org/0000-0003-4092-3195>)

<sup>2</sup> Assoc. Prof. Dr. Department of Analytical Chemistry, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye (<https://orcid.org/0000-0002-6973-4323>)

## 1. Introduction

Loratadine and cetirizine are second-generation antihistaminic drugs with low sedative effects that are widely used in treatment of diseases such as urticaria, allergic rhinitis, allergic conjunctivitis, and atopic dermatitis by blocking H<sub>1</sub> receptors. Owing to their long duration of action, these agents provide effective therapeutic options without substantially impairing daily activities (Simons & Simons, 2011). Loratadine binds with high affinity to peripheral H<sub>1</sub> receptors and exhibits minimal effects on the central nervous system. Cetirizine, on the other hand, is an active metabolite of hydroxyzine and is characterized by favorable pharmacokinetic properties, including high bioavailability and rapid absorption (Lieberman, 2011).

Accurate determination of these drugs in pharmaceutical formulations and monitoring of their therapeutic efficacy are of great importance in terms of drug safety and treatment success. Conventional analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), and spectrophotometry are commonly employed; however, these methods are often time-consuming, require extensive sample preparation, and involve high operational costs (Joseph, 2000). In contrast, electroanalytical methods have gained increasing attention in modern analytical chemistry for quantitative and qualitative determination due to their short analysis time, high sensitivity, high accuracy, and low cost. Electroanalytical chemistry focuses on the investigation of the oxidation and reduction behaviors of chemical species and the analytical exploitation of these processes. In particular, voltammetry analyzes the current–potential relationship arising from redox reactions of analytes occurring at the electrode surface in solution. Voltammetric techniques include differential pulse voltammetry (DPV), cyclic voltammetry (CV), square-wave voltammetry (SWV), and stripping voltammetry, which enable the determination of analytes at very low concentration levels (Bard et al., 2022).

Recent studies have demonstrated that electrodes modified with carbon nanotubes, polymer coatings, graphene, and metal oxides significantly enhance the selectivity and sensitivity of the voltammetric determination of pharmaceutical compounds such as loratadine and cetirizine. These advanced electrode materials generate much stronger electrochemical responses due to their high electrical conductivity and large effective surface areas (Pushpanjali et al., 2023; Raeisi-Kheirabadi et al., 2021).

As low-sedative H<sub>1</sub> receptor antagonist antihistamines are frequently prescribed, the selective and sensitive determination of loratadine and cetirizine in pharmaceutical preparations and

biological samples has become increasingly important. In this context, voltammetric methods offer an effective analytical approach by exploiting the electroactive properties of these drugs, while providing advantages such as short analysis time, high sensitivity, and low cost. In recent years, nanomaterial- and polymer-modified electrodes have markedly improved the voltammetric signals of loratadine and cetirizine, leading to lower detection limits and enhanced selectivity in complex matrices. Consequently, electroanalytical studies employing various electrode modification strategies present a robust, rapid, and sensitive alternative for the reliable analysis of these antihistamines, while also making significant contributions to pharmaceutical quality control and clinical applications.

## **2. Histamine**

Histamine, a biogenic amine, is widely distributed in nature and has been shown to exert important biological and pharmacological activities in living organisms. In tissues, histamine is synthesized from the amino acid histidine through the removal of its carboxyl group by the enzyme histidine decarboxylase. It serves as a key chemical mediator in the development of allergic diseases. Histamine is stored in granules bound to heparin and ATP within mast cells, basophilic leukocytes, and various other tissues throughout the body. In contrast, in enterochromaffin cells, the skin, and certain tissues, histamine is present in an extramastocytic form. In the central nervous system, histamine exists as neuronal histamine, with particularly high concentrations reported in hypothalamus (Maintz & Novak, 2007).

Histamine elicits its biological effects by interacting with four distinct G protein–coupled receptors (GPCRs). GPCRs constitute a major receptor family responsible for a wide range of biological activities in humans and mediate the actions of numerous signaling molecules, including epinephrine, acetylcholine, histamine, and leukotrienes. The biological responses to histamine differ depending on the receptor subtype involved. Although the majority of histamine-mediated effects associated with allergic rhinitis are primarily linked to the H<sub>1</sub> receptor, signaling through H<sub>2</sub>, H<sub>3</sub>, and H<sub>4</sub> receptors may also contribute. In the central nervous system (CNS), histamine binding to H<sub>1</sub>, H<sub>2</sub>, and H<sub>3</sub> receptors plays a regulatory role in wakefulness, attention, and reaction time (Lieberman, 2011).

### **2.1. Histamine Receptors**

Histamine receptors are categorized into four distinct subtypes, namely H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, and H<sub>4</sub>. The H<sub>1</sub> receptor is the principal mediator of allergic rhinitis symptoms and is mainly expressed in

blood vessels and sensory nerves, where its activation increases vascular permeability, stimulates airway sensory nerves, and promotes eosinophil chemotaxis, leading to sneezing, nasal congestion, and rhinorrhea (Panula et al., 2015). The H<sub>2</sub> receptor is best known for regulating gastric acid secretion but is also present in vascular tissues and nasal mucosal glands, where it enhances secretory activity and may augment H<sub>1</sub>-mediated vascular effects (Parsons & Ganellin, 2006). The H<sub>3</sub> receptor acts primarily as a presynaptic autoreceptor that inhibits norepinephrine release and increases glandular secretion, thereby contributing to nasal symptoms through modulation of peripheral sympathetic signaling (Panula & Nuutinen, 2013). The H<sub>4</sub> receptor is expressed in numerous immune cells and tissues and promotes mast cell chemotaxis and chemokinesis, suggesting a role in amplifying inflammatory responses associated with allergic rhinitis (Thurmond, 2015).

### **3. Antihistamines**

Antihistaminic drugs, also known as histamine receptor blockers, exert their effects by competitively antagonizing histamine at its receptors, particularly H<sub>1</sub> receptors. H<sub>1</sub> antihistamines are classified into first-generation (sedating) and second-generation (non-sedating) agents and are primarily used to relieve ocular, nasal, and throat itching, sneezing, rhinorrhea, lacrimation, and mild nasal congestion. However, they are generally less effective against nasal obstruction associated with the late inflammatory phase of allergic reactions (Panula et al., 2015). Clinically, these drugs are most commonly prescribed for allergic rhinitis and urticaria. Allergic rhinitis, a noninfectious inflammatory disorder of the nasal mucosa, is categorized as seasonal or perennial depending on allergen exposure, whereas urticaria results from histamine-mediated mast cell activation leading to pruritic wheals. H<sub>1</sub> antihistamines are also used in the management of allergic conjunctivitis, atopic dermatitis, motion sickness, Ménière's disease, and the common cold (Bousquet et al., 2020).

#### **3.1. Classical Antihistamines**

First-generation (classical) H<sub>1</sub> receptor antagonists exhibit a rapid onset of action, with peak plasma concentrations generally attained within 1–2 hours and exhibiting an average duration of action of 4–6 hours, which may be extended up to 12 hours with sustained-release formulations. These agents are classified into six main chemical groups, allowing alternative treatment options in cases of tolerance development. Classical antihistamines are generally well absorbed and highly lipophilic, displaying a high affinity for central H<sub>1</sub> receptors. Although their effects begin rapidly, their duration of action is relatively short, and they are predominantly

metabolized in the liver and eliminated via renal or gastrointestinal pathways, resulting in a high potential for drug–drug interactions, particularly with other hepatically metabolized drugs. The high lipophilicity and low molecular weight of first-generation antihistamines facilitate their penetration of the blood–brain barrier and widespread distribution in the central nervous system. Consequently, sedation, fatigue, dizziness, impaired psychomotor coordination, reduced attention, and agitation may occur even at therapeutic doses. In addition, their anticholinergic and  $\alpha$ -adrenergic effects may cause adverse reactions such as dry mouth, blurred vision, urinary retention, and orthostatic hypotension, significantly limiting their clinical use (Simons & Simons, 2011). Based on their chemical structure, classical antihistamines are categorized into ethanolamines, ethylenediamines, alkylamines, piperazine derivatives, phenothiazines, and piperidine derivatives (Rang et al., 2011).

### **3.2. Non-sedating Antihistamines**

Second-generation (non-sedating) H<sub>1</sub> antihistamines were developed to minimize the central nervous system adverse effects commonly associated with first-generation agents. Their greater selectivity for peripheral H<sub>1</sub> receptors, limited penetration across the blood–brain barrier, and active efflux via P-glycoprotein transporters result in minimal central receptor interaction and substantially reduced risks of sedation, psychomotor impairment, and cognitive dysfunctions (Simons & Simons, 2011). In addition, most second-generation antihistamines exhibit longer durations of action, allowing once-daily dosing, which improves patient adherence and supports effective symptom control without interfering with daily activities. Commonly used representatives of this class include loratadine, cetirizine, desloratadine, fexofenadine, and levocetirizine, with loratadine and cetirizine widely regarded as first-line therapies for allergic rhinitis and urticaria due to their rapid onset, high peripheral receptor affinity, and favorable safety profiles (Simons, 2004).

Although generally safer with respect to CNS-related adverse effects, mild sedation has occasionally been reported—particularly with cetirizine at higher doses or in sensitive individuals—yet such effects remain significantly less pronounced than those observed with classical antihistamines and are typically clinically tolerable (Kleine-Tebbe, 2011). Given their widespread clinical use and the presence of electroactive functional groups in their molecular structures, cetirizine and loratadine represent important analytical targets. Their ability to undergo electrochemical oxidation or reduction reactions enables sensitive, selective, and rapid

determination using voltammetric techniques, making them highly convenient for pharmaceutical quality control and biological monitoring applications.

### 3.2.1. Voltammetric Techniques for Determination of Cetirizine

Cetirizine is a second-generation H<sub>1</sub> receptor antagonist characterized by high selectivity for peripheral H<sub>1</sub> receptors and a generally low sedative potential. It is rapidly absorbed following oral administration, reaching peak plasma concentrations within a short time and exhibiting high bioavailability. A substantial proportion of cetirizine is excreted unchanged via the renal route, resulting in limited hepatic metabolism and a reduced risk of drug–drug interactions. Cetirizine is widely prescribed for the treatment of allergic rhinitis, chronic urticaria, and allergic conjunctivitis, providing effective relief from histamine-mediated symptoms such as sneezing, rhinorrhea, and pruritus. Although mild sedation has been reported in susceptible individuals, cetirizine is generally well tolerated and, owing to its favorable efficacy and safety profile, is considered a first-line antihistamine that requires accurate analytical determination in pharmaceutical and biological matrices (Pushanpajali et al., 2023).

Voltammetric methods were developed for the sensitive and selective determination of the antihistaminic drug cetirizine using a poly(bromocresol purple) (poly-BCP) film–modified glassy carbon electrode (poly-BCP/GCE). The poly-BCP film was successfully deposited onto the electrode surface by electropolymerization in 0.1 M phosphate buffer solution (PBS) at pH 6.0 over 50 cycles, and the modification was confirmed to significantly increase the effective surface area and reduce the charge transfer resistance, as evidenced by electrochemical impedance spectroscopy (EIS) and CV results. Under optimized conditions, linear concentration ranges of 0.2–100 µM and 0.1–100 µM were obtained for cetirizine using DPV and SWV, respectively, with corresponding limits of detection (LOD) of 21.8 nM for DPV and 15.1 nM for SWV. The accuracy and precision of the proposed methods were evaluated through recovery studies in pharmaceutical formulations and artificial urine samples, yielding recovery values between 100.12% and 100.90% and relative standard deviation (%RSD) values below 1%, demonstrating excellent accuracy and repeatability. Furthermore, the developed sensor exhibited good long-term stability, satisfactory fabrication reproducibility, and high selectivity against potential interferences in biological matrices. These results indicate that the poly-BCP/GCE-based voltammetric methods provide a robust, reliable, and practical alternative for the routine pharmaceutical and biological analysis of cetirizine. (Sofu et al., 2025)

Pushpanjali et al. developed a poly(glutamine)-modified multiwalled carbon nanotube paste electrode (PGAMCNTPE) for the sensitive and reliable voltammetric determination of the antihistaminic drug cetirizine. The electrode surface was functionalized via electropolymerization of the glutamine monomer, resulting in a marked enhancement of the effective surface area and electrical conductivity. CV measurements performed in PBS (pH 7.0) revealed that cetirizine undergoes an irreversible, adsorption-controlled oxidation process at the modified electrode. pH-dependent studies indicated the involvement of an equal number of protons and electrons in the electrochemical reaction, while kinetic analyses confirmed that the oxidation process is dominated by a single-electron transfer. Under optimized conditions, a linear concentration range of 2–50  $\mu\text{M}$  was obtained for cetirizine, with the LOD and limit of quantification (LOQ) calculated as 0.12  $\mu\text{M}$  and 0.40  $\mu\text{M}$ , respectively. The analytical performance of the method was validated in terms of repeatability (RSD  $\approx$  2.7%), reproducibility (RSD  $\approx$  3.6%), and short-term stability. The developed PGAMCNTPE was successfully applied to tablet formulations and human serum/urine samples using the standard addition method, yielding recovery values in the range of 95–102%. These results demonstrate that the proposed sensor platform offers an effective, rapid, sensitive, and low-cost electroanalytical approach for the determination of cetirizine in pharmaceutical and biological matrices. (Pushpanjali et al., 2023)

For the sensitive and selective determination of cetirizine in biological and pharmaceutical samples, a carbon paste electrode (CPE) modified with a  $\text{Li}_2\text{CoMn}_3\text{O}_8$ /carbon-doped graphitic carbon nitride ( $\text{CC}_3\text{N}_4$ ) nanocomposite and the ionic liquid ( $\text{CC}_3\text{N}_4/\text{LCMO}/\text{IL}/\text{CPE}$ ) was developed. The electrocatalytic properties of the modified electrode were evaluated using CV, DPV, and EIS. The results demonstrated a significant decrease in charge-transfer resistance, attributed to the high surface area of the nanocomposite and the enhanced conductivity provided by the ionic liquid. Using DPV, two wide linear concentration ranges for cetirizine were obtained (0.03–0.9  $\mu\text{M}$  and 3–300  $\mu\text{M}$ ), with a LOD of 11.8 nM. The accuracy and precision of the method were validated through standard addition experiments conducted in human plasma, urine, and tablet samples, yielding recovery values between 98.4% and 102.4% with low RSD values, confirming the reliability of the proposed approach. Furthermore, the developed sensor enabled the simultaneous determination of cetirizine, acetaminophen, and phenylephrine, demonstrating high selectivity and applicability in complex matrices. (Ziaie & Shabani-Nooshabadi, 2023)

A novel electrochemical sensor based on a GCE modified with hierarchical carbon nanofibers/carbon nanotubes decorated with NiCo alloy nanoparticles (eCNF/CNT/NiCo-GCE) was developed for the highly sensitive determination of the antihistaminic drug cetirizine hydrochloride. The hierarchical nanocomposite architecture provided a large active surface area, high electrical conductivity, and strong electrocatalytic activity, resulting in an approximately eightfold enhancement of the cetirizine signal compared with the bare GCE. Using DPV, multiple linear concentration ranges were obtained for cetirizine (0.05–6, 7–32, and 42–112  $\mu\text{M}$ ), while the LOD and LOQ were calculated as 14 nM and 42 nM, respectively. The repeatability and fabrication reproducibility of the sensor were confirmed with %RSD values of 3.4% ( $n = 9$ ) and 9.7% ( $n = 5$ ), respectively. The accuracy of the established method was assessed through standard addition experiments performed on pharmaceutical tablet formulations as well as biological samples (urine and plasma), yielding recovery values in the range of 97–115%, thereby demonstrating the method's reliability and applicability in complex matrices. These findings indicate that the eCNF/CNT/NiCo-GCE-based sensor offers an effective and practical alternative for the routine pharmaceutical and biological analysis of cetirizine (Górska et al., 2022).

Sawkar et al. developed a CPE modified with a zinc oxide nanoparticle–graphene nanocomposite (ZnO–Gr/CPE) for the sensitive and reliable voltammetric determination of the antihistaminic drug cetirizine. Scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and X-ray diffraction (XRD) analyses confirmed the homogeneous distribution of ZnO nanoparticles on the graphene surface and demonstrated that the nanocomposite provides a high effective surface area. Electrochemical studies revealed that the oxidation of cetirizine is a pH-dependent, diffusion-controlled, irreversible process involving the transfer of two protons and two electrons. Using DPV, a linear concentration range of 0.05–4  $\mu\text{M}$  was obtained, with LOD and LOQ calculated as 28 nM and 91 nM, respectively. The accuracy and selectivity of the developed sensor were validated through interference studies, which demonstrated that common pharmaceutical excipients exert negligible effects on the analytical signal. The applicability of the method was further confirmed by standard addition experiments performed on tablet formulations and urine samples, yielding recovery values in the range of 92.7–99.8% with low %RSD values. These results indicate that the ZnO–Gr/CPE-based sensor offers a stable, reproducible, and effective analytical platform for the determination of cetirizine in real samples (Sawkar et al., 2022).

A voltammetric method based on a cetyltrimethylammonium bromide (CTAB)-immobilized multiwalled carbon nanotube paste electrode (CTAB/MWCNTPE) was developed for the sensitive and selective determination of the antihistaminic drug cetirizine dihydrochloride. Adsorption of the cationic surfactant onto the nanotube surface significantly increased the electroactive surface area, reduced the charge-transfer resistance, and markedly enhanced the electrocatalytic oxidation of cetirizine. CV measurements performed in PBS (pH 7.0) yielded a linear concentration range of 10–100  $\mu\text{M}$  for cetirizine, while the LOD and LOQ were calculated as 0.27  $\mu\text{M}$  and 0.92  $\mu\text{M}$ , respectively. Kinetic studies demonstrated that the electrochemical processes are adsorption-controlled and irreversible, and the repeatability, reproducibility, and stability of the developed electrode were confirmed by low %RSD values. The analytical applicability of the proposed method was evaluated through standard addition experiments conducted on pharmaceutical tablet samples, yielding recovery values between 99.3% and 99.9%. These results indicate that the CTAB/MWCNTPE-based sensor provides a reliable and effective platform for the routine pharmaceutical analysis of cetirizine (Pushpanjali, Manjunatha, Sreeharsha et al., 2021).

In another study, a carbon nanotube paste electrode (CNTPE) modified with an electropolymerized poly(L-leucine) layer (poly(L-Leu)/CNTPE) was developed for the sensitive and selective determination of the antihistaminic drug cetirizine in the presence of the coexisting analyte paracetamol. The formation of a thin and conductive polymer film via the electrochemical polymerization of L-leucine on the carbon nanotube surface significantly increased the electroactive surface area of the electrode, reduced the charge-transfer resistance, and resulted in an approximately sevenfold enhancement of the cetirizine oxidation signal. Under optimized conditions, CV yielded a linear concentration range of 5–50  $\mu\text{M}$  for cetirizine, with a LOD of 0.17  $\mu\text{M}$ . Kinetic studies indicated that the electrochemical process is diffusion-controlled and irreversible, while pH-dependent investigations revealed that the numbers of electrons and protons involved in the reaction are approximately equal. The developed electrode enabled the simultaneous determination of cetirizine and paracetamol, exhibiting good selectivity. The accuracy and applicability of the method were validated through standard addition experiments performed on pharmaceutical tablet formulations and biological samples (human serum and urine), yielding recovery values in the range of 96.49–99.85%. These findings demonstrate that the poly(L-Leu)/CNTPE-based sensor provides an effective, stable, and reliable analytical platform for the routine determination of cetirizine in pharmaceutical and biological matrices (Pushpanjali, Manjunatha, Hareesha et al., 2021).

For the rapid, low-cost, and practical determination of cetirizine hydrochloride, a single-use electrochemical sensor based on SWV was developed using a pretreated graphite pencil electrode (p-GP). Comparative studies with various carbon-based electrodes demonstrated that the p-GP exhibited the lowest charge-transfer resistance and the highest oxidation current, as confirmed by EIS and CV. Cetirizine was found to undergo an irreversible oxidation process at approximately +1.0 V in Britton–Robinson buffer (BRB) at pH 2.0. Under optimized SWV conditions, a linear concentration range of 0.5–10.  $\mu\text{M}$  was achieved, with a LOD of 0.16  $\mu\text{M}$ . The analytical performance of the proposed method was evaluated in terms of linearity, sensitivity, and repeatability, and the sensor demonstrated high selectivity in the presence of common interfering species such as ascorbic acid, dopamine, and uric acid. The applicability of the method was assessed using antihistaminic syrup samples via the standard addition method, and the obtained results showed good agreement with the labeled values. These findings indicate that the p-GP-based sensor offers a simple, economical, and reliable alternative for the pharmaceutical analysis of cetirizine (Karakaya & Dilgin, 2019).

An adsorptive stripping differential pulse voltammetry (AdSDPV) method based on a CPE modified with a multiwalled carbon nanotube–platinum nanoparticle (MWCNT–PtNP) nanocomposite (MWCNT–PtNPs–CPE) was developed for the simultaneous and highly sensitive determination of paracetamol, cetirizine, and phenylephrine in pharmaceutical and biological samples. The nanocomposite structure was confirmed by transmission electron microscopy (TEM), SEM, XRD, and energy-dispersive X-ray (EDX) analyses, and the synergistic interaction between MWCNTs and PtNPs resulted in a marked enhancement of the electroactive surface area and electrical conductivity, accompanied by a significant reduction in charge-transfer resistance, as evidenced by electrochemical EIS results. Under optimized conditions, a linear concentration range of 0.19–193  $\mu\text{M}$  was obtained for cetirizine, with a LOD of 58.6 nM; similarly, wide linear ranges and low LOD values were achieved for paracetamol and phenylephrine. The accuracy, precision, and selectivity of the method were demonstrated through validation studies performed on single-, binary-, and ternary-component mixtures, confirming good peak separation during simultaneous analysis. The developed sensor was successfully applied to commercial pharmaceutical formulations as well as urine and human serum samples using the standard addition method, yielding recovery values in the range of 97–101% with low %RSD values. These results indicate that the MWCNT–PtNPs–CPE-based AdSDPV method provides a powerful and reliable electrochemical platform for the

routine and sensitive analysis of multicomponent pharmaceutical combinations in complex matrices (Kalambate & Srivastava, 2016).

A GCE modified with a MWCNT film (MWCNT/GCE) was developed for the sensitive and selective voltammetric determination of the antihistaminic drug cetirizine dihydrochloride. The MWCNT modification increased the electroactive surface area of the electrode by approximately threefold and provided a pronounced electrocatalytic effect toward cetirizine oxidation, resulting in a significant enhancement of the oxidation peak current compared with the bare GCE. CV studies conducted in PBS (pH 3.0) revealed that cetirizine undergoes an irreversible, adsorption-controlled oxidation process. Under optimized conditions, a linear concentration range of 0.5–10  $\mu\text{M}$  was obtained for cetirizine, and the LOD was calculated as 70.7 nM. The accuracy and precision of the proposed method were validated through standard addition and recovery studies performed on pharmaceutical tablet formulations and urine samples, yielding recovery values between 98.9% and 101.5% with low %RSD values. In addition, interference studies demonstrated that the effects of common excipients and biological matrix components were negligible. These findings indicate that the MWCNT/GCE-based sensor offers a sensitive, rapid, and practical analytical approach for the reliable determination of cetirizine in pharmaceutical quality control and biological samples. (Patil et al., 2011).

### **3.2.2. Voltammetric Techniques for Determination of Loratadine**

Loratadine is a second-generation  $\text{H}_1$  receptor antagonist with low sedative potential and high selectivity for peripheral  $\text{H}_1$  receptors. Its limited penetration of the blood–brain barrier and P-glycoprotein–mediated efflux result in minimal central nervous system effects. After oral administration, loratadine is rapidly absorbed and metabolized by cytochrome P450 enzymes to its active metabolite desloratadine, contributing to a long duration of action that enables effective once-daily dosing. Loratadine is widely used in the management of allergic rhinitis, chronic urticaria, and allergic conjunctivitis, providing effective relief from histamine-mediated symptoms while largely avoiding the sedative and anticholinergic adverse effects associated with first-generation antihistamines. Owing to its favorable safety profile and clinical efficacy, loratadine is regarded as a first-line antihistamine and represents an important active pharmaceutical ingredient requiring accurate analytical determination (Roushani et al., 2017).

For the simultaneous electrochemical determination of paracetamol, phenylephrine hydrochloride, and loratadine, which are commonly co-formulated in cold relief medications, an SWV-based method was developed using a low hydrogen-terminated boron-doped diamond

electrode (BDDE). Owing to the wide potential window of the BDDE, the three analytes exhibited well-resolved, diffusion-controlled, and irreversible oxidation peaks in an acidic medium (0.1 M H<sub>2</sub>SO<sub>4</sub>). The method employed both conventional linear calibration and multiple linear regression (MLR)-based calibration strategies to overcome interference arising from large concentration ratios among the analytes. The MLR approach significantly improved the accuracy, particularly for phenylephrine hydrochloride and loratadine at low concentration levels, and the results were shown to be statistically consistent with those obtained by an HPLC–UV method at the 95% confidence level. The developed SWV method demonstrated high precision (%RSD < 2.3%), good selectivity, and recovery values ranging from 92.4% to 98.8% in urine samples. With its rapid, low-cost, and solvent-free operation, the proposed method offers an environmentally friendly and effective alternative for the routine analysis of multicomponent pharmaceutical formulations (Salamanca-Neto et al., 2021).

For the sensitive and reliable determination of loratadine at low concentration levels, a SWV-based method was developed using a NiO nanoparticle-modified carbon paste electrode (NiO–CPE). The incorporation of amorphous NiO nanoparticles synthesized via a sol–gel method significantly enhanced the oxidation current of loratadine due to the electrocatalytic activity of the Ni(II)/Ni(III) redox couple, thereby improving analytical performance. Experimental conditions were systematically optimized using response surface methodology, and under optimal parameters, a linear concentration range of 30–960 nM was obtained for loratadine. The LOD and LOQ were calculated as  $1.40 \pm 0.27$  nM and  $4.60 \pm 0.40$  nM, respectively. The accuracy, precision, and selectivity of the method were validated through statistical analysis, interference studies, and long-term stability tests, demonstrating that the electrode remained stable for at least five months. The developed NiO–CPE-based sensor was successfully applied to pharmaceutical tablet formulations and human serum samples, yielding recovery values between 97% and 102%, thereby confirming its suitability as a sensitive, reliable, and practical analytical platform for loratadine determination in complex biological matrices (Raeisi-Kheirabadi et al., 2021).

For the sensitive and selective determination of the second-generation antihistamine loratadine in pharmaceutical formulations and biological samples, a linear sweep cathodic stripping voltammetry (LS-CSV) method based on a bismuth film electrode (BiFE) was developed. The BiFE was selected due to its non-toxic nature and environmentally friendly properties as an alternative to mercury electrodes. In particular, the presence of the cationic surfactant CTAB

significantly enhanced the electrochemical reduction signal of loratadine. Under optimized conditions, loratadine underwent an irreversible, diffusion-controlled reduction process governed by an adsorption mechanism at approximately  $-1.48$  V in BRB (pH 7.0, 4 mM CTAB). A linear concentration range of 1.30–14.3  $\mu\text{M}$  was obtained, with a LOD of 0.12  $\mu\text{M}$ . The accuracy and repeatability of the method were confirmed by low %RSD values, and the proposed voltammetric approach was successfully applied to tablet formulations and urine samples using the standard addition method, yielding recovery values between 95.2% and 107.5%. These results demonstrate that the BiFE/CTAB-based LS-CSV method provides a rapid, cost-effective, environmentally benign, and reliable analytical alternative for the routine pharmaceutical and biological analysis of loratadine (Önal et al., 2021).

For the selective and sensitive determination of the second-generation antihistamine loratadine, a novel electrochemical sensor based on a molecularly imprinted polymer (MIP) coated silicon carbide nanoparticle–modified glassy carbon electrode (MIP/SiCNP/GCE) was developed. The MIP was synthesized via bulk polymerization using methacrylic acid as the functional monomer and loratadine as the template molecule, creating specific binding cavities for the target analyte and thereby significantly enhancing sensor selectivity. Surface characteristics and modification steps were confirmed by SEM, FT-IR, Brunauer–Emmett–Teller (BET) analysis, and EIS, demonstrating improved electrochemical performance due to the wide potential window and low background current of SiC nanoparticles. Using DPV in BRB (pH 2.0), a linear concentration range of 1–33  $\mu\text{M}$  was obtained for loratadine, with a LOD of 0.15  $\mu\text{M}$  ( $S/N = 3$ ). The sensor exhibited good repeatability ( $\%RSD \leq 3.1\%$ ), satisfactory fabrication reproducibility, and long-term stability, along with high selectivity against structurally similar compounds, including cetirizine and hydroxyzine. The developed method was successfully applied to tablet formulations and human serum samples using the standard addition method, yielding recovery values between 94% and 101%. These findings demonstrate that the MIP/SiCNP/GCE-based sensor provides a reliable, cost-effective, and practical platform for the determination of loratadine in pharmaceutical and biological matrices. (Roushani et al., 2017)

For the simple, rapid, and environmentally friendly determination of loratadine in pharmaceutical formulations, a SWV-based method was developed using a cathodically pretreated BDDE. Owing to the wide potential window and low background current of the BDDE, loratadine was shown to undergo an irreversible, diffusion-controlled single-electron oxidation process at approximately  $+1.67$  V (Ag/AgCl), while no detectable signal was

observed at a GCE, highlighting the superiority of the BDDE. Under optimized conditions (0.5 M HClO<sub>4</sub>), a linear concentration range of 0.98–19 µM was obtained with a LOD of 0.78 µM. The repeatability of the method was confirmed by low %RSD values, and the approach enabled multiple measurements without requiring surface renewal or solution deoxygenation. The proposed SWV method was successfully applied to tablet and liquid pharmaceutical samples, yielding recovery values between 93.4% and 97.1%. In addition, the results were statistically consistent with those obtained by a comparative spectrophotometric method at the 95% confidence level, demonstrating that the BDDE-based approach provides a practical, reliable, and eco-friendly alternative for the routine quality control analysis of loratadine (Eisele & Sartori, 2015).

#### 4. Conclusions and Recommendations

In this section, the sensitive and selective determination of cetirizine and loratadine, which are second-generation, non-sedative H<sub>1</sub> receptor antagonists widely used in the treatment of allergic diseases such as urticaria, allergic rhinitis, and atopic dermatitis, has been comprehensively evaluated using voltammetric techniques. Based on the fundamental principles of electrochemical analysis, the effectiveness of voltammetric methods was investigated employing bare and variously modified electrodes, and the analytical performances of CV, DPV and SWV were systematically compared.

The literature data summarized in the comparative tables (Table 1 and Table 2) clearly demonstrate that electrochemical techniques offer significant advantages over conventional analytical methods (e.g., HPLC, LC–MS, and spectrophotometry), including low LOD, wide linear dynamic ranges, high recovery values, short analysis time, low cost, and environmentally friendly operation. Among the investigated methods, DPV emerged as the most frequently employed technique, followed by CV and SWV, primarily due to its superior sensitivity and suitability for trace-level analysis in both pharmaceutical formulations and biological matrices.

Electrode modification strategies were shown to play a crucial role in enhancing analytical performance. The voltammetric responses of cetirizine at CC<sub>3</sub>N<sub>4</sub>/LCMO/IL/CPE, eCNF/CNT/NiCo–GCE, and Poly-BCP/GCE, as well as loratadine at NiO–CPE, were markedly enhanced due to the high electrical conductivity and enlarged effective surface areas of these modified electrodes. Comparative evaluation of the tabulated results indicates that such modifications effectively lower detection limits and improve matrix tolerance. Moreover, the reported sensors were successfully applied not only to pharmaceutical dosage forms but also to

complex biological matrices such as human urine and plasma, confirming the robustness and applicability of electrochemical techniques for clinical monitoring, dosage control, and pharmaceutical quality assurance.

A comparative assessment of cetirizine and loratadine further revealed that, despite both being second-generation H<sub>1</sub> receptor antagonists, they exhibit distinct electroanalytical behaviors attributable to differences in their molecular structures. As evidenced by the tabulated data, cetirizine, due to the presence of ionizable functional groups, generally undergoes oxidation at lower potentials and follows adsorption-controlled electrochemical processes, enabling the achievement of lower LOD values, particularly when nanomaterial- and polymer-modified electrodes are employed. In contrast, loratadine, owing to its more hydrophobic nature and extended aromatic system, typically exhibits oxidation at more positive potentials and is more effectively determined using BDDEs with wide potential windows or MIP-based sensors that provide enhanced selectivity. These findings underscore the importance of drug-specific electrode design in achieving optimal analytical performance.

Based on the overall findings, the integration of electrochemical analytical techniques into clinical practice and pharmaceutical quality control processes is strongly recommended. In particular, the development of user-friendly, portable, and disposable electrochemical sensor platforms could facilitate rapid monitoring of drug levels in clinical or home settings, thereby supporting personalized treatment strategies. Furthermore, considering the environmental and biological hazards associated with mercury-based electrodes, a shift toward carbon-based and nanomaterial-supported electrodes represents a more sustainable and safer analytical approach. In conclusion, continued support for innovative electrode modification strategies and voltammetric methodologies will contribute significantly to the development of faster, more reliable, and more sensitive analytical techniques for the determination of antihistaminic drugs and other pharmaceutical compounds.

**Table 1.** Voltammetric methods for determination of cetirizine.

Electrode	Technique	Supporting Electrolyte	Linear range ( $\mu\text{M}$ )	LOD (nM)	Reference
Poly-BCP/GCE	DPV SWV	PBS, pH 6.0	0.2–100 0.1–100	21.8 15.1	Sofu et al., 2025
PGAMCNTPE	CV	PBS, pH 7.0	2–50	120	Pushpanjali et al., 2023
CC <sub>3</sub> N <sub>4</sub> /LCMO/IL/CPE	DPV	PBS, pH 7.0	0.03–0.9; 3–300	11.8	Ziaie & Shabani-Nooshabadi, 2023
eCNF/CNT/NiCo–GCE	DPV	PBS, pH 7.0	0.05–6; 7–32; 42–112	14	Górska et al., 2022
ZnO–Gr/CPE	DPV	PBS, pH 6.0	0.05–4	28	Sawkar et al., 2022
CTAB/MWCNTPE	CV	PBS, pH 7.0	10–100	270	Pushpanjali, Manjunatha, Sreeharsha et al., 2021
poly(L-Leu)/CNTPE	CV	PBS, pH 7.0	5–50	170	Pushpanjali, Manjunatha, Hareesha et al., 2021
p-GP	SWV	BRB, pH 2.0	0.5–10	160	Karakaya & Dilgin, 2019
MWCNT–PtNPs/CPE	AdSDPV	BRB, pH 5.5	0.19–193	58.6	Kalambate & Srivastava, 2016
MWCNT/GCE	CV	PBS, pH 3.0	0.5–10	70.7	Patil et al., 2011

Poly-BCP/GCE: poly(bromocresol purple

PGAMCNTPE: poly(glutamine)-modified multiwalled carbon nanotube paste electrode

CC<sub>3</sub>N<sub>4</sub>/LCMO/IL/CPE: Li<sub>2</sub>CoMn<sub>3</sub>O<sub>8</sub>/carbon-doped graphitic carbon nitride (CC<sub>3</sub>N<sub>4</sub>) nanocomposite and the ionic liquid carbon paste electrode

eCNF/CNT/NiCo–GCE: Hierarchical carbon nanofibers/carbon nanotubes decorated with NiCo alloy nanoparticles modified glassy carbon electrode

ZnO–Gr/CPE: Zinc oxide nanoparticle–graphene nanocomposite modified carbon paste electrode

CTAB/MWCNTPE: Cetyltrimethylammonium bromide (CTAB)-immobilized multiwalled carbon nanotube paste electrode

Poly(L-Leu)/CNTPE: Electropolymerized poly(L-leucine) modified carbon nanotube paste electrode

p-GP: Pretreated graphite pencil electrode

MWCNT–PtNPs/CPE: Multiwalled carbon nanotube–platinum nanoparticle modified carbon paste electrode

MWCNT/GCE: Multiwalled carbon nanotube modified glassy carbon electrode

**Table 2.** Voltammetric methods for determination of loratadine.

Electrode	Technique	Supporting Electrolyte	Linear range ( $\mu\text{M}$ )	LOD (nM)	Reference
Low hydrojen-terminated BDDE	SWV	0.1 M H <sub>2</sub> SO <sub>4</sub>	0.6–8.68	-	Salamanca-Neto et al., 2021
NiO–CPE	SWV	0.1 M NaOH	0.03–0.96	1.4	Racisi-Kheirabadi et al., 2021
BiFE	LS-CSV	BRB, pH 7.0 (4 mM CTAB)	1.305–14.3	120	Önal et al., 2021
MIP/SiCNP/GCE	DPV	BRB, pH 2.0	1–33	150	Roushani et al., 2017
Cathodically pretreated BDDE	SWV	0.5 M HClO <sub>4</sub>	0.98–19	780	Eisele & Sartori, 2015

BDDE: boron-doped diamond electrode

NiO–CPE: NiO nanoparticle-modified carbon paste electrode

BiFE: bismuth film electrode

MIP/SiCNP/GCE: molecularly imprinted polymer coated silicon carbide nanoparticle–modified glassy carbon electrode

## REFERENCES

- Bard, A. J., Faulkner, L. R., & White, H. S. (2022). *Electrochemical methods: fundamentals and applications*. John Wiley & Sons.
- Bousquet, J., Anto, J. M., Bachert, C., Baiardini, I., Bosnic-Anticevich, S., Walter Canonica, G., Melén, E., Palomares, O., Scadding, G. K., Togias, A., & Toppila-Salmi, S. (2020). Allergic rhinitis. *Nature reviews Disease primers*, 6, 95.
- Eisele, A. P. P., & Sartori, E. R. (2015). Simple and rapid determination of loratadine in pharmaceuticals using square-wave voltammetry and a cathodically pretreated boron-doped diamond electrode. *Analytical Methods*, 7, 8697-8703.
- Górska, A., Zambrzycki, M., Paczosa-Bator, B., & Piech, R. (2022). New Electrochemical Sensor Based on Hierarchical Carbon Nanofibers with NiCo Nanoparticles and Its Application for Cetirizine Hydrochloride Determination. *Materials*, 15, 3648.
- Joseph, W. (2000). Analytical electrochemistry.
- Kalambate, P. K., & Srivastava, A. K. (2016). Simultaneous voltammetric determination of paracetamol, cetirizine and phenylephrine using a multiwalled carbon nanotube-platinum nanoparticles nanocomposite modified carbon paste electrode. *Sensors and Actuators B: Chemical*, 233, 237-248.
- Karakaya, S., & Dilgin, D. G. (2019). Low-cost determination of cetirizine by square wave voltammetry in a disposable electrode: S. Karakaya, DG Dilgin. *Monatshefte für Chemie-Chemical Monthly*, 150, 1003-1010.
- Kleine-Tebbe, J. (2011). Immunological measures as potential markers of dose. *Allergy*, 66, 44-46.
- Lieberman, P. (2011). The basics of histamine biology. *Annals of Allergy, Asthma & Immunology*, 106, S2-S5.
- Maintz, L., & Novak, N. (2007). Histamine and histamine intolerance. *The American journal of clinical nutrition*, 85, 1185-1196.

- Önal, G., Altunkaynak, Y., & Levent, A. (2021). Application of BiFE for electrochemical properties and determination of loratadine by cathodic stripping voltammetry in the cationic surfactant medium. *Journal of the Iranian Chemical Society*, *18*, 3465-3475.
- Panula, P., & Nuutinen, S. (2013). The histaminergic network in the brain: basic organization and role in disease. *Nature Reviews Neuroscience*, *14*, 472-487.
- Panula, P., Chazot, P. L., Cowart, M., Gutzmer, R., Leurs, R., Liu, W. L., Stark, H., Thurmond, R. L., & Haas, H. L. (2015). International union of basic and clinical pharmacology. XCVIII. Histamine receptors. *Pharmacological reviews*, *67*, 601-655.
- Parsons, M. E., & Ganellin, C. R. (2006). Histamine and its receptors. *British journal of pharmacology*, *147*, S127-S135.
- Patil, R. H., Hegde, R. N., & Nandibewoor, S. T. (2011). Electro-oxidation and determination of antihistamine drug, cetirizine dihydrochloride at glassy carbon electrode modified with multi-walled carbon nanotubes. *Colloids and Surfaces B: Biointerfaces*, *83*, 133-138.
- Pushpanjali, P. A., Manjunatha, J. G., Hareesha, N., D'Souza, E. S., Charithra, M. M., & Prinith, N. S. (2021). Voltammetric analysis of antihistamine drug cetirizine and paracetamol at poly (L-Leucine) layered carbon nanotube paste electrode. *Surfaces and Interfaces*, *24*, 101154.
- Pushpanjali, P. A., Manjunatha, J. G., Sreeharsha, N., Asdaq, S. M. B., & Anwer, M. K. (2021). A highly responsive voltammetric methodology for the sensing of antihistamine drug cetirizine on the surface of cetrimonium bromide immobilized multi-walled carbon nanotube electrode. *Journal of Materials Science: Materials in Electronics*, *32*, 22668-22679.
- Pushpanjali, P. A., Manjunatha, J. G., Hareesha, N., Tighezza, A. M., Albaqami, M. D., & Sillanpää, M. (2023). Electrochemical analysis of the antihistamine drug cetirizine at a poly (glutamine) modified carbon nanotube paste electrode. *ChemistrySelect*, *8*, e202300818.
- Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. J., & Henderson, G. (2011). *Rang & Dale's pharmacology*. Elsevier Health Sciences.
- Raeisi-Kheirabadi, N., Nezamzadeh-Ejhih, A., & Aghaei, H. (2021). Application of NiOCPE in the quantitative determination of loratadine: Experimental design in square wave voltammetry approach. *Surfaces and Interfaces*, *27*, 101484.

- Roushani, M., Nezhadali, A., Jalilian, Z., & Azadbakht, A. (2017). Development of novel electrochemical sensor on the base of molecular imprinted polymer decorated on SiC nanoparticles modified glassy carbon electrode for selective determination of loratadine. *Materials Science and Engineering: C*, *71*, 1106-1114.
- Salamanca-Neto, C. A. R., Marcheafave, G. G., Mattos, G. J., Moraes, J. T., Schwarzova-Peckova, K., & Sartori, E. R. (2021). Boron-doped diamond film and multiple linear regression-based calibration applied to the simultaneous electrochemical determination of paracetamol, phenylephrine hydrochloride, and loratadine in fixed-dose combinations. *Microchemical Journal*, *162*, 105831.
- Sawkar, R. R., Shanbhag, M. M., Tuwar, S. M., Mondal, K., & Shetti, N. P. (2022). Zinc Oxide–Graphene nanocomposite-based sensor for the electrochemical determination of cetirizine. *Catalysts*, *12*, 1166.
- Simons, F. E. R. (2004). Advances in H1-antihistamines. *New England Journal of Medicine*, *351*, 2203-2217.
- Simons, F. E. R., & Simons, K. J. (2011). Histamine and H1-antihistamines: celebrating a century of progress. *Journal of Allergy and Clinical Immunology*, *128*, 1139-1150.
- Sofu, Ü., Öztürk, G., Reis, H. A., Erdemir, F., & Kul, D. (2025). Development of Highly Sensitive Voltammetric Methods for the Determination of Antihistamine Drug Cetirizine Using A Poly (Bromocresol Purple) Film-Based Electrochemical Sensor. *Analytical Letters*, *58*, 1652-1667.
- Thurmond, R. L. (2015). The histamine H4 receptor: from orphan to the clinic. *Frontiers in Pharmacology*, *6*, 65.
- Ziaie, N., & Shabani-Nooshabadi, M. (2023). Application of the C–C<sub>3</sub>N<sub>4</sub>/Li<sub>2</sub>CoMn<sub>3</sub>O<sub>8</sub>//IL nanocomposite for design a sensitive electrochemical sensor in order to detection of cetirizine, acetaminophen and phenylephrine in biological and pharmaceuticals samples. *Environmental Research*, *216*, 114667.



# CHAPTER 2

---

## TRANSCENDING THE BOUNDARIES OF BIOENERGETICS: MOLECULAR FOUNDATIONS OF PURINERGIC SIGNALING

*Leyla ATAÇ DOĞAN<sup>1</sup>*

---

<sup>1</sup> Dr., PhD, Lecturer, Hatay Mustafa Kemal University, Medical School, Medical Biology, Hatay, Türkiye (ORCID: 0009-0008-8923-2794)

## 1. Introduction: The Paradigm Shift in the Biochemical Identity of ATP

Within the field of biochemistry, Adenosine 5'-triphosphate (ATP) was long characterized solely as the thermodynamic fuel essential for cellular life. As the primary output of mitochondrial oxidative phosphorylation and cytosolic glycolysis, this molecule earned the designation of "universal energy currency" by driving endergonic reactions through the hydrolysis of its high-energy phosphoanhydride bonds (free energy change  $\approx -7.3$  kcal/mol). However, empirical evidence gathered over the past five decades demonstrates that ATP operates far beyond intracellular limits, serving as a central element in a sophisticated intercellular communication network. ATP and its catabolic products (Adenosine diphosphate; ADP, Adenosine monophosphate AMP, and adenosine) constitute the core components of an evolutionarily conserved and complex signaling system known as "purinergic signaling (Dunn & Grider, 2025)."

This conceptual shift originated with the "purinergic neurotransmission" hypothesis proposed by Geoffrey Burnstock in 1972 (Burnstock, 1972). Burnstock identified physiological responses in gastrointestinal and vascular tissues that persisted despite traditional cholinergic and adrenergic blockades, labeling this as "non-adrenergic, non-cholinergic" (NANC) transmission and attributing the process to ATP. Initially regarded with skepticism, this hypothesis has evolved into a fundamental biological dogma recognized across diverse fields, including neurobiology, immunology, embryology, and oncology (Burnstock, 1972).

### 1.1. Extracellular Nucleotide Dynamics and Purinergic Signal Transduction

Cellular homeostasis depends on a profound ATP concentration gradient between the internal and external environments of the plasma membrane. While ATP concentrations in the cytosol reach millimolar levels (3–10 mM), they are strictly maintained at nanomolar levels (1–10 nM) in the extracellular space. This massive million-fold gradient allows the release of ATP into the extracellular environment to be instantly recognized by adjacent cells as a signal of "emergency," "damage," or "activation" (Damage-Associated Molecular Pattern - DAMP) (Feng et al., 2020).

The translocation of ATP to the extracellular space is governed by highly specific mechanisms rather than simple passive leakage. Key pathways include the calcium-dependent exocytosis of synaptic vesicles, transport via connexin (notably Connexin 43) and pannexin (Pannexin-1) hemichannels, and the role of vesicular nucleotide transporters (VNUT). Conversely, pathological states such as mechanical stress, hypoxia, ischemia, or direct membrane rupture (necrosis) trigger an uncontrolled and substantial release of ATP, thereby initiating local inflammatory responses (Xiong et al., 2018).

Extracellular nucleotides and nucleosides exert their physiological effects through specialized receptors on the cell membrane. These are categorized into two primary superfamilies based on their structural characteristics and ligand preferences: P1 (Adenosine) and P2 (Nucleotide) receptors (**Table 1**) (Huang et al., 2021; Jacobson et al., 2012).

### 1.2. P1 Receptors: Adenosine-Mediated Immunomodulation and Signaling

P1 receptors (A1, A2A, A2B, A3), which belong to the G-protein coupled receptor (GPCR) family, are sensitive to adenosine—the final byproduct of ATP degradation by ectonucleotidases—rather than ATP itself. Adenosine signaling is typically activated during periods

of metabolic stress or energy depletion, facilitating cytoprotective and anti-inflammatory responses to maintain tissue integrity (Jacobson et al., 2012).

Intracellular signaling pathways vary depending on the specific G-protein coupled with the receptor:

**A1 and A3 Receptors (Gi/o-coupled):** Activation of these receptors inhibits adenylate cyclase, leading to a decrease in intracellular cyclic AMP (cAMP) levels. Concurrently, G-protein beta-gamma subunits trigger the opening of potassium (K<sup>+</sup>) channels, increasing K<sup>+</sup> efflux and causing membrane hyperpolarization. This mechanism results in the suppression of neuronal excitability and a reduction in heart rate (negative chronotropic effect) (Jacobson et al., 2012).

**A2A and A2B Receptors (Gs-coupled):** In contrast, this group stimulates adenylate cyclase, raising intracellular cAMP concentrations. The A2A receptor, in particular, acts as a vital "brake" for immune cells. During inflammation, elevated adenosine levels limit T-cell activation via A2A receptors and suppress the production of inflammatory cytokines, thereby promoting a resolution process that minimizes tissue damage (Jacobson et al., 2012).

### 1.3. P2 Receptors: Ionotropic and Metabotropic Signaling

The P2X and P2Y Families P2 receptors, which respond to nucleotides such as ATP, ADP, UTP, and UDP, are categorized into two distinct classes based on their signal transduction mechanisms: ionotropic (P2X) and metabotropic (P2Y) receptors (Jacobson et al., 2012).

**P2X Receptors** Functioning as ligand-gated cation channels, P2X receptors are specifically activated by extracellular ATP. In mammals, seven subunits (P2X1 through P2X7) have been identified, which assemble into either homomeric or heteromeric trimers. The binding of ATP induces a conformational change that facilitates the opening of the channel pore, allowing for the influx of sodium (Na<sup>+</sup>), K<sup>+</sup>, and notably, calcium (Ca<sup>2+</sup>) ions. This rapid ionic flux regulates physiological processes occurring within milliseconds, ranging from synaptic transmission to muscular contraction (Jacobson et al., 2012; Xiong et al., 2018).

Structural analysis reveals that P2X receptors possess a trimeric architecture, where each subunit consists of two transmembrane domains (TM1 and TM2) and an extensive extracellular ectodomain. This configuration is often described in scientific literature through a "dolphin-like" analogy, where the transmembrane regions represent the tail and the extracellular loop forms the body. The ATP-binding pocket is situated at the interface of two adjacent subunits rather than on a single subunit. Upon ligand binding, the channel opens via an "iris-like" expansion mechanism (Oken et al., 2022).

The P2X7 receptor stands out as a unique member of this family due to its low affinity, requiring high concentrations of ATP—typically associated with inflammation or cell death—for activation. Prolonged stimulation of P2X7 leads to the formation of large pores in the plasma membrane that are permeable to macromolecules. This process triggers the intracellular NLR family pyrin domain containing 3 (NLRP3) inflammasome complex, resulting in the maturation and release of potent pro-inflammatory cytokines such as Interleukin-1 $\beta$ , thereby positioning P2X7 as a central mediator in chronic inflammation (Kong et al., 2022).

**P2Y Receptors** In contrast to ionotropic channels, the P2Y family consists of GPCRs and includes eight functional subtypes: P2Y1, 2, 4, 6, 11, 12, 13, and 14. These receptors exhibit a

"serpentine" (seven-transmembrane) topology and transmit slower but more sustained signals via G-protein activation upon ligand binding (Jacobson et al., 2012).

**Gq-Coupled (P2Y1, 2, 4, 6):** These receptors activate phospholipase C (PLC), which generates inositol trisphosphate (IP3) and diacylglycerol (DAG), subsequently triggering the mobilization of intracellular Ca<sup>2+</sup> (Jacobson et al., 2012).

**Gi-Coupled (P2Y12, 13, 14):** These subtypes inhibit adenylate cyclase, thereby reducing the production of cAMP (Gachet, 2012; Jacobson et al., 2012).

The P2Y12 receptor is particularly significant from a clinical perspective. It is highly expressed on platelets and is selective for ADP. The binding of ADP initiates platelet aggregation and thrombus formation through the Gi pathway. This mechanism is the primary target for antiplatelet medications such as clopidogrel and ticagrelor, which block the receptor to decrease the risk of myocardial infarction and stroke (Gachet, 2012; Jia et al., 2015).

**Table 1:** Purinergic Receptor Superfamilies and Their Functional Characteristics

Receptor	Signaling Pathway	Physiological / Clinical Significance
A1 & A3	Gi/o-coupled (cAMP reduction, K <sup>+</sup> efflux enhancement)	Hyperpolarization, neuronal inhibition, and reduction in heart rate (Thiel et al., 2003).
A2A & A2B	Gs-coupled (Elevation of intracellular cAMP)	Immune "brake" mechanism and suppression of inflammatory responses (Thiel et al., 2003).
P2X7	Macropore formation	NLRP3 inflammasome activation and pro-inflammatory (IL-1 $\beta$ ) mediation (Kong et al., 2022).
P2Y1, 2, 4, 6	Gq-coupled (PLC $\rightarrow$ IP3/DAG $\rightarrow$ Ca <sup>2+</sup> mobilization)	Mobilization of intracellular Ca <sup>2+</sup> ions (Jacobson et al., 2012).
P2Y12	Gi-coupled (Inhibition of cAMP production)	Platelet aggregation (Primary target for Clopidogrel/Ticagrelor) (Cheng, 2012; Gachet, 2012; Jia et al., 2015).

#### 1.4.Purinergic Signal Termination: Ectonucleotidases and Enzymatic Control

Because the uncontrolled presence of extracellular ATP can lead to tissue damage, this signaling system is maintained under rigorous enzymatic oversight. Ectonucleotidase enzymes catalyze the removal of phosphate groups from ATP to convert it into adenosine; thus, the "danger" signal (ATP) is transformed into a "healing/suppressive" signal (adenosine) (Shen et al., 2025).

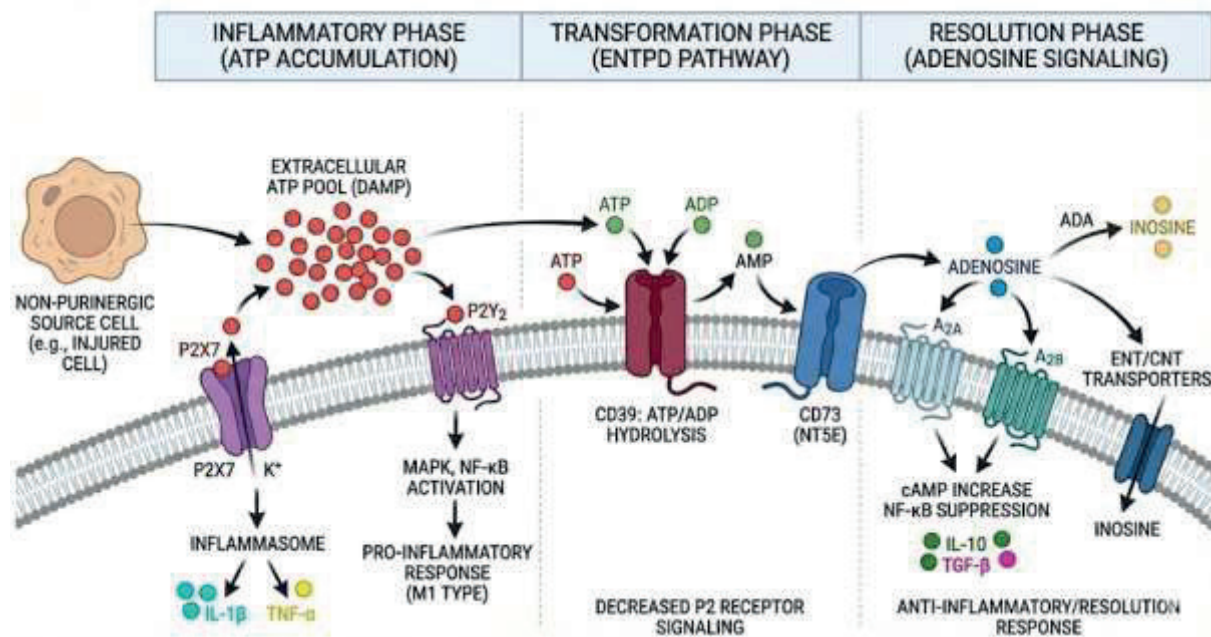
CD39 (ENTPD1) serves as the pivotal enzyme in this process, rapidly hydrolyzing ATP and ADP into AMP through its apyrase activity. Particularly prevalent on regulatory T cells (Tregs) and the vascular endothelium, CD39 functions to eliminate pro-inflammatory signals (Shen et al., 2025). The final step of this enzymatic cascade is executed by CD73 (Ecto-5'-nucleotidase), which converts AMP into adenosine. The resulting adenosine stimulates P1 receptors to terminate the inflammatory response (Shen et al., 2025). However, malignant cells can exploit this mechanism by generating a dense "adenosine halo" in their microenvironment, allowing them to evade cytotoxic T cells. The signaling process concludes when adenosine is either

recaptured into the cell or converted into inosine by adenosine deaminase (ADA) (Table 2) (Sharma et al., 2023).

**Table 2:** Mechanisms of Signal Termination and Enzymatic Conversion

Enzyme	Biochemical Function	Physiological Outcome
CD39 (ENTPD1)	Hydrolysis of ATP / ADP into AMP	Eradication of pro-inflammatory signaling (Shen et al., 2025).
CD73	Conversion of AMP into Adenosine	Induction of anti-inflammatory (P1) pathways (Shen et al., 2025).
ADA (Adenosine Deaminase)	Deamination of Adenosine to Inosine	Definitive cessation of the purinergic signal (Sharma et al., 2023).

In summary, the purinergic system is a multi-layered communication network that coordinates organismal homeostasis, danger perception, and immune responses. The delicate equilibrium between the pro-inflammatory "stimulatory" role of ATP and the anti-inflammatory "tranquilizing" effect of adenosine is vital for tissue health (Figure 1). Disruption of this balance underlies various pathologies, including thrombosis, autoimmune diseases, and cancer. Consequently, P2 receptor antagonists and CD39/CD73 modulators represent some of the most promising targets in contemporary pharmacology (Huang et al., 2021; Shen et al., 2025).



**Figure 1. The Role of Purinergic Signaling Pathways in the Regulation and Resolution of the Inflammatory Response:** The transition of the immune response from the pro-inflammatory phase to the anti-inflammatory resolution phase, driven by changes in extracellular purine concentrations following tissue injury or infection, is modulated through three primary stages: **A) Inflammatory Phase (ATP Accumulation):** As a consequence of cellular stress, injury, or necrosis, ATP is released into the extracellular space at high concentrations, where it functions as a DAMP. Extracellular ATP (eATP) initiates the pro-inflammatory cascade by activating ionotropic P2X7 and metabotropic P2Y2 receptors. P2X7 activation triggers the assembly of the NLRP3 inflammasome complex via intracellular K<sup>+</sup> efflux, leading to the maturation and secretion of the pro-inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$ . Simultaneously, P2Y2 signaling induces the Mitogen-Activated Protein Kinase (MAPK) and Nuclear Factor kappa-B (NF- $\kappa$ B) pathways, promoting M1-type macrophage

polarization and acute inflammation. **B) Transformation Phase (Ectonucleotidase Cascade):** The enzymatic hydrolysis of extracellular purines serves as a critical "metabolic switch" to bring the inflammatory process under control. Among the ectonucleotidases, CD39 (ENTPD1) hydrolyzes extracellular ATP and ADP into AMP, while CD73 (5'-nucleotidase) converts AMP into adenosine (ADO). This enzymatic conversion facilitates the termination of pro-inflammatory P2 receptor signaling and the initiation of anti-inflammatory P1 signaling. **C) Resolution Phase (Adenosinergic Signaling):** Accumulated extracellular adenosine binds to high-affinity G-protein coupled A2A and A2B receptors. This interaction increases intracellular cAMP levels, thereby suppressing pro-inflammatory NF- $\kappa$ B activity. As a result of this molecular inhibition, the expression of anti-inflammatory cytokines (IL-10 and TGF- $\beta$ ) increases, supporting tissue repair and immune homeostasis. To restore systemic homeostasis, adenosine is either converted to inosine by the ADA (Adenosine Deaminase) enzyme or removed from the extracellular space through cellular re-uptake via ENT/CNT transporters (Huang et al., 2021).

## 2. Genetic Foundations and Polymorphisms of Purinergic Signaling

The central role of the purinergic system in physiological processes dictates that genetic variations within this system directly influence disease susceptibility and pharmacological response (Huang et al., 2021). Single nucleotide polymorphisms (SNPs) can disrupt purinergic equilibrium by altering receptor affinity, enzymatic activity, and protein expression levels (Guerini et al., 2022). The human P2RX7 gene, in particular, exhibits high polymorphic diversity. Mutations occurring at the C-terminal end or the extracellular loop of the protein can dramatically shift the receptor's sensitivity to ATP or its channel-opening kinetics (Gartland et al., 2012).

Specifically, certain loss-of-function mutations in the P2X7 receptor may exert a protective effect against autoimmune conditions, such as rheumatoid arthritis, by decreasing the release of pro-inflammatory cytokines like IL-1 $\beta$  and IL-18. Conversely, gain-of-function polymorphisms are associated with heightened sensitivity to chronic pain and various neuropsychiatric disorders, including depression (Guerini et al., 2022).

In cardiovascular pharmacology, variations in the P2RY12 gene are among the most significant genetic factors. Specific haplotypes in the P2Y12 receptor can trigger an exaggerated platelet response to ADP, thereby increasing the risk of atherothrombotic events. Beyond genetic differences in the metabolism of prodrugs like clopidogrel, structural changes in the P2Y12 receptor itself contribute to "antiplatelet resistance," impacting the clinical risk of myocardial infarction and stroke (Jia et al., 2015; Nie et al., 2017).

This genetic diversity extends to the enzymatic level. Polymorphisms in the ENTPD1 (CD39) and NT5E (CD73) genes are critical determinants of ATP and adenosine concentrations within the tissue microenvironment (Adhikary et al., 2020; St Hilaire et al., 2011). Genetic variants that enhance CD39 expression can accelerate cancer progression by rapidly depleting the "danger signal" ATP and expanding the immunosuppressive adenosine halo, which facilitates evasion from cytotoxic T cells (Adhikary et al., 2020; Maloney et al., 2017). In contrast, lower genetic levels or downregulation of these enzymes in Inflammatory Bowel Diseases (IBD) allow the pro-inflammatory effects of ATP to remain dominant, delaying mucosal healing and exacerbating chronic inflammation (Shen et al., 2025).

## 3. Purinergic Dysregulation in the Immunopathogenesis of IBD Diseases

IBD, encompassing chronic pathologies such as Crohn's disease and ulcerative colitis, are systemic disorders characterized by the loss of intestinal epithelial barrier integrity (Diezmos et al., 2016). These conditions arise from complex interactions between genetic predisposition, microbiota dysbiosis, and impaired mucosal immunity. Recent mechanistic studies highlight the critical role of extracellular nucleotide imbalance in this pathological process (Diezmos et al., 2016). While homeostasis is maintained in a healthy intestinal mucosa, concentrations of ATP in luminal and interstitial compartments rise significantly above physiological limits during active disease phases. This "purinergic storm" results from a combination of passive leakage from necrotic or apoptotic enterocytes, active vesicular secretion from activated neutrophils and eosinophils, and extracellular ATP produced by commensal bacteria in the gut lumen (Longhi et al., 2017).

The high concentration of ATP accumulating in the environment functions as a classic DAMP. This molecular stimulus activates low-affinity P2X7 receptors located on macrophages and dendritic cells within the lamina propria. The activation of P2X7 receptors triggers the assembly of the NLRP3 inflammasome complex via intracellular potassium loss (K<sup>+</sup> efflux) (Kong et al., 2022). This process facilitates the conversion of pro-caspase-1 into active caspase-1, ultimately leading to the proteolytic maturation and secretion of potent pro-inflammatory cytokines, interleukin-1 beta (IL-1 $\beta$ ) and interleukin-18 (IL-18). Furthermore, overstimulation of the P2X7 receptor induces a form of inflammatory lytic cell death known as "pyroptosis" in enterocytes, further compromising epithelial barrier permeability. This promotes a "vicious cycle" of inflammation by increasing bacterial translocation (Kong et al., 2022).

Clinical data confirm that P2X7 receptor expression in colon biopsies from patients with active IBD correlates positively with the disease activity index (Cheng et al., 2021). However, the primary factor in pathological progression is not only the increase in ATP but also the deficiency of ectonucleotidase enzymes (CD39 and CD73) required to terminate this signal (Cheng et al., 2021). In normal physiology, the CD39/CD73 axis should suppress inflammation by converting ATP into anti-inflammatory adenosine; however, this axis is downregulated in the vascular endothelium and Tregs of IBD patients (Cheng et al., 2021). This deficit in adenosine production prevents the development of immune tolerance typically mediated through A2A and A2B receptors. Consequently, the pro-inflammatory ATP signal remains dominant, leading to chronic tissue damage. In this context, the use of P2X7 antagonists or the pharmacological restoration of CD39/CD73 activity provides a robust rational framework for next-generation therapies aimed at mucosal healing (Cheng et al., 2021).

#### **4. Metabolic Reprogramming in the Tumor Microenvironment: The Immunosuppressive Role of Purinergic Signaling**

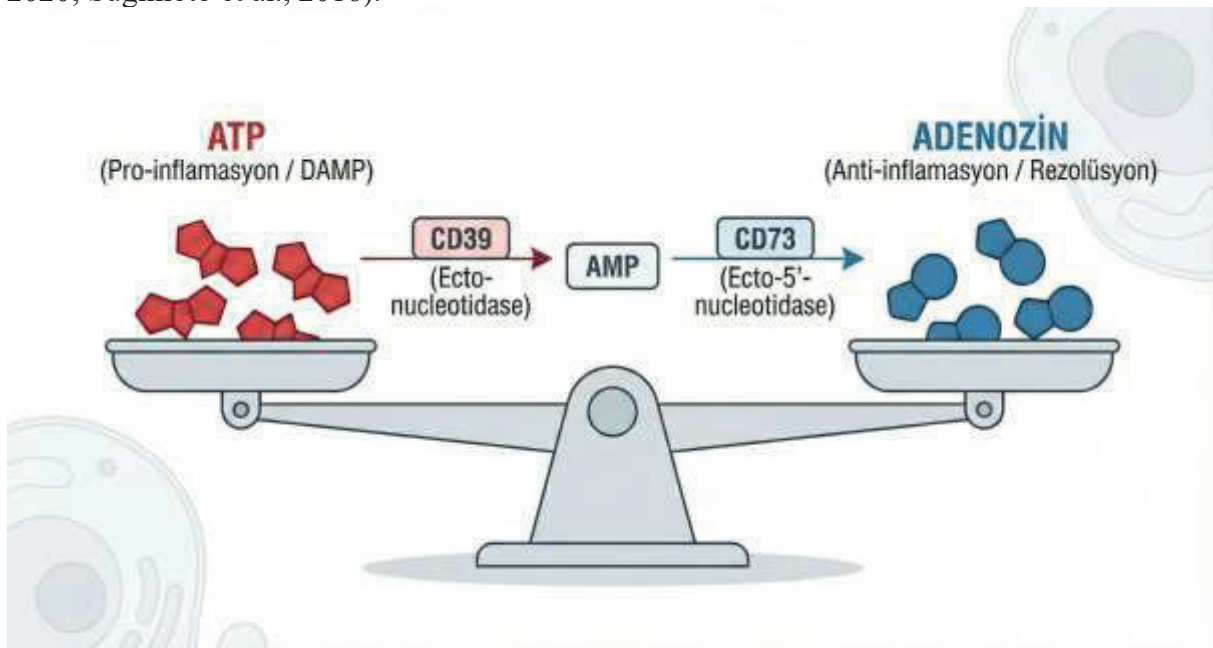
Oncological research has demonstrated that the progression of solid tumors is dictated not only by the genetic characteristics of the malignant cells but also by the dynamics of the surrounding tumor microenvironment (TME) (Gardani et al., 2024; Zanoni et al., 2022). The TME is characterized by profound hypoxia, acidosis, and nutrient deprivation, resulting from rapid cellular proliferation, inadequate vascularization, and elevated interstitial pressure. Under these rigorous conditions, mass cell death leads to the continuous and substantial release of ATP into the extracellular space (Gardani et al., 2024; Zanoni et al., 2022). While this ATP would theoretically be expected to stimulate the immune system to attack the tumor—a process known as immunogenic cell death—tumors utilize an evolutionary adaptation to subvert this mechanism for their own benefit (Gardani et al., 2024; Zanoni et al., 2022).

Tumor cells and Cancer-Associated Fibroblasts (CAF) within the tumor stroma express high levels of CD39 (ENTPD1) and CD73 (NT5E) enzymes on their surfaces, a process mediated by the hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) transcription factor (Shen et al., 2025). These enzymes hydrolyze extracellular ATP at an accelerated rate, creating a dense "adenosine halo" around the tumor (Shen et al., 2025). This adenosine-rich microenvironment directly impacts the phenotypic plasticity of Tumor-Associated Macrophages (TAM). By binding to A2A and A2B receptors on the macrophage surface, adenosine elevates cAMP levels (Di Virgilio & Adinolfi, 2017; Shen et al., 2025). High cAMP concentrations activate the Protein Kinase A (PKA) pathway, leading to the phosphorylation of the transcription factor CREB. This signaling cascade causes macrophages to lose their anti-tumoral (M1-like) characteristics and transition into a pro-tumoral (M2-like) phenotype. M2-like macrophages secrete growth factors that facilitate tumor expansion, promote angiogenesis via vascular endothelial growth factor (VEGF), and simplify tissue invasion through the secretion of matrix metalloproteinases (MMPs) (Di Virgilio & Adinolfi, 2017; Gardani et al., 2024; Shen et al., 2025; Zanoni et al., 2022)..

Furthermore, elevated adenosine levels paralyze the functions of cytotoxic T lymphocytes (CD8+) and Natural Killer (NK) cells that are essential for anti-tumor immunity. This phenomenon is currently recognized as a primary mechanism of resistance against immune checkpoint inhibitors, such as anti-PD1/PD-L1 therapies. Consequently, integrating CD73 inhibitors or A2A receptor antagonists into immunotherapy protocols holds the potential to dismantle this metabolic barrier and enhance therapeutic efficacy (Tu et al., 2022; Xia et al., 2023).

## 5. Active Purinergic Control in the Resolution of Inflammation

The traditional perspective defined the resolution of inflammation as a passive process that occurs spontaneously as pro-inflammatory signals diminish. However, contemporary molecular pathology data confirm that this phase is a highly regulated, energy-dependent, and active biochemical program, much like the initiation of inflammation itself. The purinergic signaling system functions as a central molecular switch that governs the transition of the inflammatory response from the "initiation" phase to the "healing/resolution" phase (**Figure 2**) (Fodor et al., 2020; Sugimoto et al., 2016).



**Figure 2. The Inflammatory "Switch" Mechanism: The Equilibrium Between ATP and Adenosine:** This diagram illustrates the dynamic equilibrium between extracellular ATP and adenosine, emphasizing its critical role in the modulation of inflammation. The left side of the scale represents the accumulation of ATP molecules, which are released during cellular injury or stress and function as potent "danger signals" (DAMPs), thereby initiating a pro-inflammatory response. Conversely, the right side represents adenosine molecules—"healing signals" produced through the enzymatic degradation of ATP that facilitate anti-inflammatory effects and promote tissue repair (resolution). The transition between these opposing states is regulated by the CD39 and CD73 enzymes, positioned at the center of the scale as the "metabolic switch." CD39 (ectonucleotidase) hydrolyzes ATP into ADP and subsequently into AMP. CD73 (ecto-5'-nucleotidase) then converts AMP into adenosine, effectively terminating the pro-inflammatory signal and initiating the anti-inflammatory cascade. This transition is vital for the timely cessation of the inflammatory response and the restoration of tissue homeostasis.

During the initial moments of acute tissue injury, high concentrations of released ATP attract neutrophils to the site of damage (chemotaxis) and initiate phagocytosis. However, as the inflammatory process progresses, an increase in CD39 and CD73 expression on the surface of immune cells causes the dominant signaling molecule to shift from ATP to adenosine. This temporal shift represents a critical threshold that alters the course of inflammation (Han et al., 2024; Yu et al., 2024).

The accumulation of adenosine in the microenvironment halts neutrophil infiltration via A2A receptors and promotes efferocytosis, the process by which macrophages safely clear apoptotic neutrophils (Murphy et al., 2017). Efferocytosis triggers a shift in macrophage activity, halting the production of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6) and stimulating the synthesis of mediators that support tissue repair (IL-10, TGF- $\beta$ , and resolvins). Furthermore, adenosine signaling regulates fibroblast activation, preventing wound healing from progressing into excessive fibrosis or scar tissue formation (Chi et al., 2023; Zhang et al., 2018).

Experimental models involving the silencing of CD39 or CD73 genes have demonstrated that without this enzymatic control, inflammation fails to resolve, leading to persistent tissue damage and chronicity, as seen in conditions like chronic obstructive pulmonary disease (COPD) or atherosclerosis. These findings confirm that the purinergic pathway is not merely a communication network but an essential regulatory mechanism for re-establishing systemic homeostasis (Ferrari et al., 2015).

## 6. ATP and Adenosine: From Cellular Communication to Pharmacological Targets

Due to its fundamental role in physiological processes, the purinergic signaling system has become a highly attractive area for drug development in modern pharmacology (Diezmos et al., 2016). The dual role of molecules like ATP and adenosine as both metabolic fuel and intercellular messengers makes this system unique (Burnstock, 1972). However, the ubiquitous distribution of purinergic receptors presents a pharmacological challenge; while it offers vast therapeutic potential, it also carries the risk of off-target effects in healthy tissues. Despite these challenges, drugs targeting purinergic mechanisms have achieved significant clinical success across disciplines ranging from cardiology to oncology (Bendell et al., 2023; Chi et al., 2023; Feng et al., 2020; Gardani et al., 2024).

The cardiovascular system represents the most successful application of purinergic pharmacology, particularly in the modulation of platelet function. P2Y<sub>12</sub> antagonists

(antiplatelet agents) prevent myocardial infarction and stent thrombosis by blocking the P2Y<sub>12</sub> receptor, a key mediator of platelet aggregation (Gachet, 2012). Within this class, clopidogrel and prasugrel bind irreversibly to the receptor, whereas ticagrelor and cangrelor offer reversible binding (Cheng, 2012). This reversibility allows for a faster restoration of platelet function once the medication is discontinued. In the context of adenosinergic modulation, adenosine is utilized in emergency medicine to restore normal rhythm in paroxysmal supraventricular tachycardia. Additionally, regadenoson, a potent vasodilator, is the preferred pharmacological stress agent for myocardial perfusion imaging in patients unable to perform physical exercise (Adhikary et al., 2020; Jia et al., 2015).

In neurology, the neuromodulatory properties of the purinergic system offer alternatives to traditional therapies for neurodegenerative diseases. Istradefylline improves motor control via a non-dopaminergic pathway by blocking A<sub>2A</sub> receptors in the basal ganglia. This agent is used to reduce "off" periods in Parkinson's disease patients treated with levodopa (Paton, 2020).

Specialized purinergic receptors are also targeted in the management of chronic conditions that impact quality of life. Diquafosol, a P<sub>2Y2</sub> agonist, stabilizes the tear film in dry eye syndrome by increasing water and mucin secretion on the ocular surface (Lau et al., 2014). Gefapixant reduces chronic refractory cough by blocking P<sub>2X3</sub> receptors, which are sensitive to the ATP released during cellular damage in the airways (Robbins et al., 2024).

Looking toward future therapies, promising research is expanding the boundaries of oncology and inflammation. In cancer immunotherapy, agents such as oleclumab target the CD73 enzyme to dismantle the immunosuppressive "adenosine shield" used by tumors to evade the immune system (Bendell et al., 2023). Furthermore, the blockade of the P<sub>2X7</sub> receptor—a driver of potent inflammation—is currently being evaluated in phase studies for the treatment of conditions such as rheumatoid arthritis and depression (Zablocki & Gorecki, 2023; Zhou et al., 2020).

**Table 3: Pharmacological Agents Targeting the Purinergic System**

Pharmacological Agent	Molecular Target	Pharmacodynamic Mechanism	Clinical Indication
<b>Clopidogrel / Prasugrel</b>	P2Y12 Receptor	Irreversible Antagonism	Prophylaxis of myocardial infarction and stent-related thrombosis (Jia et al., 2015).
<b>Ticagrelor / Cangrelor</b>	P2Y12 Receptor	Reversible Antagonism	Management of acute coronary syndrome and antiplatelet therapy (Cheng, 2012).
<b>Adenosine</b>	P1 (A1) Receptor	Receptor Agonism	Management of Paroxysmal Supraventricular Tachycardia (PSVT) (Parker & McCollam, 1990).
<b>Regadenoson</b>	P1 (A2A) Receptor	Receptor Agonism	Myocardial perfusion imaging (Pharmacological stress agent) (Bengalorkar et al., 2012).
<b>Istradefylline</b>	P1 (A2A) Receptor	Receptor Antagonism	Parkinson's Disease (Management of "off" episodes) (Paton, 2020).
<b>Diquafosol</b>	P2Y2 Receptor	Receptor Agonism	Treatment of Dry Eye Syndrome (Lau et al., 2014).
<b>Gefapixant</b>	P2X3 Receptor	Receptor Antagonism	Management of chronic refractory cough (Robbins et al., 2024).
<b>Oleclumab</b>	CD73 Enzyme	Enzymatic Inhibition	Cancer Immunotherapy (Investigational phase clinical trials) (Bendell et al., 2023).

## 7. Future Perspectives and Therapeutic Potential of Purinergic Signaling

Purinergic signaling, which transcends the conventional boundaries of bioenergetics, is currently established as a central regulatory network governing both physiological homeostasis and pathological progression. The paradigm shift initiated by the identification of ATP as an intercellular signaling molecule, rather than merely a metabolic energy currency, has unveiled novel therapeutic avenues across numerous medical disciplines. The delicate equilibrium between the pro-inflammatory "danger" signal (DAMP) function of ATP and the anti-inflammatory "healing" role of adenosine remains vital for maintaining tissue integrity (Burnstock, 1972; Diezmos et al., 2016; Ferrari et al., 2020).

The most promising future dimension of this system lies in its potential integration into personalized medicine. It is well-documented that polymorphisms in the genes encoding P2X7 and P2Y12 receptors directly modulate individual susceptibility to autoimmune disorders and clinical responses to antiplatelet therapies. Future analysis of these genetic variations will facilitate the development of more efficacious therapeutic protocols with minimized adverse effects (Gachet, 2012; Jia et al., 2015).

In the field of oncology, the conversion of ATP into an immunosuppressive "adenosine halo" within the TME via CD39 and CD73 enzymes represents a significant barrier to contemporary immunotherapies. In this regard, CD73 inhibitors and A2A receptor antagonists offer a critical therapeutic vision for dismantling tumor-mediated immune evasion mechanisms. Similarly,

restoring purinergic balance in inflammatory bowel diseases (IBD) through P2X7 antagonists or ectonucleotidase modulators provides a rational target for terminating chronic inflammation and promoting mucosal recovery (Shen et al., 2025).

Beyond established successes in cardiovascular medicine, the application of P2X3 antagonists like Gefapixant for chronic cough and Istradefylline for Parkinson's disease underscores the broad-spectrum impact of the system in neurological and respiratory pathologies (Paton, 2020; Robbins et al., 2024). In conclusion, the purinergic system functions not merely as a communication network, but as a biochemical switch that actively coordinates the resolution of inflammation and tissue regeneration. Mastery over this molecular switch is poised to become one of the most potent tools for restructuring tissue homeostasis in future pharmacological strategies.

## 8. Bibliography

- Adhikary, S. R., Cuthbertson, P., Turner, R. J., Sluyter, R., & Watson, D. (2020). A single-nucleotide polymorphism in the human ENTPD1 gene encoding CD39 is associated with worsened graft-versus-host disease in a humanized mouse model. *Immunol Cell Biol*, 98(5), 397–410. <https://doi.org/10.1111/imcb.12328>
- Bendell, J., LoRusso, P., Overman, M., Noonan, A. M., Kim, D. W., Strickler, J. H., Kim, S. W., Clarke, S., George, T. J., Grimison, P. S., Barve, M., Amin, M., Desai, J., Wise-Draper, T., Eck, S., Jiang, Y., Khan, A. A., Wu, Y., Martin, P.,...Patel, S. P. (2023). First-in-human study of oclumab, a potent, selective anti-CD73 monoclonal antibody, alone or in combination with durvalumab in patients with advanced solid tumors. *Cancer Immunol Immunother*, 72(7), 2443–2458. <https://doi.org/10.1007/s00262-023-03430-6>
- Bengalorkar, G. M., Bhuvana, K., Sarala, N., & Kumar, T. N. (2012). Regadenoson. *J Postgrad Med*, 58(2), 140–146. <https://doi.org/10.4103/0022-3859.97177>
- Burnstock, G. (1972). Purinergic nerves. *Pharmacol Rev*, 24(3), 509–581. <https://www.ncbi.nlm.nih.gov/pubmed/4404211>
- Cheng, J. W. (2012). Ticagrelor: oral reversible P2Y<sub>12</sub> receptor antagonist for the management of acute coronary syndromes. *Clin Ther*, 34(6), 1209–1220. <https://doi.org/10.1016/j.clinthera.2012.04.005>
- Cheng, N., Zhang, L., & Liu, L. (2021). Understanding the Role of Purinergic P2X<sub>7</sub> Receptors in the Gastrointestinal System: A Systematic Review. *Front Pharmacol*, 12, 786579. <https://doi.org/10.3389/fphar.2021.786579>
- Chi, L., Huan, L., Zhang, C., Wang, H., & Lu, J. (2023). Adenosine receptor A<sub>2b</sub> confers ovarian cancer survival and PARP inhibitor resistance through IL-6-STAT3 signalling. *J Cell Mol Med*, 27(15), 2150–2164. <https://doi.org/10.1111/jcmm.17802>
- Di Virgilio, F., & Adinolfi, E. (2017). Extracellular purines, purinergic receptors and tumor growth. *Oncogene*, 36(3), 293–303. <https://doi.org/10.1038/onc.2016.206>
- Diezmos, E. F., Bertrand, P. P., & Liu, L. (2016). Purinergic Signaling in Gut Inflammation: The Role of Connexins and Pannexins. *Front Neurosci*, 10, 311. <https://doi.org/10.3389/fnins.2016.00311>
- Dunn, J., & Grider, M. H. (2025). Physiology, Adenosine Triphosphate. In *StatPearls*. <https://www.ncbi.nlm.nih.gov/pubmed/31985968>
- Feng, L. L., Cai, Y. Q., Zhu, M. C., Xing, L. J., & Wang, X. (2020). The yin and yang functions of extracellular ATP and adenosine in tumor immunity. *Cancer Cell Int*, 20, 110. <https://doi.org/10.1186/s12935-020-01195-x>
- Ferrari, D., la Sala, A., Milani, D., Celeghini, C., & Casciano, F. (2020). Purinergic Signaling in Controlling Macrophage and T Cell Functions During Atherosclerosis Development. *Front Immunol*, 11, 617804. <https://doi.org/10.3389/fimmu.2020.617804>

- Ferrari, D., Vitiello, L., Idzko, M., & la Sala, A. (2015). Purinergic signaling in atherosclerosis. *Trends Mol Med*, 21(3), 184–192. <https://doi.org/10.1016/j.molmed.2014.12.008>
- Fodor, P., White, B., & Khan, R. (2020). Inflammation-The role of ATP in pre-eclampsia. *Microcirculation*, 27(1), e12585. <https://doi.org/10.1111/micc.12585>
- Gachet, C. (2012). P2Y(12) receptors in platelets and other hematopoietic and non-hematopoietic cells. *Purinergic Signal*, 8(3), 609–619. <https://doi.org/10.1007/s11302-012-9303-x>
- Gardani, C. F. F., Diz, F. M., Donde, L. B., Rockenbach, L., Laufer, S., & Morrone, F. B. (2024). The potential role of purinergic signaling in cancer therapy: perspectives on anti-CD73 strategies for prostate cancer. *Front Immunol*, 15, 1455469. <https://doi.org/10.3389/fimmu.2024.1455469>
- Gartland, A., Skarratt, K. K., Hocking, L. J., Parsons, C., Stokes, L., Jorgensen, N. R., Fraser, W. D., Reid, D. M., Gallagher, J. A., & Wiley, J. S. (2012). Polymorphisms in the P2X7 receptor gene are associated with low lumbar spine bone mineral density and accelerated bone loss in post-menopausal women. *Eur J Hum Genet*, 20(5), 559–564. <https://doi.org/10.1038/ejhg.2011.245>
- Guerini, F. R., Agliardi, C., Bolognesi, E., Zanzottera, M., Caputo, D., Pasanisi, M. B., Rovaris, M., & Clerici, M. (2022). Two Single Nucleotide Polymorphisms in the Purinergic Receptor P2X7 Gene Are Associated with Disease Severity in Multiple Sclerosis. *Int J Mol Sci*, 23(23). <https://doi.org/10.3390/ijms232315381>
- Han, S., Kim, B., Hyeon, D. Y., Jeong, D., Ryu, J., Nam, J. S., Choi, Y. H., Kim, B. R., Park, S. C., Chung, Y. W., Shin, S. J., Lee, J. Y., Kim, J. K., Park, J., Lee, S. W., Kim, T. B., Cheon, J. H., Cho, H. J., Kim, C. H.,...Ryu, J. H. (2024). Distinctive CD39(+)CD9(+) lung interstitial macrophages suppress IL-23/Th17-mediated neutrophilic asthma by inhibiting NETosis. *Nat Commun*, 15(1), 8628. <https://doi.org/10.1038/s41467-024-53038-2>
- Huang, Z., Xie, N., Illes, P., Di Virgilio, F., Ulrich, H., Semyanov, A., Verkhatsky, A., Sperlagh, B., Yu, S. G., Huang, C., & Tang, Y. (2021). From purines to purinergic signalling: molecular functions and human diseases. *Signal Transduct Target Ther*, 6(1), 162. <https://doi.org/10.1038/s41392-021-00553-z>
- Jacobson, K. A., Balasubramanian, R., Deflorian, F., & Gao, Z. G. (2012). G protein-coupled adenosine (P1) and P2Y receptors: ligand design and receptor interactions. *Purinergic Signal*, 8(3), 419–436. <https://doi.org/10.1007/s11302-012-9294-7>
- Jia, M., Li, Z., Chu, H., Li, L., & Chen, K. (2015). Novel oral P2Y12 inhibitor prasugrel vs. clopidogrel in patients with acute coronary syndrome: evidence based on 6 studies. *Med Sci Monit*, 21, 1131–1137. <https://doi.org/10.12659/MSM.893914>
- Kong, H., Zhao, H., Chen, T., Song, Y., & Cui, Y. (2022). Targeted P2X7/NLRP3 signaling pathway against inflammation, apoptosis, and pyroptosis of retinal endothelial cells in

- diabetic retinopathy. *Cell Death Dis*, 13(4), 336. <https://doi.org/10.1038/s41419-022-04786-w>
- Lau, O. C., Samarawickrama, C., & Skalicky, S. E. (2014). P2Y2 receptor agonists for the treatment of dry eye disease: a review. *Clin Ophthalmol*, 8, 327–334. <https://doi.org/10.2147/OPTH.S39699>
- Longhi, M. S., Moss, A., Jiang, Z. G., & Robson, S. C. (2017). Purinergic signaling during intestinal inflammation. *J Mol Med (Berl)*, 95(9), 915–925. <https://doi.org/10.1007/s00109-017-1545-1>
- Maloney, J. P., Branchford, B. R., Brodsky, G. L., Cosmic, M. S., Calabrese, D. W., Aquilante, C. L., Maloney, K. W., Gonzalez, J. R., Zhang, W., Moreau, K. L., Wiggins, K. L., Smith, N. L., Broeckel, U., & Di Paola, J. (2017). The ENTPD1 promoter polymorphism -860 A > G (rs3814159) is associated with increased gene transcription, protein expression, CD39/NTPDase1 enzymatic activity, and thromboembolism risk. *FASEB J*, 31(7), 2771–2784. <https://doi.org/10.1096/fj.201600344R>
- Murphy, P. S., Wang, J., Bhagwat, S. P., Munger, J. C., Janssen, W. J., Wright, T. W., & Elliott, M. R. (2017). CD73 regulates anti-inflammatory signaling between apoptotic cells and endotoxin-conditioned tissue macrophages. *Cell Death Differ*, 24(3), 559–570. <https://doi.org/10.1038/cdd.2016.159>
- Nie, X. Y., Li, J. L., Zhang, Y., Xu, Y., Yang, X. L., Fu, Y., Liang, G. K., Lu, Y., Liu, J., & Shi, L. W. (2017). Haplotype of platelet receptor P2RY12 gene is associated with residual clopidogrel on-treatment platelet reactivity. *J Zhejiang Univ Sci B*, 18(1), 37–47. <https://doi.org/10.1631/jzus.B1600333>
- Oken, A. C., Krishnamurthy, I., Savage, J. C., Lisi, N. E., Godsey, M. H., & Mansoor, S. E. (2022). Molecular Pharmacology of P2X Receptors: Exploring Druggable Domains Revealed by Structural Biology. *Front Pharmacol*, 13, 925880. <https://doi.org/10.3389/fphar.2022.925880>
- Parker, R. B., & McCollam, P. L. (1990). Adenosine in the episodic treatment of paroxysmal supraventricular tachycardia. *Clin Pharm*, 9(4), 261–271. <https://www.ncbi.nlm.nih.gov/pubmed/2184971>
- Paton, D. M. (2020). Istradefylline: adenosine A2A receptor antagonist to reduce "OFF" time in Parkinson's disease. *Drugs Today (Barc)*, 56(2), 125–134. <https://doi.org/10.1358/dot.2020.56.2.3098156>
- Robbins, J. A., Sands, S., Maganti, L., Crumley, T., Fox-Bosetti, S., Hussain, A., Schwartz, H., Safirstein, B., Ahmad, M., Dragone, L., Nussbaum, J., Kushida, C., Iwamoto, M., & Stoch, S. A. (2024). Gefapixant as a P2X3 receptor antagonist treatment for obstructive sleep apnea: a randomized controlled trial. *J Clin Sleep Med*, 20(12), 1905–1913. <https://doi.org/10.5664/jcsm.11272>
- Sharma, V., Deo, P., & Sharma, A. (2023). Deficiency of adenosine deaminase 2 (DADA2): Review. *Best Pract Res Clin Rheumatol*, 37(1), 101844. <https://doi.org/10.1016/j.berh.2023.101844>

- Shen, J., Liao, B., Gong, L., Li, S., Zhao, J., Yang, H., Gong, Y., & Li, Y. (2025). CD39 and CD73: biological functions, diseases and therapy. *Mol Biomed*, 6(1), 97. <https://doi.org/10.1186/s43556-025-00345-9>
- St Hilaire, C., Ziegler, S. G., Markello, T. C., Brusco, A., Groden, C., Gill, F., Carlson-Donohoe, H., Lederman, R. J., Chen, M. Y., Yang, D., Siegenthaler, M. P., Arduino, C., Mancini, C., Freudenthal, B., Stanescu, H. C., Zdebik, A. A., Chaganti, R. K., Nussbaum, R. L., Kleta, R.,...Boehm, M. (2011). NT5E mutations and arterial calcifications. *N Engl J Med*, 364(5), 432–442. <https://doi.org/10.1056/NEJMoa0912923>
- Sugimoto, M. A., Sousa, L. P., Pinho, V., Perretti, M., & Teixeira, M. M. (2016). Resolution of Inflammation: What Controls Its Onset? *Front Immunol*, 7, 160. <https://doi.org/10.3389/fimmu.2016.00160>
- Thiel, M., Caldwell, C. C., & Sitkovsky, M. V. (2003). The critical role of adenosine A2A receptors in downregulation of inflammation and immunity in the pathogenesis of infectious diseases. *Microbes Infect*, 5(6), 515–526. [https://doi.org/10.1016/s1286-4579\(03\)00068-6](https://doi.org/10.1016/s1286-4579(03)00068-6)
- Tu, E., McGlinchey, K., Wang, J., Martin, P., Ching, S. L., Floc'h, N., Kurasawa, J., Starrett, J. H., Lazdun, Y., Wetzel, L., Nuttall, B., Ng, F. S., Coffman, K. T., Smith, P. D., Politi, K., Cooper, Z. A., & Streicher, K. (2022). Anti-PD-L1 and anti-CD73 combination therapy promotes T cell response to EGFR-mutated NSCLC. *JCI Insight*, 7(3). <https://doi.org/10.1172/jci.insight.142843>
- Xia, C., Yin, S., To, K. K. W., & Fu, L. (2023). CD39/CD73/A2AR pathway and cancer immunotherapy. *Mol Cancer*, 22(1), 44. <https://doi.org/10.1186/s12943-023-01733-x>
- Xiong, Y., Sun, S., Teng, S., Jin, M., & Zhou, Z. (2018). Ca(2+)-Dependent and Ca(2+)-Independent ATP Release in Astrocytes. *Front Mol Neurosci*, 11, 224. <https://doi.org/10.3389/fnmol.2018.00224>
- Yu, Y., Zhang, C., Dong, B., Zhang, Z., Li, X., Huang, S., Tang, D., Jing, X., Yu, S., Zheng, T., Wu, D., & Tai, S. (2024). Neutrophil extracellular traps promote immune escape in hepatocellular carcinoma by up-regulating CD73 through Notch2. *Cancer Lett*, 598, 217098. <https://doi.org/10.1016/j.canlet.2024.217098>
- Zablocki, K., & Gorecki, D. C. (2023). The Role of P2X7 Purinoceptors in the Pathogenesis and Treatment of Muscular Dystrophies. *Int J Mol Sci*, 24(11). <https://doi.org/10.3390/ijms24119434>
- Zanoni, M., Pegoraro, A., Adinolfi, E., & De Marchi, E. (2022). Emerging roles of purinergic signaling in anti-cancer therapy resistance. *Front Cell Dev Biol*, 10, 1006384. <https://doi.org/10.3389/fcell.2022.1006384>
- Zhang, S., Chuah, S. J., Lai, R. C., Hui, J. H. P., Lim, S. K., & Toh, W. S. (2018). MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and

modulating immune reactivity. *Biomaterials*, 156, 16–27.  
<https://doi.org/10.1016/j.biomaterials.2017.11.028>

Zhou, Y., Fei, M., Zhang, G., Liang, W. C., Lin, W., Wu, Y., Piskol, R., Ridgway, J., McNamara, E., Huang, H., Zhang, J., Oh, J., Patel, J. M., Jakubiak, D., Lau, J., Blackwood, B., Bravo, D. D., Shi, Y., Wang, J.,...Yan, M. (2020). Blockade of the Phagocytic Receptor MerTK on Tumor-Associated Macrophages Enhances P2X7R-Dependent STING Activation by Tumor-Derived cGAMP. *Immunity*, 52(2), 357–373 e359. <https://doi.org/10.1016/j.immuni.2020.01.014>



# CHAPTER 3

---

## ORAL POTENTIALLY MALIGNANT DISORDERS

*Hatice BİLGİÇ<sup>1</sup> , Emre KÖSE<sup>2</sup> , Selime CHAUSH<sup>3</sup>*

---

<sup>1</sup> Research Assistant, Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Aydın Adnan Menderes University, Aydın, 09010, Turkey dt.haticebilgic@gmail.com, ORCID ID: 0000-0003-1805-3605

<sup>2</sup> Associate Professor, Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Aydın Adnan Menderes University, Aydın, 09010, Turkey emre.kose@adu.edu.tr ORCID ID: 0000-0002-0659-7157

<sup>3</sup> Dentist, Selime İbryam Cahaush, Peperstraat 7 bus 101 9120 Beveren was Belgie, dtscavus@gmail.com ORCID ID :0009-0005-5408-3694

In recent years, the World Health Organization (WHO) has categorized a diverse group of conditions and lesions as Oral Potentially Malignant Disorders (OPMD). In its publications, OPMDs are listed based on the available evidence regarding their worldwide prevalence and risk of malignant transformation. The OPMDs with the highest clinical relevance and prevalence are included in the WHO Classification of Head and Neck Tumors series (1), whereas a more extensive list, encompassing less common OPMDs, is available in the WHO Oral Cancer Collaboration Center consensus documents (2). According to the 5th edition of the WHO Classification of Head and Neck Tumors, Chapter 5, "Tumors of the Oral Cavity and Mobile Tongue," and the WHO 2020 Oral Cancer Collaboration Center reports, OPMDs include (3):

### **1. Leukoplakia**

Leukoplakia has been defined in multiple ways over the past 40 years, with definitions evolving continuously. The most widely accepted current definition was proposed by the WHO Oral Cancer Collaboration Center in 2007 and reaffirmed in 2020. Leukoplakia is described as "a predominantly white plaque with a suspicious risk, excluding known diseases or disorders that do not carry a cancer risk" (2). The term is applied only when other white lesions of the oral mucosa such as oral lichen planus, frictional keratosis, white spongy nevus, or hairy leukoplakia are excluded (4). By definition, leukoplakia is a clinical diagnosis without a specific histopathological feature (5).

This condition is relatively common, affecting about 4% of the global population (6). Among OPMDs, leukoplakia is the most frequent. It occurs more often in men compared with women and is typically observed in individuals over 40 years old. Its prevalence varies geographically, often higher in Southeast Asia compared to Western countries, likely due to differing etiological factors (7).

The causes of leukoplakia appear similar to those of oral cancer, including tobacco use in all forms, betel nut chewing, and alcohol consumption. Nevertheless, it is notable that older women who have never smoked can also develop leukoplakia, and these lesions may carry a greater risk of malignant alteration compared to those in patients with known risk factors (2).

Clinically, leukoplakia can be grouped into two types: homogeneous and heterogeneous. This distinction is based entirely on clinical features such as surface, color, and texture (2):

*Homogeneous leukoplakia:* presents as smooth-edged, thin, flat white plaques or spots, sometimes with fine cracks in the keratin layer (8).

*Heterogeneous leukoplakia:* exhibits an irregular surface, potentially with superficial ulceration and poorly defined borders (8, 9).

Heterogeneous leukoplakia may present with various clinical appearances (2, 10):

*Specular:* mixed red and white areas, predominantly white, also called erythro-leukoplakia.

*Nodular:* small, round, polypoid projections that can be white or red.

*Exophytic or Verrucous:* furrowed or wrinkled surface pattern.

The risk of malignant transformation in leukoplakia can vary depending on clinical, histological, and molecular characteristics. Key risk factors for cancer development include (4):

- Irregular clinical appearance
- Female gender
- Long duration of the lesion
- Idiopathic leukoplakia (non-smoker/non-chewer)
- Location on the floor of the mouth or lateral side of tongue
- Lesion size > 200 mm<sup>2</sup>
- Occurrence of epithelial dysplasia
- Existence of *Candida albicans* (11)

The severity of dysplastic changes correlates with cancer risk, with more pronounced dysplasia carrying the highest transformation potential. In non-smokers, oral epithelial dysplasia (OED) is associated with approximately a twofold higher risk of progression to cancer (12).

## **2. Verrucous Proliferative Leukoplakia**

Verrucous proliferative leukoplakia (PVL) represents an aggressive subtype of leukoplakia. It initially appears as keratosis without dysplasia but can gradually evolve

sometimes over 20 years into confluent, multifocal oral keratosis (13). According to the WHO, PVL is defined as "a persistent, irreversible, and progressive disorder marked by multiple leukoplakic lesions," which frequently develops a verrucous appearance (2).

PVL is usually seen in women with a female-to-male ratio of 4:1 (14). The mandibular gingiva, buccal mucosa and tongue are the most frequently affected sites (15). The exact cause of PVL is unclear, and unlike many other oral lesions, tobacco use does not appear to be a primary factor, as PVL occurs in both smokers and non-smokers (14). Human papillomavirus (HPV) has been detected in PVL lesions, with 89% of specimens testing positive and HPV 16 identified in most cases (16). Additionally, Epstein-Barr virus (EBV) was present in 60% of PVL samples (17). Among all OPMDs, PVL has the highest malignant transformation rate, reaching 49.5% (18).

Diagnosis of PVL relies on both clinical and histological evaluation. The disease progresses from early hyperkeratosis to epithelial hyperplasia and atypia, potentially advancing to verrucous carcinoma and finally, squamous cell carcinoma (SCC). This progression underscores the need for multiple biopsies and ongoing follow-up (19). Histopathological features vary depending on disease stage, biopsy site, and lesion location. Lesions typically show hyperkeratosis and acanthosis with lymphocytic infiltration of the lamina propria. As lesions evolve, histology may reveal hyperplasia, with or without dysplasia, and irregular surface morphology (20).

### **3. Erythroplakia**

Erythroplakia was originally defined by the WHO as "oral mucosal lesions appearing as bright red velvety plaques that cannot be classified as any other pathologically or clinically identifiable condition" (21). The most often cited definition in the scientific literature describes it as a bright red patch that cannot be categorized as any other pathologically or clinically identifiable lesion (22). Similar to leukoplakia, erythroplakia is defined mainly by exclusion (21).

The exact cause of erythroplakia is not fully clarified. Known contributing factors include chewing tobacco, betel quid, cigarette smoking, and alcohol intake (21). Erythroplakia is most commonly observed in middle-aged individuals, especially in the sixth and seventh decades of

life, and affects both sexes equally. Its prevalence ranges from 0.02% to 0.83%, with an average of 0.11% in the general population (23).

A meta-analysis and systematic review published in 2022 reported a malignant transformation rate of 12.7% (24). Lesions with moderate to severe dysplasia carry a significantly higher risk of progressing to cancer (21).

Clinically, erythroplakia appears as a bright red macule or patch with a soft velvety surface. The lesions are usually soft upon palpation, with induration present only when malignancy is involved. Borders can be irregular, though they are often well demarcated, and the surface may occasionally appear granular (25).

The most commonly affected sites include the floor of the mouth, soft palate, ventral side of tongue, tonsillar fossa, and buccal alveolar mucosa. Affected areas can vary between studies, with tongue involvement reported less frequently. Lesions are generally small (<1.5 cm), though larger lesions (>4 cm) have been documented. Involvement of multiple sites is rare (21). Symptoms are usually absent, but some patients report vague pain or a burning sensation at the lesion site. A metallic taste has also been described (26).

Histopathologically, erythroplakia is characterized by thin, atrophic epithelium, absence of keratin, and epithelial hyperplasia. These features account for the deep red appearance of the lesions. The absence of surface keratin, which normally diminishes red coloration, is particularly notable. Prompt biopsy is recommended to exclude neoplastic changes. Histological evaluation reveals mild to severe dysplasia or SCC in 60–90% of cases (21).

#### **4. Oral Submucosal Fibrosis**

Oral Submucosal Fibrosis (OSMF) is a chronic fibrotic disorder of the oral mucosa described by epithelial immune cell intrusion, followed by fibroelastic changes in the submucosa and lamina propria, ultimately leading to stiffening of the oral mucosa (27). Clinically, it has been described as "a debilitating, irreversible, progressive collagen metabolic disorder caused by commercial preparations and chronic areca nut chewing; affecting the oral and pharynx mucosa; resulting in functional impairment and mucosal stiffness with a potential for malignant transformation (28).

The global prevalence of OSMF is approximately 4.96%, with a clear geographical distribution, being largely confined to South and Southeast Asia. The condition is strongly associated with paan (betel quid) chewing and areca nut consumption; over 90% of OSMF patients have a history of paan chewing (29).

The rate of malignant transformation in OSMF ranges between 1% and 9%, with higher rates observed in patients who also present with oral leukoplakia. Oral cancers developing from OSMF may be less frequent but more aggressive compared to those arising from other oral lesions (30).

Clinically, OSMF is recognized by restricted tongue mobility, blanching of the oral mucosa, leathery texture, loss of papillae, progressive reduction in mouth opening, and depigmentation. Staging of OSMF early, intermediate, and advanced guides both treatment planning and patient management (31).

Histopathological examination confirms the clinical diagnosis, revealing features such as loss of rete ridges, epithelial atrophy, hyalinization of the lamina propria, and alterations in the underlying muscle layer (32).

## **5. Oral Lichen Planus**

Oral Lichen Planus (OLP) is a chronic inflammatory disorder of the oral basal epithelium mediated by T lymphocytes, particularly CD8<sup>+</sup> cells. The WHO defines OLP as "a chronic inflammatory disorder of unclear etiology, characterized by white reticular lesions with recurrent remissions and exacerbations, which may be accompanied by atrophic, ulcerative, plaque-type, or erosive areas. Lesions are often bilaterally symmetrical (2).

OLP is a moderately common mucocutaneous disorder, with a prevalence of 1–3% in the general population. It is more frequently observed in women than men. Although the exact etiology remains unclear, genetic predisposition, immunological disorders, nutritional deficiencies, psychological stress, and infectious agents have been proposed as contributing factors (33).

Despite being classified as a potentially malignant disorder, the definitive diagnostic criteria for OLP and its malignant transformation potential remain under debate, with conflicting reports in the literature (34).

Six classical clinical subtypes of OLP have been described, which may occur individually or in combination: reticular, atrophic/erythematous, plaque-like, ulcerative /erosive, bullous and papular forms. The reticular and erosive subtypes are the most frequently observed. Atrophic and erosive forms are most commonly associated with malignant transformation, possibly due to the atrophic nature of the mucosa rather than the disease itself (35, 36).

*Reticular:* The most common type, characterized by fine, shiny, white lines known as Wickham striae, often surrounded by an erythematous line. The buccal mucosa is the most frequently affected site, with lesions typically bilateral and symmetrical (37–40).

*Plaque-like:* These lesions resemble leukoplakia, appearing as homogeneous white patches. They may be slightly raised or irregular and are often multifocal, affecting the dorsum of the tongue and buccal mucosa (40).

*Atrophic:* Widespread, red lesions often surrounded by fine white striae, typically involving adjacent gingiva. This form is commonly stated as "chronic desquamative gingivitis" and may present with symmetrical patchy distribution across all quadrants (40).

*Erosive:* Irregularly shaped lesions covered by fibrous pseudomembranes or plaques. The periphery may display reticular lines. Disruption of the pseudomembrane or plaque causes pain. Erosive OLP is considered to carry a higher risk for malignant transformation (41).

*Papular:* Small white papules (~0.5 mm) that may be overlooked during routine examination due to their size. Rarely observed (40).

*Bullous:* Small vesicle or bullae that ruptures easily, leaving painful ulcerated surfaces. Nikolsky's sign may be positive. Lesions most commonly appear on the buccal mucosa of the region adjacent to the second and third molars, with less frequent involvement of the lateral tongue, gingiva, or inner lips (40, 42).

Histopathological findings alone may not be sufficient for diagnosis, as discrepancies between clinical and microscopic features are possible. Therefore, OLP diagnosis requires correlation between clinical presentation and histopathology (43).

OLP must be distinguished from OLP-like lesions, including lichenoid reactions, frictional keratosis, lupus erythematosus, leukoplakia, pemphigus, erythematous candidiasis, mucous membrane pemphigoid, graft-versus-host disease (GVHD), and chronic ulcerative stomatitis. Among these, drug-induced lichenoid reactions are the most challenging to differentiate clinically and histopathologically from OLP (42, 44).

## **6. Oral Lichenoid Lesions**

Oral Lichenoid Lesions (OLL) are intraoral lesions appearing as white or red patches with a reticular streaked pattern similar to Oral Lichen Planus (OLP), but they are linked to identifiable triggers. OLL can be categorized into oral lichenoid contact lesions (OLCL), drug-induced OLL, and GVHD-associated OLL (45).

The term OLCL refers to oral lesions that resemble OLP both clinically and histopathologically but are believed to result from localized hypersensitivity reactions (delayed immune-mediated) to dental restorative materials, particularly amalgam, or other contact agents such as cinnamon. Other restorative substances, including gold, nickel, and acrylic resin, may also contribute to lichenoid lesion formation (46, 47).

OLCL typically appears as white or mixed red-and-white lesions and can sometimes be ulcerated. These lesions are generally less symmetrical and more often unilateral compared to OLP, and they lack the classic reticular pattern, instead presenting as plaques or atrophic areas (48).

Clinically and histologically, OLCL can be indistinguishable from OLP. A key diagnostic feature is the lesion's location, which is directly related to the suspected triggering agent. The lateral tongue and buccal mucosa are the most frequently affected sites (48). Although OLP may also occur near dental restorations, these lesions are usually more widespread, affecting additional areas. The duration of contact between the agent and the oral mucosa is considered important in OLCL development (45).

Diagnosis primarily relies on clinical observation, and lesion resolution following removal of the suspected causative material confirms the diagnosis. Biopsy is recommended when clinical features are atypical or when malignancy cannot be excluded (48).

Removal of neighboring amalgam fillings or those in direct contact leads to complete healing in 39–89% of cases, whereas lesions where restorations are left in place heal in only 0–29% of cases (49).

Drug-induced OLL are uncommon and arise from exposure to various medications. Systemic drugs linked to OLL include non-steroidal anti-inflammatory drugs, antihypertensives, oral hypoglycemics, antifungals, monoclonal antibodies and antibiotics. Usually, there is a temporal relationship between drug exposure and lesion onset; however, reactions can occur even years after drug use (50). These lesions may have an uncertain clinical appearance, but unilateral presentation can assist in diagnosis (51).

GVHD occurs in recipients of bone marrow or hematopoietic stem cell transplants (25). Acute GVHD rarely affects the oral cavity, but in chronic GVHD, the mouth is frequently involved. Oral manifestations may include papules, white plaques, and hyperkeratotic lines resembling Wickham striae, alongside erythema and pseudomembranous ulcers (52).

## **7. Palatal Lesions in Reverse Smokers**

Reverse smoking is an uncommon smoking habit in which the burning end of the cigarette or bidi is placed inside the mouth while inhaling the smoke. This practice is particularly prevalent in the Indian subcontinent (53). Temperatures in the oral cavity can reach up to 120°C, and the products of combustion increase the occurrence of oral lesions compared to conventional smoking. However, epidemiological and controlled studies examining these lesions are relatively limited (53). Thermal injury combined with prolonged exposure to various carcinogens causes changes in the palatal mucosa (54).

In 2020, the WHO described palatal lesions in palatal lesions in reverse smokers (PLRS) as "white and/or red patches on the hard palate mucosa often stained with nicotine" (2).

Clinical features in reverse smokers differ from those seen in regular smokers. The primary sites affected are the palate and tongue. Lesions range from palatal keratosis, exfoliative lesions, leukoplakia, and ulcers to frank malignancy. Other observed abnormalities include mucosal thickening, fissures, pigmentation, nodularity, erythema, and ulceration (55).

Palatal hyperpigmentation gives a gray-black appearance due to increased melanin production by melanocytes. This melanin synthesis is a protective mechanism against heat and acts as an

antioxidant against toxic compounds generated during reverse smoking. In some cases, patchy depigmented areas surrounded by hyperpigmented mucosa have been reported. These findings are often associated with heavy alcohol use and smoking. Depigmentation has also been linked to reduced melanin production, and hyperchromasia has been detected in the basal epithelial layer (55).

Given the malignant potential of PLRS, biopsy of these lesions is essential and may reveal various stages of dysplasia or carcinoma. Histological evaluation may also show ductal epithelial hyperplasia (55).

For differential diagnosis, conditions such as OLP, oral lupus and oral candidiasis should be considered. A thorough history regarding reverse smoking habits can help rule out these conditions. Oral candidiasis can be excluded through culture, and other risk factors like immunosuppression should also be evaluated (53).

## **8. Discoid Lupus Erythematosus**

Discoid Lupus Erythematosus (DLE) is a chronic inflammatory disorder that affects both the skin and oral mucosa (25). It is the most prevalent form of chronic cutaneous lupus, representing about 80% of cases. The disease pathophysiology is multifactorial, involving genetics, environmental triggers, and both innate and adaptive immune responses (56). Key environmental triggers include ultraviolet radiation, certain medications, radiotherapy, and smoking (57).

DLE is more common in women, with a female-to-male ratio of 3:1. While it can occur at any age, it most often presents between 20 and 40 years old (58). Oral lesions without skin involvement are uncommon, reported in roughly 10% of patients (59).

Oral involvement occurs in approximately 20% of cases, usually affecting the lips, buccal alveolar mucosa and hard palate (60). Lesions typically appear as hyperkeratotic papules or spreading striae surrounding central erythema or ulceration, sometimes with peripheral telangiectasia. Chronic lesions may show a “honeycomb” pattern. Mucosal lesions can precede or occur independently of skin lesions. Lip lesions may extend to adjacent skin, blurring the vermilion border, and desquamative gingivitis may be present on the upper and/or lower gingiva (61). Healing may result in post-inflammatory hyperpigmentation. Common

symptoms include burning sensation (66.6%), photosensitivity (57.1%), dryness (23.8%), tenderness (14.3%) and pain (4.8%), although some lesions remain asymptomatic (59).

The WHO classifies DLE as a potentially malignant oral disorder, but malignant transformation is uncommon (62). When it does occur, it is usually in lesions on the vermilion border of the lips, more frequently affecting the lower lip. Risk factors for malignant transformation include prolonged UV exposure, chronic scarring, HPV infection, and long-term immunosuppression. The interval from DLE onset to lip cancer development is shorter than for cancers arising from DLE in other locations (63).

Differential diagnosis of oral DLE includes OLP, OLL, leukoplakia, and actinic keratosis (when the lower lip is involved) (64). OLP lesions are typically more extensive and symmetrical, with a more distinct reticular pattern. OLP appears as white striae in areas directly contacting dental restorations and resolves after removal of the restoration. Oral leukoplakia does not show the characteristic hyperkeratotic striae or central atrophy. Actinic keratosis generally affects the lower lip and presents with scaling rather than striae (64).

## **9. Graft Versus Host Disease**

Graft Versus Host Disease (GVHD) is an immune-mediated condition that can develop after allogeneic hematopoietic stem cell transplantation. GVHD may present with both acute (aGVHD) and chronic (cGVHD) oral manifestations. Clinically and histopathologically, GVHD lesions resemble those seen in OLP (2).

In aGVHD, oral involvement is uncommon, and lesions are usually nonspecific. They may present as gingivitis, mucositis, erythema, or ulceration on the oral mucosa or lips (65).

In contrast, more than 70% of patients with cGVHD have oral involvement. Clinical features include mucosal inflammation, atrophy, and lichenoid hyperkeratotic changes such as striae, papules, plaques and patches. Additional findings may include pseudomembranous ulceration, perioral fibrosis and mucocoeles. Other characteristic changes include vasculitis-like patterns, telangiectasia, and sclerotic alterations, which can reduce mouth opening. Patients often report oral pain, sensitivity to normally tolerated foods or substances, dry mouth, and sometimes altered taste (66).

Diagnosis of oral GVHD can be made clinically when lesions display typical lichenoid features. If lesions present as erythema, ulceration, or white plaques without the classic white striations, biopsy is required to confirm the diagnosis (67).

## **10. Human Papilloma Virus-Associated Dysplasia**

The 2022 WHO classification recognizes Human Papilloma Virus (HPV)-associated oral epithelial dysplasia (OED) as a distinct OPMD category (68). The morphological characteristics of HPV-OED are now well documented (69, 70). Although HPV-positive SCC cases are relatively rare, a subset of OED is strongly associated with HPV infection. HPV-OED predominantly affects males (M:F = 6:1) and typically occurs in the sixth decade, although it can present across a wide age range (71).

The most frequently affected sites are the ventral and lateral side of tongue and the floor of the mouth, though lesions can also appear on the buccal mucosa, palate, lip mucosa, and gingiva (69). Clinically, HPV-OED usually appears as a flat, well-defined patch ranging from white to red, resembling other oral leukoplakias. Its etiology is mainly linked to high-risk HPV types, primarily HPV 16. The pathogenesis is not fully understood but is considered similar to HPV-associated cervical dysplasia. Invasive squamous cell carcinoma develops in approximately 5–15% of cases (72).

## **11. Familial Cancer Syndromes**

Some case reports suggest that oral cancer can occur in families (73). Several genetic syndromes are linked to increased susceptibility to oral cancer. Individuals with conditions such as dyskeratosis congenita (DC), Fanconi anemia (FA), xeroderma pigmentosum, Bloom syndrome, ataxia-telangiectasia, Li-Fraumeni and Cowden syndromes have a higher risk of oral cancer due to genomic instability (43).

Dyskeratosis congenita (DC) is a rare multisystem genetic disorder affecting the skin, characterized by chromosomal instability and reduced telomerase activity (74). DC usually presents between ages 5 and 12 and is more common in males (75). Zinsser described the classic triad of DC in 1906 (76):

- Reticular atrophy with cutaneous hyperpigmentation
- Nail dystrophy

- Oral leukoplakia

Because of chromosomal instability, DC patients have a markedly increased risk of developing cancers in multiple organ systems. Children with DC have been reported to have a 100-fold higher risk of Acute Myeloid Leukemia. Additionally, the risk of squamous cell carcinoma (SCC) in the head and neck, cervix, and anogenital regions is elevated (77). Leukoplakia in DC progresses to cancer in roughly 30% of cases between ages 10 and 30 (78).

Fanconi Anemia (FA) is a rare autosomal recessive disorder involving defects in DNA repair, especially cross-link repair. FA is associated with congenital physical abnormalities (e.g., skeletal malformations), aplastic anemia, and progressive pancytopenia. Patients with FA are at risk of bone marrow failure, leukemia, and solid tumors, including head and neck cancers (2).

Overall, the risk of head and neck cancers in FA patients is estimated to be 500–700 times higher than in the general population (79), and these cancers often occur at a young age. Young individuals presenting with head and neck SCC should be evaluated for FA. FA patients may also show potentially malignant lesions, such as leukoplakia (80).

## **Conclusion**

Potential malignant oral disorders include various lesions and conditions. Sometimes these lesions may be too vague or small to be noticed. During routine examinations, the oral mucosa should be thoroughly examined, and in the presence of suspicious lesions, potential malignant disorders of the mouth and their differential diagnoses should be considered. These patients should be informed about risk factors. Observed lesions should be monitored and biopsied when necessary.

## REFERENCES

- El-Naggar AK, Chan JKC, Rubin Grandis J, Takata T, Slootweg PJ, editors. WHO classification of head and neck tumours. 4th ed. Lyon: IARC Press; 2017.
- Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, Bagan JV, González-Moles MA, Kerr AR, et al. Oral potentially malignant disorders: a consensus report from an international seminar on nomenclature and classification. *Oral Dis.* 2021;27(8):1862-80.
- Muller S, Tilakaratne WM. Update from the 5th edition of the WHO classification of head and neck tumors: tumors of the oral cavity and mobile tongue. *Head Neck Pathol.* 2022;16(1):54-62.
- Lodi G, Franchini R, Warnakulasuriya S, Varoni EM, Sardella A, Kerr AR, et al. Interventions for treating oral leukoplakia to prevent oral cancer. *Cochrane Database Syst Rev.* 2016;(7):CD001829.
- Amagasa T, Yamashiro M, Uzawa N. Oral premalignant lesions: from a clinical perspective. *Int J Clin Oncol.* 2011;16(1):5-14.
- Mello FW, Miguel AFP, Dutra KL, Porporatti AL, Warnakulasuriya S, Guerra ENS, et al. Prevalence of oral potentially malignant disorders: a systematic review and meta-analysis. *J Oral Pathol Med.* 2018;47(7):633-40.
- Muller S, Tilakaratne WM. Oral potentially malignant disorders. In: WHO Classification of Head and Neck Tumours. 5th ed. Lyon: IARC; 2022.
- van der Waal I. Oral leukoplakia: proposal for simplification and consistency of clinical classification. *Med Oral Patol Oral Cir Bucal.* 2019;24(6):e799-803.
- Awadallah M, Idle M, Patel K, Kademani D. Management update of potentially premalignant oral epithelial lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2018;125(6):628-36.
- Sundberg J, Korytowska M, Holmberg E, Bratel J, Wallström M, Kjellström E, et al. Recurrence rates after surgical removal of oral leukoplakia. *PLoS One.* 2019;14(12):e0225682.
- van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa. *Oral Oncol.* 2009;45(4-5):317-23.
- Rock LD, Rosin MP, Zhang L, Chan B, Shariati B, Laronde DM. Characterization of epithelial oral dysplasia in non-smokers. *Oral Oncol.* 2018;78:119-25.

- Murrah VA, Batsakis JG. Proliferative verrucous leukoplakia and verrucous hyperplasia. *Ann Otol Rhinol Laryngol.* 1994;103(8):660-3.
- Abadie WM, Partington EJ, Fowler CB, Schmalbach CE. Optimal management of proliferative verrucous leukoplakia. *Otolaryngol Head Neck Surg.* 2015;153(4):504-11.
- Bagan JV, Jimenez Y, Sanchis JM, Poveda R, Milian MA, Murillo J, et al. Proliferative verrucous leukoplakia. *J Oral Pathol Med.* 2003;32(7):379-82.
- Palefsky JM, Silverman S Jr, Abdel-Salaam M, Daniels TE, Greenspan JS. HPV-16 and proliferative verrucous leukoplakia. *J Oral Pathol Med.* 1995;24(5):193-7.
- Began J, Jiménez Y, Murillo J, Poveda R, Diaz JM, Sanchis J, et al. Epstein-Barr virus in proliferative verrucous leukoplakia. *Med Oral Patol Oral Cir Bucal.* 2008;13(2):E110-3.
- Iocca O, Sollecito TP, Alawi F, Weinstein GS, Newman JG, De Virgilio A, et al. Malignant transformation rate by subtype. *Head Neck.* 2020;42(3):539-55.
- Staines K, Rogers H. Oral leukoplakia and proliferative verrucous leukoplakia. *Br Dent J.* 2017;223(9):655-61.
- Morton TH, Cabay RJ, Epstein JB. Proliferative verrucous leukoplakia progression. *J Oral Pathol Med.* 2007;36(5):315-8.
- Reichart PA, Philipsen HP. Oral erythroplakia—a review. *Oral Oncol.* 2005;41(6):551-61.
- Pindborg JJ, Reichart PA, Smith CJ, van der Waal I. *Histological typing of cancer and precancer of the oral mucosa.* 2nd ed. Berlin: Springer; 1997.
- Reichart PA. Erythroplakia—clinical markers. *Oral Oncol Suppl.* 2005;1(1):47.
- Lorenzo-Pouso AI, Lafuente-Ibáñez de Mendoza I, Pérez-Sayáns M, Pérez-Jardón A, Chamorro-Petronacci CM, Blanco-Carrión A, et al. Oral erythroplakia systematic review. *J Oral Pathol Med.* 2022;51(7):585-93.
- Warnakulasuriya S. Clinical features of oral potentially malignant disorders. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2018;125(6):582-90.
- Villa A, Villa C, Abati S. Oral erythroplakia update. *Aust Dent J.* 2011;56(3):253-6.
- Abidullah M, Kumar GK, Mawardi H, Alyami Y, Arif SM, Qureshi Y. Oral submucous fibrosis correlation study. *J Evol Med Dent Sci.* 2018;7:2227-30.

- More CB, Rao NR. Proposed clinical definition for oral submucous fibrosis. *J Oral Biol Craniofac Res.* 2019;9(4):311-4.
- Kujan O, Mello FW, Warnakulasuriya S. Malignant transformation of oral submucous fibrosis. *Oral Dis.* 2021;27(8):1936-46.
- Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation in oral submucous fibrosis. *Community Dent Oral Epidemiol.* 1985;13(6):340-1.
- Rao NR, Villa A, More CB, Jayasinghe RD, Kerr AR, Johnson NW. Oral submucous fibrosis narrative review. *J Otolaryngol Head Neck Surg.* 2020;49(1):3.
- Wollina U, Verma SB, Ali FM, Patil K. Oral submucous fibrosis update. *Clin Cosmet Investig Dermatol.* 2015;8:193-204.
- Torrente Castells E, Barbosa de Figueiredo RP, Berini Aytés L, Gay Escoda C. Clinical features of oral lichen planus. *Med Oral Patol Oral Cir Bucal.* 2010;15(5):e685-90.
- van der Meij EH, Mast H, van der Waal I. Premalignant character of oral lichen planus. *Oral Oncol.* 2007;43(8):742-8.
- Oliveira Alves MG, Almeida JD, Balducci I, Cabral LAG. Oral lichen planus retrospective study. *BMC Res Notes.* 2010;3:157.
- Pogrel MA, Weldon LL. Carcinoma arising in erosive lichen planus. *Oral Surg Oral Med Oral Pathol.* 1983;55(1):62-6.
- Scully C, El-Kom M. Lichen planus pathogenesis. *J Oral Pathol Med.* 1985;14(6):431-58.
- Strassburg M, Knolle G. Diseases of the oral mucosa. *Quintessence Int.* 1974;5(2):67-72.
- Regezi JA, Sciubba JJ, Jordan RCK. *Oral pathology: clinical pathologic correlations.* 7th ed. St Louis: Elsevier; 2016.
- Bricker SL. Oral lichen planus: a review. *Semin Dermatol.* 1994;13(2):87-92.
- Marder MZ, Deesen KC. Transformation of oral lichen planus to SCC. *J Am Dent Assoc.* 1982;105(1):55-60.
- Gorouhi F, Davari P, Fazel N. Cutaneous and mucosal lichen planus review. *Sci World J.* 2014;2014:742826.
- Albuquerque R, Brailo V, Carey B, Diniz-Freitas M, Fricain JC, Lodi G, et al. Oral potentially malignant disorders: healthcare professional training. 2022.

- Lavanya N, Jayanthi P, Rao UK, Ranganathan K. Oral lichen planus update. *J Oral Maxillofac Pathol.* 2011;15(2):127-32.
- Al-Hashimi I, Schifter M, Lockhart PB, Wray D, Brennan M, Migliorati CA, et al. Diagnostic and therapeutic considerations. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007;103:S25.e1-12.
- Epstein JB, Wan LS, Gorsky M, Zhang L. Oral lichen planus malignant potential. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2003;96(1):32-7.
- Elhadad MA, Gaweesh Y. Hawley retainer and lichenoid reaction. *BMC Oral Health.* 2019;19:1-5.
- Carrozzo M, Porter S, Mercadante V, Fedele S. Oral lichen planus spectrum. *Periodontol* 2000. 2019;80(1):105-25.
- Baccaglini L, Thongprasom K, Carrozzo M, Bigby M. Urban legends: lichen planus. *Oral Dis.* 2013;19(2):128-43.
- McCartan BE, McCreary CE. Oral lichenoid drug eruptions. *Oral Dis.* 1997;3(2):58-63.
- Van den Haute V, Antoine JL, Lachapelle JM. Lichenoid drug eruption vs idiopathic LP. *Dermatology.* 1989;179(1):10-3.
- Kuten-Shorrer M, Woo SB, Treister NS. Oral graft-versus-host disease. *Dent Clin North Am.* 2014;58(2):351-68.
- Dharmavaram AT, Nallakunta R, Reddy SR, Chennoju SK. Smoking observational study. *J Clin Diagn Res.* 2016;10(4):ZC94-8.
- Alvarez Gómez GJ, Alvarez Martínez E, Jiménez Gómez R, Mosquera Silva Y, Gaviria Núñez AM, Garcés Agudelo A, et al. Reverse smokers and oral mucosa changes. *Med Oral Patol Oral Cir Bucal.* 2008;13:E1-8.
- Bharath TS, Kumar NGR, Nagaraja A, Saraswathi TR, Babu GS, Raju PR. Palatal changes in reverse smokers. *J Oral Maxillofac Pathol.* 2015;19(2):182-7.
- Stannard JN, Kahlenberg JM. Cutaneous lupus erythematosus updates. *Curr Opin Rheumatol.* 2016;28(5):453-9.
- Szczęch J, Samotij D, Werth VP, Reich A. Trigger factors of CLE. *Lupus.* 2017;26(8):791-807.
- Nico MMS, Vilela MAC, Rivitti EA, Lourenço SV. Oral lesions in lupus erythematosus. *Eur J Dermatol.* 2008;18(4):376-81.

- Ranginwala AM, Chalishazar MM, Panja P, Buddhdev KP, Kale HM. Oral discoid lupus erythematosus. *J Oral Maxillofac Pathol.* 2012;16(3):368-73.
- Shafer WG, Hine MK, Levy BM. *A textbook of oral pathology.* 4th ed. Philadelphia: Saunders; 1983.
- Kranti K, Seshan H, Juliet J. Discoid lupus erythematosus of gingiva. *J Indian Soc Periodontol.* 2012;16(1):126-8.
- Arvanitidou IE, Nikitakis NG, Georgaki M, Papadogeorgakis N, Tzioufas AG, Sklavounou A. Multiple primary SCC in discoid lupus. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2018;125(2):e22-30.
- Makita E, Akasaka E, Sakuraba Y, Korekawa A, Aizu T, Kaneko T, et al. SCC arising from discoid lupus. *Eur J Dermatol.* 2016;26(4):395-6.
- Naik V, Prakash S. Oral discoid lupus erythematosus case report. *Indian J Dent Adv.* 2018;9(4):221-5.
- Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. NIH consensus criteria for chronic GVHD. *Biol Blood Marrow Transplant.* 2005;11(12):945-56.
- Mays JW, Fassil H, Edwards DA, Pavletic SZ, Bassim CW. Oral chronic GVHD. *Oral Dis.* 2013;19(4):327-46.
- Lee SJ, Wolff D, Kitko C, Koreth J, Inamoto Y, Jagasia M, et al. Measuring therapeutic response in chronic GVHD. *Biol Blood Marrow Transplant.* 2015;21(6):984-99.
- Ogawa K, Langlais RP, McDavid WD, Noujeim M, Seki K, Okano T, et al. New panoramic radiographic system. *Dentomaxillofac Radiol.* 2010;39(1):47-53.
- Lerman MA, Almazrooa S, Lindeman NI, Hall DL, Villa A, Woo SB. HPV-16 in oral epithelial dysplasia. *Mod Pathol.* 2017;30(12):1646-54.
- Woo SB, Cashman EC, Lerman MA. HPV-associated oral intraepithelial neoplasia. *Mod Pathol.* 2013;26(10):1288-97.
- Alsabbagh A, Robins TL, Harriman A, Jackson-Boeters L, Darling MR, Khan ZA, et al. Surrogate markers for high-risk HPV. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2020;129(3):246-59.e1.
- Hendawi N, Niklander S, Allsobrook O, Khurram SA, Bolt R, Doorbar J, et al. HPV infection in dysplastic oral mucosa. *Histopathology.* 2020;76(4):592-602.

- Ankathil R, Mathew A, Joseph F, Nair MK. Is oral cancer susceptibility inherited? *Eur J Cancer B Oral Oncol.* 1996;32(1):63-7.
- Dokal I. Dyskeratosis congenita. *Hematology Am Soc Hematol Educ Program.* 2011;2011:480-6.
- Sinha S, Trivedi V, Krishna A, Rao N. Dyskeratosis congenita case report. *Oman Med J.* 2013;28(4):281-3.
- Zinsser F. Reticular cutaneous atrophy with nail dystrophy and oral leukoplakia. *Ikonogr Dermatol.* 1910;5:219-23.
- Alter BP, Giri N, Savage SA, Peters JA, Loud JT, Leathwood L, et al. Malignancies in inherited bone marrow failure syndromes. *Br J Haematol.* 2010;150(2):179-88.
- Karunakaran A, Ravindran R, Arshad M, Ram MK, Laxmi MS. Dyskeratosis congenita report. *Case Rep Dent.* 2013;2013:845125.
- Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, et al. High incidence of head and neck SCC in Fanconi anemia. *Arch Otolaryngol Head Neck Surg.* 2003;129(1):106-12.
- Amenábar JM, Torres-Pereira CC, Tang KD, Punyadeera C. Oral cancer in Fanconi anemia update. *Cancer.* 2019;125(22):3936-46.



# CHAPTER 4

---

## ACCOUNTABILITY IN THE UNITED KINGDOM PUBLIC HEALTH SYSTEM

*Kenan YURTSAL<sup>1</sup>*

---

<sup>1</sup> Dr.Öğr.Üyesi, Sivas Cumhuriyet Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu, Sivas <https://orcid.org/0000-0002-9330-258X>

## **Introduction**

Accountability is a central principle of governance in modern public institutions, particularly within healthcare systems where policy decisions directly affect human well-being and societal welfare. In the context of health systems, accountability encompasses the obligation of governments, institutions, and professionals to justify their actions, demonstrate responsible stewardship of resources, and ensure that services meet the needs of the populations they serve. As healthcare systems grow more complex and resource-intensive, the need for robust accountability mechanisms becomes increasingly important for maintaining public trust, improving service quality, and promoting equitable access to care (Bovens, 2007; Brinkerhoff, 2004).

The English healthcare system, organized primarily through the National Health Service (NHS), provides an important case study for examining the dynamics of accountability in a large publicly funded health system. Established in 1948, the NHS was founded on principles of universality, equity, and accessibility, with healthcare services provided largely free at the point of delivery and financed through general taxation. Over the decades, the NHS has evolved into one of the largest and most complex public healthcare systems in the world, serving more than 55 million people in England and employing more than one million staff members (Appleby et al., 2020; Greer et al., 2016).

This scale and complexity require sophisticated governance arrangements to ensure that healthcare organizations remain accountable for their decisions and performance. Accountability in the NHS operates through a multi-layered framework involving political oversight, administrative regulation, professional governance, and public participation. Government bodies set national policy and allocate funding, independent regulators monitor quality and safety, healthcare providers report performance metrics, and patients participate in feedback and oversight processes. Together, these mechanisms form a comprehensive accountability system designed to balance organizational autonomy with transparency and oversight (Bevan & Hood, 2006; Smith et al., 2019).

However, despite the presence of extensive accountability mechanisms, the NHS continues to face significant challenges. Persistent regional inequalities in healthcare access, financial pressures resulting from demographic change and technological advancements, and increasing demand for services all place strain on existing governance structures. Furthermore, critics have

questioned whether performance targets and regulatory frameworks always lead to genuine improvements in quality or whether they sometimes encourage bureaucratic compliance rather than meaningful organizational learning (Hood, 2010; Timmins, 2019).

This chapter examines the concept and practice of accountability within the English health system. It begins by outlining the conceptual foundations of accountability in healthcare governance and discussing the different forms that accountability can take. It then analyzes the institutional architecture of the NHS and the mechanisms through which accountability is implemented, including vertical and horizontal oversight structures, professional regulation, and performance measurement systems. The chapter also explores the roles of transparency and public engagement in strengthening accountability and examines key challenges facing the NHS, including inequalities and financial sustainability. Finally, the chapter considers opportunities for improving accountability mechanisms in the future.

## **Conceptual Framework of Accountability in Healthcare**

### **Defining Accountability**

Accountability is a fundamental concept in public administration and governance, referring to the obligation of individuals or organizations to explain and justify their actions to those with the authority to evaluate and potentially sanction them. Bovens (2007) describes accountability as a relationship between an actor and a forum in which the actor must provide information about their conduct, explain and justify their actions, and accept consequences based on the evaluation of their performance.

Similarly, Mulgan (2000) defines accountability as the requirement for public officials to report on their actions and accept responsibility for them. Roberts (2008) further emphasizes the ethical dimension of accountability, arguing that it involves not only compliance with formal rules but also responsiveness to social expectations and professional standards.

Within healthcare systems, accountability takes on additional significance because decisions made by healthcare providers and policymakers can directly affect patient safety, health outcomes, and public trust. As a result, accountability frameworks in healthcare must address

a wide range of responsibilities, including financial management, clinical quality, patient safety, and equitable access to services (Brinkerhoff, 2004).

Healthcare accountability is therefore inherently multidimensional. It requires mechanisms that ensure organizations use public resources responsibly, adhere to professional and ethical standards, and deliver services that meet the needs of patients and communities.

### **Dimensions of Healthcare Accountability**

Scholars often categorize healthcare accountability into several distinct dimensions, including political, administrative, professional, and social accountability (Saltman & Ferroussier-Davis, 2000; Brinkerhoff, 2004).

Political accountability refers to the responsibility of elected officials and government institutions to citizens. In democratic systems, this form of accountability is exercised through elections, legislative oversight, and public reporting. Governments are expected to justify policy decisions, demonstrate effective management of public funds, and respond to public concerns about healthcare services.

Administrative accountability focuses on the internal governance of public organizations. It involves mechanisms that ensure institutions operate efficiently, comply with regulations, and achieve policy objectives. Administrative accountability often relies on hierarchical oversight, performance management systems, and audit processes.

Professional accountability is particularly important in healthcare because many decisions are made by highly trained professionals whose expertise gives them significant autonomy. Professional regulatory bodies establish standards of practice, oversee licensing and certification, and investigate complaints related to professional misconduct (Freidson, 2001).

Finally, social accountability refers to the involvement of citizens, patients, and communities in monitoring healthcare services and influencing policy decisions. Public participation mechanisms such as patient surveys, advisory councils, and complaint systems enable individuals to hold healthcare institutions accountable for service quality and responsiveness (Brinkerhoff, 2004).

These different forms of accountability interact with one another to create a comprehensive governance framework within health systems.

## **Structural Features of the English Health System**

### **Historical Development of the NHS**

The establishment of the NHS represented a major transformation in British social policy. Prior to 1948, healthcare in the United Kingdom was delivered through a fragmented system of voluntary hospitals, municipal services, and private providers. Access to care was often limited by income, employment status, or geographic location.

Following the recommendations of the Beveridge Report in 1942, the British government introduced the NHS as part of a broader welfare state initiative aimed at addressing social inequalities and improving population health. The NHS was founded on three core principles: healthcare should be universal, comprehensive, and free at the point of use (Greer & Lillvis, 2021).

Over time, the NHS has undergone numerous reforms aimed at improving efficiency, quality, and accountability. In the 1990s, the introduction of the internal market created a separation between purchasers and providers of healthcare services. Later reforms introduced performance targets, expanded regulatory oversight, and promoted greater patient choice (Bevan & Hood, 2006; Hood, 2010).

More recent reforms have focused on integrating health and social care services through the development of Integrated Care Systems (ICSs). These regional partnerships aim to improve coordination between healthcare providers and local authorities while maintaining accountability for service delivery (NHS England, 2022).

### **Institutional Architecture**

The governance structure of the NHS involves multiple organizations with distinct roles and responsibilities. At the national level, the Department of Health and Social Care (DHSC) sets policy priorities and allocates funding. The Secretary of State for Health and Social Care is accountable to Parliament for the overall performance of the NHS.

NHS England is responsible for implementing national health policies, commissioning specialized services, and overseeing the performance of healthcare providers. It plays a key role in managing the operational aspects of the health system and ensuring that services meet national standards (Appleby et al., 2020).

Integrated Care Systems operate at the regional level and are responsible for coordinating healthcare planning across hospitals, primary care providers, community services, and local authorities. These systems aim to improve collaboration and address population health needs more effectively.

Independent regulatory bodies also play an essential role in the accountability framework. The Care Quality Commission (CQC) inspects healthcare providers and evaluates the quality and safety of services, while the National Institute for Health and Care Excellence (NICE) develops evidence-based clinical guidelines and conducts health technology assessments (Legido-Quigley et al., 2018).

Together, these institutions create a complex governance structure that supports multiple forms of accountability.

## **Accountability Mechanisms in Practice**

### **Vertical Accountability**

Vertical accountability refers to the mechanisms through which public institutions are held accountable to citizens and political authorities. In the NHS, vertical accountability is primarily exercised through parliamentary oversight and public reporting.

Parliamentary committees regularly examine healthcare policies and scrutinize government spending on health services. Ministers and senior officials are required to explain policy decisions and provide evidence regarding the performance of the NHS.

Public transparency initiatives also support vertical accountability. NHS organizations publish detailed information about service performance, including waiting times, clinical outcomes, and patient satisfaction. These data allow citizens, journalists, and researchers to evaluate the effectiveness of healthcare services (NHS England, 2021).

### **Horizontal Accountability**

Horizontal accountability refers to oversight relationships between institutions operating at similar levels of authority. In the English health system, regulatory agencies perform this function by monitoring healthcare providers and enforcing quality standards.

The Care Quality Commission conducts inspections of hospitals, clinics, and community health services to evaluate their performance. Providers are rated based on criteria such as safety, effectiveness, and responsiveness to patient needs.

NICE contributes to horizontal accountability by developing clinical guidelines that establish evidence-based standards of care. These guidelines help ensure consistency across the NHS and support cost-effective decision-making (Legido-Quigley et al., 2018).

### **Professional Accountability**

Professional accountability is maintained through regulatory bodies such as the General Medical Council (GMC) and the Nursing and Midwifery Council (NMC). These organizations regulate professional conduct, maintain registers of qualified practitioners, and investigate complaints about clinical practice.

Professional accountability plays a crucial role in healthcare because clinical decision-making often requires specialized expertise and independent judgment. By enforcing professional standards, regulatory bodies help protect patients and maintain trust in the healthcare system (Freidson, 2001).

### **Performance Measurement and Quality Standards**

Performance measurement has become a central component of accountability in the NHS. Indicators are used to monitor a wide range of outcomes, including waiting times, treatment effectiveness, financial performance, and patient satisfaction.

Targets such as the 18-week referral-to-treatment standard and the four-hour emergency department waiting time target provide measurable benchmarks for evaluating healthcare performance (Bevan & Hood, 2006).

However, the use of performance targets has also generated debate. Some scholars argue that strict targets can encourage organizations to focus on meeting numerical goals rather than improving underlying service quality. This phenomenon, often referred to as “gaming,” can lead to unintended consequences such as data manipulation or the prioritization of easily measurable outcomes over more complex aspects of care (Hood, 2010).

Despite these concerns, performance measurement remains an important tool for promoting accountability and transparency within healthcare systems.

### **Transparency and Public Engagement**

Transparency and public participation are increasingly recognized as essential components of healthcare accountability.

The NHS has implemented extensive open data initiatives that make information about service performance and financial expenditures publicly available. These datasets allow independent researchers, journalists, and civil society organizations to analyze healthcare performance and identify areas requiring improvement (Timmins, 2019).

Public engagement mechanisms also play an important role. Patient experience surveys, community advisory boards, and complaint procedures provide opportunities for individuals to share their perspectives and influence healthcare policy.

These mechanisms help ensure that healthcare institutions remain responsive to the needs and expectations of the communities they serve.

### **Challenges and Accountability**

Despite the presence of comprehensive accountability mechanisms, the English health system faces several significant challenges.

Regional inequalities remain a persistent issue, with variations in healthcare access and outcomes across different parts of the country (Marmot et al., 2020).

Socioeconomic disparities also affect health outcomes, highlighting the need for accountability frameworks that prioritize equity alongside efficiency.

In addition, financial pressures resulting from population aging, technological innovation, and rising demand for services place strain on healthcare organizations and complicate accountability relationships (Appleby et al., 2020).

### **Opportunities for Strengthening Accountability**

Future reforms in the English health system may focus on improving integration between healthcare providers, expanding digital transparency tools, and strengthening community participation in governance.

Emerging technologies such as electronic health records and data analytics offer new opportunities for monitoring performance and improving transparency.

At the same time, policymakers must ensure that accountability mechanisms promote meaningful improvements in healthcare quality rather than simply encouraging bureaucratic compliance.

### **Conclusion**

Accountability is a central component of governance within the English health system. Through a combination of political oversight, regulatory monitoring, professional governance, and public participation, the NHS has developed a complex framework designed to ensure transparency, quality, and responsiveness.

However, maintaining effective accountability requires continuous adaptation in response to evolving challenges. Financial pressures, demographic change, and persistent health inequalities highlight the need for innovative governance strategies that balance efficiency with equity.

Strengthening accountability mechanisms will be essential for ensuring that the NHS continues to deliver high-quality healthcare while maintaining public trust in the years ahead.

## References

- Appleby, J., Thompson, J., & Jabbal, J. (2020). *The NHS in numbers*. The King's Fund.
- Bevan, G., & Hood, C. (2006). What's measured is what matters. *Public Administration*, 84(3), 517–538.
- Bevan, G., & Wilson, D. (2013). Does “naming and shaming” work? *Public Administration*, 91(2), 333–350.
- Bovens, M. (2007). Analysing and assessing accountability. *European Law Journal*, 13(4), 447–468.
- Brinkerhoff, D. (2004). Accountability and health systems. *Health Policy and Planning*, 19(6), 371–379.
- Checkland, K., Coleman, A., Harrison, S., & McDermott, I. (2016). National policy, local problems. *Health Policy*, 120(10), 1170–1177.
- Freidson, E. (2001). *Professionalism: The third logic*. University of Chicago Press.
- Greer, S., Wismar, M., & Figueras, J. (2016). *Strengthening health system governance*. Open University Press.
- Greer, S., & Lillvis, D. (2021). Beyond leadership in health policy. *Public Administration Review*.
- Hood, C. (2010). *The blame game*. Princeton University Press.
- Legido-Quigley, H., McKee, M., & Nolte, E. (2018). Assuring the quality of health care in the EU. European Observatory.
- Marmot, M., Allen, J., Boyce, T., Goldblatt, P., & Morrison, J. (2020). *Health equity in England*. Institute of Health Equity.
- Mulgan, R. (2000). Accountability. *Public Administration*, 78(3), 555–573.
- NHS England. (2021). *NHS performance statistics*.

NHS England. (2022). *Integrated care systems: Design framework*.

NICE. (2022). *Developing NICE guidelines*.

Roberts, J. (2008). Limits of transparency. *Accounting, Organizations and Society*, 34(8), 957–970.

Saltman, R., & Ferroussier-Davis, O. (2000). Stewardship in health policy. *Bulletin of the WHO*, 78(6), 732–739.

Smith, P., Mossialos, E., Papanicolas, I., & Leatherman, S. (2019). *Performance measurement for health system improvement*. Cambridge University Press.

Timmins, N. (2019). *The five giants*. HarperCollins.

# CHAPTER 5

---

## BIOELECTRICAL SIGNALING IN CANCER: A BIOPHYSICAL PERSPECTIVE

*Cagatay Han TURKSEVEN<sup>1</sup>*

---

<sup>1</sup> Dr. Mersin University Medicine of Faculty, Department of Biophysics, Mersin, Turkey. E-Mail: cagatayhanturkseven1923@gmail.com ; turkseven@mersin.edu.tr ORCID: <https://orcid.org/0000-0002-0584-0661>

## Introduction

Cancer has traditionally been conceptualized as a genetic and biochemical disease; however, emerging evidence increasingly supports the notion that cancer is also fundamentally a biophysical disorder characterized by alterations in electrical, mechanical, and electrochemical properties of cells and tissues (Maqoud et al., 2023). Among these properties, bioelectrical signaling—primarily governed by membrane potential ( $V_m$ ), ion fluxes, and channel activity—has gained significant attention as a central regulator of tumor initiation, progression, and therapeutic response (Doldi et al., 2024). Membrane potential, defined as the voltage difference across the plasma membrane, integrates ionic gradients and channel activity to orchestrate key cellular processes including proliferation, differentiation, migration, and apoptosis (Maqoud et al., 2023). In cancer cells,  $V_m$  is typically depolarized relative to normal cells, a state that has been consistently associated with enhanced proliferative capacity and resistance to programmed cell death (Maqoud et al., 2023). This depolarized state is not merely a passive consequence of oncogenic transformation but is associated with alterations in intracellular signaling pathways, including PI3K/AKT, MAPK, and STAT cascades (Zhou et al., 2025). Importantly, bioelectrical signaling extends beyond cancer cells themselves and encompasses the entire tumor microenvironment (TME), including immune cells, fibroblasts, and endothelial cells (Zhang et al., 2025). Cancer-associated fibroblasts (CAFs), for example, exhibit altered electrophysiological properties characterized by increased  $Na^+$ ,  $Ca^{2+}$ , and  $K^+$  conductance, which enhances their contractility, extracellular matrix remodeling, and tumor-promoting functions (Doldi et al., 2024). Similarly, tumor-associated neutrophils (TANs) and macrophages contribute to tumor progression through osmotic and signaling mechanisms involving channels such as aquaporins (Xu et al., 2025). Mechanical and microenvironmental factors influence intracellular signaling and tumor progression through integrated biochemical and structural pathways (Zhang et al., 2025). At the intracellular level, ion fluxes—particularly  $Ca^{2+}$  signaling—is closely associated with the regulation of cell fate decisions, including proliferation, apoptosis, ferroptosis, and emerging forms of cell death such as calcicoptosis (Yang et al., 2025). Disruption of  $Ca^{2+}$  homeostasis can induce mitochondrial dysfunction, endoplasmic reticulum stress, and oxidative damage, ultimately leading to cell death or adaptation depending on the context (Fernandez-Alarcon et al., 2025). Recent advances in nanotechnology and bioengineering have enabled targeted manipulation of these bioelectric processes, allowing simultaneous induction of multiple death pathways such as ferroptosis and pyroptosis (Wu et al., 2025). These findings underscore the concept that cancer cells can be reprogrammed

through controlled perturbation of their electrical and ionic states. In addition to intracellular signaling, immune responses within the tumor microenvironment are modulated by cytokine-mediated signaling pathways (Garcia-Lacarte et al., 2025). For instance, tumor-derived IL-10 signaling plays a critical role in shaping the lymphoma microenvironment and modulating response to immunotherapy (Garcia-Lacarte et al., 2025). Furthermore, bioelectric interventions such as irreversible electroporation can reshape the tumor microenvironment by inducing immunogenic cell death and promoting pro-inflammatory immune responses (Imran et al., 2024). Collectively, these studies suggest that cancer should be redefined as a “multicellular channelopathy,” in which dysregulated ion channel activity and membrane potential dynamics orchestrate tumor behavior across multiple cellular compartments (Maqoud et al., 2023). This perspective provides a unifying framework that integrates electrical, biochemical, and mechanical aspects of tumor biology, opening new avenues for diagnosis and therapy (Doldi et al., 2024). Therefore, understanding the biophysical basis of bioelectrical signaling in cancer is essential for the development of next-generation therapeutic strategies targeting ion channels, membrane potential, and electrophysiological networks within tumors.

### **Membrane Potential and Cancer Cells**

Membrane potential ( $V_m$ ) represents a dynamic and integrative biophysical parameter that reflects the collective activity of ion channels, transporters, and pumps, thereby orchestrating cancer cell behavior at multiple levels (Maqoud et al., 2023). In malignant cells,  $V_m$  is characteristically depolarized compared to normal counterparts, a state that has been associated with enhanced proliferative capacity and reduced differentiation (Maqoud et al., 2023). This depolarized electrical state is associated with changes in signaling pathways related to cell cycle regulation by modulating intracellular signaling pathways and ion fluxes (Zhou et al., 2025). Furthermore, depolarization alters the electrochemical driving force for  $Ca^{2+}$  entry, thereby reshaping intracellular calcium signaling networks that regulate gene expression and metabolic reprogramming (Yang et al., 2025). Importantly,  $V_m$  is not a static parameter but undergoes spatiotemporal fluctuations that may encode regulatory information governing tumor growth and organization (Maqoud et al., 2023). These electrical gradients can propagate across cell populations through gap junctions, allowing coordinated behavior within tumor tissues (Zhang et al., 2025). In addition, cancer-associated fibroblasts exhibit altered  $V_m$  and increased electrical conductance, which contribute to extracellular matrix remodeling and tumor stiffness (Doldi et al., 2024). This suggests that bioelectrical dysregulation may extend beyond tumor cells but represents a multicellular phenomenon involving the tumor microenvironment (Xu et

al., 2025). Moreover, microenvironmental and structural factors influence tumor progression through integrated signaling networks and extracellular matrix interactions (Zhang et al., 2025). These findings collectively support the concept that membrane potential functions as a key integrator of biochemical and biophysical signals in cancer biology.

### **Ion Channels ( $\text{Ca}^{2+}$ , $\text{K}^+$ , $\text{Na}^+$ )**

Ion channels are central regulators of bioelectrical signaling and contribute to tumor progression by controlling ion fluxes and membrane excitability (Maqoud et al., 2023). Among these,  $\text{Ca}^{2+}$  channels are closely involved in intracellular signaling cascades associated with proliferation, apoptosis, and metabolic adaptation (Yang et al., 2025). Sustained  $\text{Ca}^{2+}$  influx has been associated with activation of signaling pathways such as PI3K/AKT and MAPK, which are known to promote tumor cell survival and growth (Zhou et al., 2025). Potassium channels, particularly ATP-sensitive  $\text{K}^+$  channels, link cellular metabolic status to electrical activity and contribute to the maintenance of depolarized membrane states observed in cancer cells (Maqoud et al., 2023). Alterations in  $\text{K}^+$  channel activity influence cell cycle regulation by modulating membrane potential and intracellular ionic balance (Maqoud et al., 2023). Voltage-gated sodium channels have been associated with increased cellular motility and invasiveness through their effects on cytoskeletal dynamics and extracellular matrix interactions (Doldi et al., 2024). In addition, tumor progression is also influenced by interactions within the tumor microenvironment, including macrophage polarization dynamics that regulate tumor-associated immune responses (Qiu et al., 2025). Similarly, aquaporins such as AQP9 contribute to tumor progression by modulating osmotic balance and activating signaling pathways such as STAT3 (Xu et al., 2025). These findings indicate that ion channels function not only as passive conduits for ion transport but also as regulators of complex signaling networks involved in cancer progression.

### **Electrophysiological Mechanisms of Proliferation**

Electrophysiological signaling is associated with cancer cell proliferation through modulation of intracellular signaling pathways and gene expression programs (Zhou et al., 2025). Changes in membrane potential and ion fluxes are linked to key oncogenic pathways such as PI3K/AKT and MAPK, which regulate cell cycle progression (Zhou et al., 2025). Calcium-dependent signaling further modulates cyclin expression and checkpoint control, thereby influencing proliferative capacity (Yang et al., 2025). In addition to intracellular signaling, tumor proliferation is influenced by interactions with the tumor microenvironment, including immune and stromal components (Mousset et al., 2023). Neutrophil extracellular traps have been shown

to promote tumor growth and therapy resistance through activation of TGF- $\beta$  signaling pathways (Mousset et al., 2023). Furthermore, tumor-immune interactions, including CD8<sup>+</sup> T cell-mediated cytotoxicity, contribute to the regulation of tumor progression and therapeutic response (Huang et al., 2024). Tumor progression is also shaped by macrophage polarization dynamics within the microenvironment, which influence inflammatory signaling and tumor growth (Qiu et al., 2025). Overall, electrophysiological mechanisms represent an important regulatory axis through which cancer cells integrate intracellular and microenvironmental signals to sustain proliferation.

### **Apoptosis and Electrical Signals**

Bioelectrical signaling is closely associated with the regulation of programmed cell death through modulation of ion homeostasis and mitochondrial function (Yang et al., 2025). Disruption of ion balance can induce mitochondrial dysfunction, leading to loss of membrane potential, cytochrome c release, and activation of apoptotic pathways (Fernandez-Alarcon et al., 2025). This process highlights the close relationship between electrical signaling and metabolic regulation in cancer cells (Fernandez-Alarcon et al., 2025). Beyond apoptosis, bioelectrical perturbations can induce additional forms of regulated cell death such as ferroptosis and pyroptosis (Wu et al., 2025). Targeted modulation of Ca<sup>2+</sup> signaling has been associated with the induction of synergistic cell death pathways, including calcicoptosis and ferroptosis, thereby enhancing anti-tumor efficacy (Yang et al., 2025). Hyperthermia-induced changes in membrane properties can further promote apoptosis through signaling pathways such as Hippo/YAP (Li et al., 2025). Additionally, immune-mediated mechanisms contribute to tumor cell death through cytokine signaling and microenvironmental regulation (Garcia-Lacarte et al., 2025).

### **Bioelectricity as a Therapeutic Target**

Targeting bioelectrical signaling is considered a promising strategy for cancer therapy by modulating ion channels and membrane potential (Doldi et al., 2024). Pharmacological approaches have shown potential in reprogramming cancer-associated fibroblasts and suppressing tumor progression (Doldi et al., 2024). Physical approaches such as irreversible electroporation induce tumor cell death by disrupting membrane integrity and promoting anti-tumor immune responses (Imran et al., 2024). These interventions may contribute to reshaping the tumor microenvironment by enhancing pro-inflammatory signaling and immune activation (Imran et al., 2024). Advances in nanotechnology enable targeted modulation of bioelectrical and ionic states, improving therapeutic precision and efficacy (Zhang et al., 2025). For example,

ultrasound-activated platforms can induce immunogenic cell death through activation of the cGAS-STING pathway (Wu et al., 2025). Targeting tumor–immune interactions, including macrophage polarization pathways, represents an emerging therapeutic strategy for improving treatment outcomes (Qiu et al., 2025).

## **Conclusions**

Bioelectrical signaling represents an emerging dimension of cancer biology by integrating electrical, chemical, and microenvironmental processes that regulate tumor behavior (Maqoud et al., 2023). The concept of cancer as a multicellular channelopathy highlights the role of ion channels and membrane potential in tumor progression (Doldi et al., 2024). This perspective complements traditional genetic models by incorporating dynamic biophysical processes that influence cell fate and tissue organization (Xu et al., 2025). Recent advances demonstrate that bioelectrical pathways may be targeted through pharmacological, physical, and nanotechnological approaches to enhance therapeutic efficacy (Imran et al., 2024). The integration of bioelectric signaling with intracellular and immune pathways provides new opportunities for precision oncology (Zhou et al., 2025). Future research should focus on quantitative mapping of membrane potential dynamics and the translation of bioelectric therapies into clinical practice.

## References

- Doldi, V., Tortoreto, M., Colecchia, M., Maffezzini, M., Percio, S., Giammello, F., Brandalise, F., Gandellini, P., & Zaffaroni, N. (2024). Repositioning of antiarrhythmics for prostate cancer treatment: A novel strategy to reprogram cancer-associated fibroblasts towards a tumor-suppressive phenotype. *Journal of Experimental & Clinical Cancer Research*, 43, 161. <https://doi.org/10.1186/s13046-024-03081-0>
- Fernandez-Alarcon, J., Cladera, M. A., Rodriguez-Camenforte, N., Sitia, G., Guerra-Rebollo, M., Borros, S., & Fornaguera, C. (2025). Regulation of mitochondrial apoptosis via siRNA-loaded metallo-alginate hydrogels: A localized and synergistic antitumor therapy. *Biomaterials*, 318, 123164. <https://doi.org/10.1016/j.biomaterials.2025.123164>
- Garcia-Lacarte, M., Grijalba, S. C., Melchor, J., Pascual, M., Goñi, E., Clemente-Larramendi, I., Morales-Sánchez, S., Burrell, M. A., Blanco, O., Arnaiz-Leché, A., Berrozpe, B. S., Amann, M., Klein, C., Umaña, P., Canales, M., Martínez-Climent, J. Á., Lasarte, J. J., Sarobe, P., Novo, F. J., & Roa, S. (2025). IL-10 from tumoral B cells modulates the diffuse large B-cell lymphoma microenvironment and response to immunotherapy. *Blood*, 145(23), 2746–2761. <https://doi.org/10.1182/blood.2024025755>
- Huang, H., Pan, Y., Mai, Q., Zhang, C., Du, Q., Liao, Y., Qin, S., Chen, Y., Huang, J., Li, J., Liu, T., Zhou, Y., Yuan, L., Wang, W., Liang, Y., Pan, C. Y., Liu, J., & Yao, S. (2024). Targeting CDCP1 boosts CD8<sup>+</sup> T cell-mediated cytotoxicity in cervical cancer via the JAK/STAT signaling pathway. *Journal for ImmunoTherapy of Cancer*, 12, e009416. <https://doi.org/10.1136/jitc-2024-009416>
- Imran, K. M., Brock, R. M., Beitel-White, N., Powar, M., Orr, K., Aycock, K. N., Alinezhadbalalami, N., Salameh, Z. S., Eversole, P., Tintera, B., Markov Madanick, J., Hendricks-Wenger, A., Coutermarsh-Ott, S., Davalos, R. V., & Allen, I. C. (2024). Irreversible electroporation promotes a pro-inflammatory tumor microenvironment and anti-tumor immunity in a mouse pancreatic cancer model. *Frontiers in immunology*, 15, 1352821. <https://doi.org/10.3389/fimmu.2024.1352821>
- Jeong, H., Koh, J., Kim, S., Song, S. G., Lee, S. H., Jeon, Y., Lee, C. H., Keam, B., Lee, S. H., Chung, D. H., & Jeon, Y. K. (2024). Epithelial–mesenchymal transition induced by tumor cell-intrinsic PD-L1 signaling predicts a poor response to immune checkpoint inhibitors in PD-L1-high lung cancer. *British Journal of Cancer*, 131, 23-36. <https://doi.org/10.1038/s41416-024-02698-4>

- Li, J., Qin, Y., Yang, Y., Chen, H., Li, M., Liu, Y., Liu, B., Shang, J., Zhang, Y., Han, T., Hu, Y., & Ren, F. (2025). Photothermal Hyperthermia Suppresses Liver Tumor Growth Via Hippo Signaling Pathway-Dependent Inhibition of Cell Proliferation and Induction of Apoptosis. *Biological procedures online*, 27(1), 22. <https://doi.org/10.1186/s12575-025-00282-5>
- Maquod, F., Zizzo, N., Attimonelli, M., Tinelli, A., Passantino, G., Antonacci, M., Ranieri, G., & Tricarico, D. (2023). Immunohistochemical, pharmacovigilance, and omics analyses reveal the involvement of ATP-sensitive K<sup>+</sup> channel subunits in cancers: role in drug-disease interactions. *Frontiers in pharmacology*, 14, 1115543. <https://doi.org/10.3389/fphar.2023.1115543>
- Mousset, A., Lecorgne, E., Bourget, I., Lopez, P., Jenovai, K., Cherfils-Vicini, J., Dominici, C., Rios, G., Girard-Riboulleau, C., Liu, B., Spector, D. L., Ehmsen, S., Renault, S., Hego, C., Mechta-Grigoriou, F., Bidard, F. C., Terp, M. G., Egeblad, M., Gaggioli, C., & Albregues, J. (2023). Neutrophil extracellular traps formed during chemotherapy confer treatment resistance via TGF- $\beta$  activation. *Cancer Cell*, 41(4), 757–775. <https://doi.org/10.1016/j.ccell.2023.03.008>
- Qiu, J. G., Sang, P., Zhou, F. M., Zhang, X. Q., Wang, W., Qian, Y. C., Zhang, Y., Wang, L., Liu, L. Z., Xue, Y. Q., Zhao, X., Shan, Q., Huang, P., Zheng, S. G., & Jiang, B. H. (2025). Novel role of MKRN2 in regulating tumor growth through host microenvironment and macrophage M1 to M2 switch. *Cancer letters*, 633, 218035. Advance online publication. <https://doi.org/10.1016/j.canlet.2025.218035>
- Xu, W., Zhu, G., Sheng, Y., Zhang, W., Wang, S., & Wu, Q. (2025). Tumor-Associated Neutrophils Regulate Breast Cancer Progression Through the AQP9/STAT3 Signaling Pathway. *Cancer science*, 116(9), 2374–2387. <https://doi.org/10.1111/cas.70121>
- Yang, L., Wang, K., Dong, J., Zhang, X., Liu, X., Cui, J., Liu, J., Zhou, M., & Wang, K. (2025). Biomaterialized microspheres trigger synergistic calcicoptosis-ferroptosis for enhanced non-small cell lung cancer therapy. *Acta biomaterialia*, 207, 617–632. <https://doi.org/10.1016/j.actbio.2025.09.053>
- Zhang, Y., Wang, T. W., Tamatani, M., Zeng, X., Nakamura, L., Omori, S., Yamaguchi, K., Hatakeyama, S., Shimizu, E., Yamazaki, S., Furukawa, Y., Imoto, S., Johmura, Y., & Nakanishi, M. (2025). Signaling networks in cancer stromal senescent cells establish

- malignant microenvironment. *Proceedings of the National Academy of Sciences*, 122, e2412818122. <https://doi.org/10.1073/pnas.2412818122>
- Zhang, Y., Liu, C., Jin, S., Xie, L., Xiao, Q., & Yao, J. (2025). Ultrasound-Targeted Nanobubbles Codelivering NKP-1339 and miR-142-5p for Synergistic Mitochondrial Immunogenic Cell Death and PD-L1 Inhibition in Cancer Therapy. *Biomaterials research*, 29, 0232. <https://doi.org/10.34133/bmr.0232>
- Zhou, J., Cheng, A., Guo, J., Liu, Y., Li, X., Chen, M., Hu, D., & Wu, J. (2025). Targeting PRDX2 to inhibit tumor growth and metastasis in triple-negative breast cancer: the role of FN1 and the PI3K/AKT/SP1 pathway. *Journal of translational medicine*, 23(1), 434. <https://doi.org/10.1186/s12967-025-06441-2>
- Zhou, Z., Chen, B., Liu, J., Feng, W., Ren, Z., Jin, K., Li, Y., Ju, L., Chen, L., Zhu, G., & Wang, F. (2025). tRNA-Derived Small RNA Accelerates Tumorigenesis through Crosstalk with Tumor-Associated Macrophages, and Downregulation with Cell Membrane-Modified Polymer Nanoparticles Enables Treatment Response. *ACS applied materials & interfaces*, 17(29), 41610–41625. <https://doi.org/10.1021/acsami.5c05698>
- Wu, Y., Zhao, Z., Ma, M., Zhang, W., Liu, W., Liang, X., Zhao, T., Luo, Y., Wang, Y., Li, M., Li, T., Liu, C., Luo, X., Wang, S., Li, W., Zeng, W., Wang, H., Li, W., Wu, T., Ke, Z., & Luo, F. (2025). Ultrasound-activated erythrocyte membrane-camouflaged Pt(II) layered double hydroxide enhances PD-1 inhibitor efficacy in triple-negative breast cancer through cGAS-STING pathway-mediated immunogenic cell death. *Theranostics*, 15, 1456–1477. <https://doi.org/10.7150/thno.102284>



# CHAPTER 6

---

## EVALUATION OF PUBLIC HEALTH SYSTEMS IN SCANDINAVIAN COUNTRIES: SWEDEN, NORWAY AND FINLAND

*Kenan YURTSAL<sup>1</sup>*

---

<sup>1</sup> Dr.Öğr.Üyesi, Sivas Cumhuriyet Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu, Sivas <https://orcid.org/0000-0002-9330-258X>

## **Introduction**

Healthcare services are among the most important public services in modern welfare states. Healthcare systems are not limited to treating diseases but also have broader objectives such as disease prevention, ensuring access to healthcare, and improving public health (World Health Organization) . Organization [WHO], 2023).

Healthcare systems in different countries vary significantly in terms of funding, management, and service delivery. These differences are generally related to the countries' economic structures, social policy approaches, and historical development processes ( Blank & Burau , 2017).

Sweden, Norway, and Finland, collectively known as the Scandinavian countries, are frequently studied in international literature due to their strong social policy structures and predominantly public healthcare systems . In these countries, healthcare services are largely financed through a tax-based model, and access to healthcare is considered a fundamental civic right ( Saltman , Bankauskaite , & Vrangbaek , 2007).

Scandinavian healthcare systems are often evaluated within the framework of the Beveridge model . In this model, healthcare services are financed through general taxes, and the public sector plays a dominant role in service delivery ( Wendt , Frisina , & Rothgang , 2009).

Healthcare systems are one of the fundamental institutional structures that determine the well-being of societies. Sweden, Norway, and Finland, known as the Scandinavian countries, hold a significant place in the world healthcare system literature due to their strong welfare state structures and universal healthcare concepts . In these countries, healthcare services are largely publicly funded, and equal access to healthcare for citizens is adopted as a fundamental policy objective. The aim of this study is to comparatively examine the healthcare systems of Sweden, Norway, and Finland and to evaluate their key characteristics.

## **2. The Welfare State and Scandinavian Health Policies**

The Scandinavian countries represent some of the most advanced examples of the welfare state model. The welfare state is a social policy model in which the state plays an active role in health, education, and social services to ensure the social security of its citizens ( Esping -Andersen, 1990).

the Nordic welfare state can be summarized as follows:

- Universal social rights
- Policies aimed at reducing income inequality.
- high government spending
- strong public services
- social solidarity

In this model, healthcare is considered a fundamental social right, and the state guarantees citizens' access to healthcare (Greve, 2019).

The basic principles of healthcare systems in Scandinavian countries are as follows:

- Universal inclusivity
- Equal access
- Public finance
- The active role of local governments
- Prioritizing preventive health services.

In line with these principles, health systems aim to improve both individual and public health levels.

### **3. Swedish Public Health System**

Healthcare systems are considered one of the most fundamental elements of a state's social welfare policies. The accessibility, financing, and organization of healthcare services develop in different models depending on the economic, political, and social structures of each country. In this context, Scandinavian welfare states have developed healthcare systems notable for their universal healthcare provision and strong public funding. Sweden, in particular, stands out among these countries with its tax-based financing model, decentralized governance approach, and universal health coverage.

The Swedish healthcare system is fundamentally based on the principles of equality, need, and human dignity. These principles guide the planning and distribution of healthcare services. The system aims to ensure equal access to healthcare for all citizens and individuals living in the country ( Anell , Glenngård , & Merkur , 2012). Healthcare services are largely publicly funded, and service delivery is largely managed by regional administrations.

The Swedish healthcare system has long been among the top-performing systems in international comparisons. High life expectancy, low infant mortality rates, and strong public health indicators are considered indicators of its success. However, the system also faces various structural problems such as long waiting times, shortages of healthcare personnel, and an aging population.

This section comprehensively examines the historical development, institutional structure, financing model, service delivery, and current reforms of the Swedish public health system.

### **3.1. Historical Development of the Swedish Healthcare System**

The development of the modern healthcare system in Sweden dates back to the early 20th century. However, its institutional strengthening occurred after World War II with the widespread adoption of welfare state policies. Reforms in the field of social policy during the 1940s and 1950s ensured that healthcare services were considered a public responsibility.

The Hospital Act of the 1960s marked a significant turning point in the organization of healthcare services. This regulation largely transferred the planning and management of healthcare services to local governments. This approach formed the basis of the decentralized model of the Swedish healthcare system, which continues to this day ( Saltman , Bankauskaite , & Vrangbæk , 2007).

In the 1970s, the scope of health services was expanded, and significant progress was made in accessing healthcare. During this period, policies were implemented particularly aimed at strengthening primary healthcare services.

In the 1990s, reforms were implemented in the Swedish healthcare system to increase efficiency and control costs. These reforms included the introduction of competitive service delivery in some regions, performance-based payment models, and limited inclusion of private service providers in the system .

In the 2000s, the main focus of the healthcare system became improving service quality, patient-centered care, and digital transformation in healthcare services.

### **3.2. Fundamental Principles of the Swedish Healthcare System**

The organization of the Swedish healthcare system is based on three fundamental ethical principles:

- The Principle of Human Dignity

According to this principle, there should be no discrimination in the provision of health services between individuals based on factors such as economic status, social status, or age. All individuals have the right to equal access to health services.

- The Principle of Need and Solidarity

This principle stipulates that priority should be given to individuals with high health needs in the distribution of health services. The aim is thus to ensure a more equitable distribution of health service resources.

- Cost-Effectiveness Principle

The goal of health policy planning is to utilize existing resources in the most efficient way. Therefore, economic evaluations and cost-effectiveness analyses play an important role in the health system.

### **3.3. Institutional Structure of the Health System**

The Swedish healthcare system has a three-tier governance model:

- National Level

The Ministry of Health and Social Affairs is responsible for determining national health policies. The Ministry sets the general framework for health policies and prepares legal regulations concerning the organization of the health system.

Some important institutions operating at the national level include: the National Board of Health and Welfare, the Public Health Agency, and the Medical Devices Agency.

These institutions undertake tasks such as monitoring the implementation of health policies, developing quality standards, and analyzing health data.

- Regional Level

One of the most important features of the Swedish health system is that health services are largely administered by 21 regional administrations ( regions ). These regions are responsible for the financing, planning and delivery of health services (WHO, 2023).

Regional administrations perform the following tasks:

- Hospital management
- specialized health services

- creation of a health budget
- quality control of healthcare services

Regional governments also have the authority to collect local income tax to fund healthcare services.

- Local Level

In Sweden, approximately 290 municipalities are responsible for a significant portion of health and social care services. These municipalities primarily provide the following services:

- elderly care services
- home care services
- rehabilitation services
- school health services

integration between healthcare and social care services .

### **3.3. Financing of the Health System**

The Swedish healthcare system is largely funded by a tax-based model. Approximately 80–86% of total healthcare spending comes from public funds (WHO, 2023; OECD, 2023).

Funding sources can generally be grouped under four main headings:

- Taxes

The most important source of funding for the healthcare system is local and regional taxes. Regional governments have the authority to collect income tax to finance healthcare services.

- Central Government Transfers

The central government provides various financial transfers to reduce economic disparities between regions. These transfers support the provision of healthcare services at equal standards throughout the country.

- Patient Co-payments

The Swedish healthcare system includes patient co-payments. However, these co-payments are limited and have an annual upper limit. This practice aims to prevent financial barriers to accessing healthcare services.

- Private Health Insurance

In Sweden, the role of private health insurance is quite limited and is used by only a small portion of the population. Private insurance is generally preferred for supplementary services.

### **3.4. Provision of Health Services**

The Swedish healthcare system consists of three main service levels:

#### **1. Primary Healthcare Services**

Primary healthcare services are considered the entry point into the healthcare system. These services are generally provided through health centers ( vårdcentral ).

The healthcare professionals working at these centers include:

- family doctors
- nurses
- physiotherapists
- psychologists

The basic functions of primary healthcare services are as follows:

- early diagnosis of diseases
- management of chronic diseases
- preventive health services

In recent years, Sweden has implemented various reforms aimed at strengthening primary healthcare services.

#### **2. Secondary Healthcare Services**

Secondary healthcare services encompass specialist services offered at regional hospitals. These hospitals provide diagnostic and treatment services, as well as emergency medical services.

#### **3. Tertiary Healthcare Services**

Tertiary healthcare encompasses advanced treatment services offered at university hospitals. These hospitals also play a significant role in medical education and research.

### **3.5. Health Technologies and Digital Health Applications**

The Swedish healthcare system is one of the leading countries in Europe in the use of digital health technologies. In particular, electronic health records, telemedicine applications, and digital health platforms contribute to the more efficient delivery of healthcare services.

Thanks to digital health systems:

- Patient data can be managed more effectively.
- Access to healthcare services is becoming easier.
- healthcare services is being increased.

### **3.6. Performance of the Swedish Healthcare System**

The Swedish healthcare system has achieved very successful results in terms of international health indicators. It particularly excels in the following areas:

- high life expectancy
- low infant mortality rates
- effective management of chronic diseases

However, the performance of the health system can vary from region to region.

### **3.7. Problems Encountered in the Healthcare System**

Despite its many strengths, the Swedish healthcare system faces some significant challenges.

- Waiting Times

One of the most frequently discussed issues in the Swedish healthcare system is the long waiting times for some healthcare services.

- Healthcare Personnel Shortage

In recent years, the shortage of nurses and specialist doctors has become a significant problem in the healthcare sector.

- Demographic Change

The aging population of Sweden is leading to increased demand for healthcare services. This is creating a significant policy debate regarding the sustainability of the healthcare system.

### **3.8. Current Health Reforms**

In recent years, various reforms have been implemented in the Swedish healthcare system. The main aim of these reforms is to increase access to healthcare services and improve the efficiency of the system.

The reforms focus on the following:

- strengthening primary healthcare services
- Increasing digitalization in healthcare services
- reducing waiting times
- Development of quality measurement systems in healthcare services

## **4. Norwegian Public Health System**

Norway, part of the Scandinavian welfare state model, stands out with its strong public finances, universal healthcare coverage, and high health indicators. The Norwegian healthcare system is fundamentally based on the principles of equality, accessibility, and high quality.

In Norway, healthcare services are largely publicly funded and organized. The system has a multi-layered governance structure based on the division of labor between the central government and local authorities. Planning and financing of healthcare services are largely carried out by public authorities, with a limited role for the private sector. This structure aims to ensure that healthcare services are provided equally and accessibly to the entire population ( Ringard , Sagan, Sperre) . Saunes , & Lindahl , 2013).

The Norwegian healthcare system is among the top-performing systems in international comparisons. High life expectancy, low infant mortality rates, and advanced

healthcare infrastructure are key indicators of its success. However, issues such as rising healthcare costs, an aging population, and a shortage of healthcare personnel remain on the agenda of healthcare policies.

This section examines the historical development, institutional structure, financing model, organization of health services, and current reforms of the Norwegian public health system.

#### **4.1. Historical Development of the Norwegian Healthcare System**

The foundations of the modern healthcare system in Norway were laid in the mid-20th century. Following World War II, with the strengthening of welfare state policies in Europe, the concept of healthcare as a public responsibility became widespread. During this period, Norway implemented various reforms to increase access to healthcare and expand its social security system.

The National Insurance Act, enacted in 1956, marked a significant turning point in the financing of healthcare. With this law, healthcare services became largely publicly funded. Furthermore, local governments assumed an important role in organizing healthcare services.

During the 1980s and 1990s, various structural reforms were implemented in the Norwegian healthcare system. The main aim of these reforms was to increase the efficiency of healthcare services and strengthen the management structure.

The Hospital Reform of 2002 is considered one of the most significant reforms to the Norwegian healthcare system. This reform transferred ownership and management of hospitals to the central government and established a new governance model for the organization of healthcare services (Magnussen, Vrangbæk, & Saltman, 2009).

This reform aims to manage hospital services more effectively and increase coordination among healthcare services.

#### **4.2. Fundamental Principles of the Norwegian Healthcare System**

The Norwegian healthcare system is based on three fundamental policy principles:

- Equality

Providing healthcare services equally to all individuals is one of the fundamental aims of the system. In Norway, access to healthcare is not dependent on an individual's income level or social status.

- Universal Inclusion

The Norwegian healthcare system is a universal system covering the entire population. All individuals living in the country have the right to access public healthcare services.

- Public Responsibility

The financing and organization of healthcare services are largely carried out by public authorities. This ensures that healthcare services are provided equally throughout society.

#### **4.2.1. Institutional Structure of the Health System**

The Norwegian healthcare system has a multi-layered governance model. This structure consists of three main levels: National level, Regional level, and Local level.

- National Level

The Norwegian Ministry of Health and Care Services is responsible for setting national health policies. The Ministry defines the general framework of health policies and prepares legal regulations concerning the organization of the health system.

Some important institutions operating at the national level include:

- Norwegian Institute of Public Health
- Norwegian Directorate of Health
- Norwegian Medical Products Agency

These institutions undertake tasks such as monitoring the implementation of health policies, analyzing health data, and evaluating the quality of health services.

- Regional Level

In Norway, hospital services are managed by four regional health authorities. These authorities are responsible for the planning, financing, and coordination of health services.

Regional health authorities:

- It provides management of hospitals.
- health services.
- It ensures coordination among health services.

This structure aims to ensure that healthcare services are provided equally across the country.

- Local Level

In Norway, municipalities are responsible for a significant portion of healthcare services. The main responsibilities of municipalities include:

- primary healthcare services
- family medicine services
- home care services
- elderly care services
- rehabilitation services

This structure allows healthcare services to be planned in a way that suits local needs.

### **4.3 Financing of the Norwegian Health System**

The Norwegian healthcare system is largely funded by public resources. A significant portion of total healthcare expenditure comes from taxes and social security contributions (OECD, 2023).

The main sources of funding for the health system are:

- Taxes

The most important source of funding for the Norwegian healthcare system is general taxes. The central government plays a significant role in financing healthcare services.

- Social Security System

In Norway, the National Insurance System makes a significant contribution to the financing of healthcare services.

- Patient Co-payments

In Norway, limited patient co-payments apply to some healthcare services. However, these co-payments are subject to a certain upper limit.

- Private Health Insurance

The role of private health insurance in the Norwegian healthcare system is quite limited and is generally used for supplementary services.

#### **4.4. Organization of Health Services**

The Norwegian healthcare system consists of three basic service levels:

- Primary Healthcare Services

Primary healthcare services are considered the entry point into the healthcare system. These services are organized by municipalities.

The essential components of primary care services are as follows:

- family medicine services
- public health services
- preventive health services

Norway has a very strong family doctor system, and a large proportion of the population is registered with a family doctor.

- Secondary Healthcare Services

Secondary healthcare services encompass specialist services offered in hospitals. These services are managed by regional health authorities.

- Tertiary Healthcare Services

Tertiary healthcare services include advanced treatment and research services. University hospitals play a significant role in providing these services.

#### **4.5. Digital Health in the Norwegian Health System**

Norway's healthcare system is one of the leading countries in Europe in the use of digital health applications. Electronic health records and telemedicine applications contribute to the more efficient delivery of healthcare services.

Thanks to digital health systems:

- patient data is becoming easier.
- Coordination among healthcare services is increasing.
- healthcare services is becoming easier.

#### **4.6. Performance of the Health System**

Norway's healthcare system performs highly according to international health indicators. Life expectancy in Norway is above the OECD average, and infant mortality rates are quite low.

These indicators show that the Norwegian health system is performing strongly.

#### **4.7. Problems Encountered in the Norwegian Healthcare System**

Despite its strengths, the Norwegian healthcare system faces some structural problems.

- Waiting Times

Waiting times are considered a significant problem for some specialized services.

- Increase in Healthcare Spending

An aging population and advancements in healthcare technologies are leading to increased healthcare costs.

- Healthcare Personnel Shortage

The shortage of healthcare personnel, particularly in rural areas, is considered a significant policy issue.

#### **4.8. Current Health Reforms**

The Norwegian government is implementing various reforms to improve the sustainability of its healthcare system. The main objectives of these reforms are as follows:

- strengthening primary healthcare services
- digital transformation in healthcare
- increasing coordination among health services

- increasing the efficiency of healthcare services

## **5. The Finnish Public Health System**

Finland stands out with its comprehensive public health system and strong local governance model. The Finnish health system is fundamentally based on universal healthcare coverage, public funding, and the strong role of local governments. All individuals living in the country have the right to access healthcare services, and healthcare services are largely publicly funded. The Finnish health system has a multi-layered governance structure shared among the central government, regional administrations, and municipalities ( Tynkynen et al ., 2019).

The Finnish healthcare system generally performs well in international comparisons. The country has a high life expectancy, widespread access to healthcare, and a well-developed healthcare infrastructure. However, issues such as an aging population, rising healthcare costs, and regional inequalities in healthcare access are among the important agenda items of health policies.

This section examines the historical development, institutional structure, financing model, organization of health services, system performance, and current reforms of the Finnish public health system.

### **5.1. Historical Development of the Finnish Healthcare System**

The development of the modern healthcare system in Finland began in the mid-20th century. Following World War II, with the strengthening of welfare state policies in Europe, significant steps were taken in Finland to address healthcare services as a public responsibility.

Significant reforms were implemented in the 1960s and 1970s that strengthened the institutional structure of the Finnish healthcare system. In particular, the Primary Healthcare Services Act of 1972 is considered a crucial turning point for the Finnish healthcare system. This law enabled municipalities to assume a central role in the organization of healthcare services ( Vuorenkoski , Mladovsky , & Mossialos , 2008).

Since the 1970s, primary healthcare services provided through health centers have become a fundamental element of the Finnish healthcare system. These centers play a vital role in the delivery of community health services.

The economic crisis of the 1990s led to reforms in the Finnish healthcare system. During this period, various policy changes were implemented to control healthcare spending and improve the efficiency of healthcare services.

In the 2000s, the main focus of the healthcare system became the integration of healthcare services, improving service quality, and developing digital health applications.

## **5.2. Fundamental Principles of the Finnish Healthcare System**

The Finnish healthcare system is built on a number of fundamental policy principles. These principles play a crucial role in the planning and delivery of healthcare services.

- Equality

One of the fundamental principles of the Finnish healthcare system is ensuring equality in access to healthcare services. Healthcare services are provided regardless of individuals' income level or social status.

- Universal Health Service

In Finland, all citizens and individuals residing in the country have the right to access public health services. This ensures universal coverage of healthcare.

- Public Responsibility

The financing and organization of healthcare services are largely carried out by public authorities. This approach aims to ensure that healthcare services are provided equally throughout society.

### **5.2.1 Institutional Structure of the Health System**

The Finnish healthcare system has a multi-layered governance model. This structure consists of three basic levels.

#### National Level

The Finnish Ministry of Social Affairs and Health is responsible for setting national health policies. The Ministry determines the overall strategies of the health system and oversees the implementation of health policies.

Key institutions operating at the national level include:

- Finnish Institute for Health and Welfare

- Finnish Medicines Agency
- National Health Insurance Corporation

These institutions undertake tasks such as regulating the health system, analyzing health data, and evaluating health policies.

### Regional Level

In Finland, a significant portion of healthcare services are organized by regional administrations. With the healthcare reform implemented in 2023, the management of healthcare services was transferred to wellbeing zones . services They were transferred to new administrative units called counties .

These regions are responsible for the following services:

- hospital services
- Specialized healthcare services
- emergency medical services

This reform aims to increase coordination among health services.

### Local Level

In Finland, municipalities have played a significant role in organizing healthcare services for many years. They have been particularly responsible for the delivery of primary healthcare services.

Although some responsibilities have been transferred to regional administrations with the new health reform, municipalities still play an important role in planning health services.

## **5.3. Financing of the Health System**

The Finnish healthcare system has a mixed financing model. Healthcare services are largely funded by public resources. A significant portion of total healthcare expenditure is covered through taxes and social security contributions (OECD, 2023).

The main sources of funding for the health system are:

- Taxes

The most important source of funding for the Finnish healthcare system is general taxes. The central government and local authorities play significant roles in financing healthcare services.

- Social Health Insurance

In Finland, the social health insurance system plays a significant role in financing healthcare services. This system specifically covers the cost of medications and certain healthcare treatments.

- Patient Co-payments

In Finland, patient co-payments are applied for some healthcare services. However, these co-payments are limited and subject to a certain upper limit.

- Private Health Insurance

The role of private health insurance in the Finnish healthcare system is limited and it is generally used for supplementary services.

#### **5.4. Organization of Health Services**

The Finnish healthcare system consists of three main service levels:

1. Primary healthcare services
2. Secondary healthcare services
3. Tertiary healthcare services

##### **Primary Healthcare Services**

Primary healthcare forms the backbone of the Finnish healthcare system. These services are provided through health centers.

The basic functions of primary care services are as follows:

- preventive health services
- early diagnosis of diseases
- management of chronic diseases

- public health services

### Secondary Healthcare Services

Secondary healthcare services encompass specialist services offered at regional hospitals. These hospitals provide diagnostic and treatment services, as well as emergency medical services.

### Tertiary Healthcare Services

Tertiary healthcare services include advanced treatment and research services. University hospitals play a vital role in providing these services.

## **5.5. Digital Health in the Finnish Healthcare System**

Finland is one of the leading countries in Europe in the development of digital health applications. Electronic health records and digital health platforms are increasing the effectiveness of healthcare services.

Thanks to these systems:

- Patient data can be managed more effectively.
- Coordination is being ensured among health services.
- healthcare services is becoming easier.

## **5.6. Performance of the Finnish Health System**

Finland's healthcare system demonstrates successful results in terms of international health indicators. Life expectancy in the country is above the OECD average, and access to healthcare services is widespread.

However, differences in access to healthcare services can be observed in some regions.

### **5.7. Problems Encountered in the Healthcare System**

The Finnish healthcare system faces various structural problems.

- **Aging Population**

Finland has one of the fastest aging populations in Europe, which leads to increased demand for healthcare services.

- **Healthcare Personnel Shortage**

The shortage of healthcare personnel is seen as a significant problem, especially in rural areas.

- **Regional Inequalities**

Access to healthcare services varies in different regions.

### **5.8. Current Health Reforms**

The Finnish government is implementing various reforms to improve the sustainability of its healthcare system.

The main objectives of these reforms are as follows:

- increasing the integration of health services
- strengthening primary healthcare services
- Developing digital transformation in healthcare services
- reducing regional inequalities in health services

### **Conclusion**

literature due to their universal access to healthcare, strong public funding, and high health indicators . Although the health systems of Sweden, Norway, and Finland are based on common welfare state principles, they differ in terms of governance structure, financing model, and service organization.

Sweden's healthcare system is notable for its strong regional governance structure, while Norway has adopted a more centralized healthcare management model. Finland, on the other hand, aims to strengthen regional integration in healthcare services through reforms implemented in recent years .

Sweden, Norway, and Finland are successful healthcare system models built on the principles of strong public funding, universal coverage, and equitable access.

In these countries, the predominantly public nature of healthcare services and strong primary care systems contribute to the efficient and equitable delivery of healthcare.

Scandinavian healthcare systems serve as an important example for many countries, particularly in terms of the universal healthcare model.

In the future, demographic changes, rising healthcare costs, and the need for healthcare personnel are among the most important factors that could affect the sustainability of Scandinavian healthcare systems. Therefore, digital transformation in healthcare systems, strengthening primary healthcare services, and increasing service integration stand out as important policy priorities.

## References

- Anell , A. , Glenngård , A.H., & Merkur , S. (2012). Sweden : Health system Review . Health Systems in Transition , 14(5), 1–159.
- Blank , R. , & Burau , V. (2017). comparative health policy (4th ed.). Palgrave Macmillan .
- Esping -Andersen, G. (1990). the three worlds of welfare capitalism Princeton University Press .
- Greve, B. (2019). Welfare states and social policies . Routledge .
- Magnussen , J. Vrangbæk , K., & Saltman , R. (2009). Nordic health resort systems : Recent reforms and current policy challenges . McGraw-Hill .
- OECD. (2023). Health at a glance 2023: OECD indicators . OECD Publishing.
- OECD. (2023). Health statistics database .
- Ringard , Å ., Sagan, A., Saunes , I., & Lindahl , A. (2013). Norway : Health system Review . Health Systems in Transition .
- Saltman , R. , Bankauskaite , V., & Vrangbæk , K. (2007). Decentralization in healthcare care : Strategies and outcomes . McGraw-Hill .
- Tynkkynen , L. , Keskimäki , I., & Lehto , J. (2019). Finland health system Review . Health Systems in Transition , 21(2), 1–166.
- Vuorenkoski , L. , Mladovsky , P., & Mossialos , E. (2008). Finland : Health system Review . Health Systems in Transition , 10(4), 1–168.
- Wendt , C. , Frisina , L., & Rothgang , H. (2009). healthcare system types .
- World Health Organization . (2023). Sweden health system summary . WHO Regional Office for Europe.

# CHAPTER 7

---

## MALE BREAST CANCER: THREE CASE STUDIES

*Selim YALÇIN*<sup>1</sup>

## **Introduction**

Breast cancer in men is a rare malignancy, with an estimated incidence of 1 in 100,000 men per year, accounting for approximately 0.5%–1% of all breast cancers [1]. While the incidence in women has stabilized or slightly decreased in some regions, the incidence of MBC has shown a slow but steady rise over the past few decades [2].

Risk factors for MBC include older age (average age at diagnosis is 67), genetic mutations (especially BRCA2 and BRCA1), family history of breast cancer, radiation exposure, and conditions that alter the estrogen-to-androgen ratio, such as Klinefelter syndrome, liver cirrhosis, and obesity [3].

Pathologically, most cases of MBC are invasive ductal carcinoma (IDC), with increased expression of estrogen receptor (ER) and progesterone receptor (PR) compared to female breast cancer (FBC) [4]. Other subtypes, like invasive lobular carcinoma (ILC), are extremely rare in men, making up only about 1% of cases, mainly due to the absence of acini and lobules in the normal male breast. However, lobular development can occur in conditions of chronic estrogen exposure or Klinefelter syndrome.

Because male breast tissue is located subareolarly and screening programs are lacking, MBC often presents with skin or nipple involvement at an earlier stage than in women [5]. The absence of a protective fatty envelope in male breasts allows tumors to invade the pectoral fascia and skin more quickly. Despite this, diagnosis is often delayed, with a median time from symptom onset to diagnosis of approximately 6 to 10 months. This paper aims to present three cases illustrating the clinical spectrum of MBC, highlighting the demographic diversity and the critical role of individualized multidisciplinary management.

## **Case Presentations**

### **Case 1: Typical Presentation in an Older Patient**

Demographics and History:

A 72-year-old man presented with a 4-month history of a painless, firm mass in his left breast. He had no significant medical history, no history of radiation exposure, and no family history of breast or ovarian cancer. Physical examination revealed a 2.5 cm firm, non-tender, mobile mass in the subareolar region of the left breast. There was no nipple discharge or skin ulceration. No palpable axillary lymphadenopathy was noted.

Diagnosis:

Mammography and ultrasonography showed a highly suspicious, irregular, hypoechoic mass (BI-RADS 5). A core needle biopsy confirmed Grade 2 invasive ductal carcinoma (IDC). Immunohistochemistry (IHC) showed strong positivity for ER (90%) and PR (80%), while HER2 was negative (Score 1+). The Ki-67 index was 15%.

Treatment:

The patient underwent a left modified radical mastectomy (MRM). Histopathology confirmed a 2.2 cm IDC with negative surgical margins. Two of twelve axillary lymph nodes were positive for macrometastasis (pT2N1aM0, Stage IIB). Following surgery, he received adjuvant chemotherapy (4 cycles of Adriamycin and Cyclophosphamide followed by Paclitaxel) and adjuvant radiotherapy to the chest wall and regional nodes. He was started on Tamoxifen. (20 mg daily) for a planned duration of five years.

## **Case 2: Genetic Predisposition in a Younger Patient**

Demographics and History:

A 48-year-old male presented with a 2-month history of nipple retraction and occasional bloody discharge from the right breast. His family history was significant; his mother and sister had been diagnosed with breast cancer at ages 45 and 42, respectively. Physical examination revealed a 1.5 cm subareolar mass with associated nipple inversion.

Diagnosis:

Diagnostic mammography showed a spiculated mass with microcalcifications. Core needle biopsy confirmed Grade 3 IDC. IHC results indicated ER-positive (70%), PR-positive (60%), and HER2-negative status. Due to his young age and family history, the patient was referred for genetic counseling. Genetic testing detected a pathogenic germline mutation in the BRCA2 gene.

Treatment:

The patient opted for a bilateral total mastectomy with sentinel lymph node biopsy (SLNB) on the right side to treat his current cancer and mitigate the risk of contralateral disease. The SLNB was negative for metastasis. The final staging was pT1cN0M0 (Stage IA). He was started on adjuvant Tamoxifen. Additionally, the patient was enrolled in a high-risk screening program for other BRCA2-associated cancers, including prostate and pancreatic cancer.

### **Case 3: Advanced and Neglected Presentation**

#### Demographics and History:

An 80-year-old man presented with a large, ulcerated mass in his right breast that had been present for over a year. He delayed seeking medical care due to embarrassment and the belief that "men don't get breast cancer." He also reported weight loss and persistent lower back bone pain. Examination revealed a 6 cm fixed, ulcerated mass involving the skin and nipple-areolar complex. Multiple enlarged, hard, fixed axillary lymph nodes were palpable.

#### Diagnosis:

Biopsy of the ulcerated mass confirmed Grade 3 IDC, ER/PR positive, and HER2-negative. A CT scan of the chest, abdomen, and pelvis, along with a bone scan, revealed multiple osteoblastic lesions in the lumbar spine and pelvis, as well as several small nodules in the lungs. The final diagnosis was Stage IV (metastatic) MBC.

#### Treatment:

Due to the metastatic nature of the disease, the primary goal was palliative. The patient was started on systemic therapy with an aromatase inhibitor (Letrozole) combined with a CDK4/6 inhibitor (Palbociclib), alongside a GnRH agonist to suppress testicular androgen production (a necessary step when using aromatase inhibitors in men). He also received denosumab for bone protection and palliative radiotherapy to the painful spinal lesions. The local ulcerated mass was managed with wound care and eventually showed partial regression with systemic therapy.

### **Discussion**

#### **Demographic and Clinical Characteristics**

The cases presented here highlight the diverse demographic and clinical profiles of MBC. While Case 1 depicts the typical older patient with a hormone-sensitive tumor, Case 2 emphasizes the crucial role of genetic factors, especially in younger men. Case 3 illustrates a common and tragic theme in MBC: delayed diagnosis caused by lack of awareness and social stigma [6]. Studies indicate that men are much more likely to present with Stage III or IV disease than women, often due to misinterpreting breast masses as just a bump or muscle strain in the male psyche.

The demographic characteristics of MBC are changing. Although the median age at diagnosis remains higher than in women, the rising rates of obesity and liver disease—both of which can

elevate estrogen levels—may lead to a younger age of onset in some populations. Moreover, the psychological impact of MBC is significant. Men often feel isolated in female-dominated breast cancer support groups and may feel emasculated after a mastectomy, which highlights the need for support systems tailored specifically for men.

### Diagnostic Challenges

In all three cases, the initial presentation was a subareolar mass, the most common site due to the concentration of ductal tissue in this area in men. Mammography continues to be the gold standard for imaging, with sensitivity exceeding 90% in men [8]. However, because the disease is rare, many men do not have regular screening, and symptoms like nipple retraction or skin changes may be dismissed as benign conditions such as gynecomastia.

### Treatment Paradigms

Treating MBC largely relies on guidelines derived from female breast cancer, though there are notable differences.

- **Surgery:** Mastectomy (either simple or modified radical) is the standard treatment for most men, given the small size of the male breast and the central location of most tumors, which makes breast-conserving surgery (BCS) difficult to achieve with good cosmetic results [9]. However, recent studies suggest BCS may be suitable for a select group of men with smaller, peripheral tumors if followed by radiation therapy. Sentinel lymph node biopsy (SLNB) has become the standard for axillary staging in clinically node-negative men, replacing the more invasive axillary lymph node dissection (ALND) in many cases.
- **Endocrine Therapy:** Since over 90% of MBC cases are ER-positive, endocrine therapy is a key part of adjuvant treatment. Tamoxifen remains the preferred agent. Aromatase inhibitors (AIs) are less effective as sole therapy in men because they do not block testicular estrogen production, so GnRH analogs are often added when AIs are used [10].
- **Genetic Testing:** As seen in Case 2, all men diagnosed with breast cancer should undergo genetic testing for BRCA1/2 mutations, regardless of family history, because mutation prevalence is much higher in men—up to 10%—compared to 2-3% in women [11].

## **Conclusion**

Male breast cancer, although rare, presents significant clinical challenges. The three cases discussed emphasize the need for greater awareness among men and healthcare providers to ensure early diagnosis. While treatment methods are currently based on female-focused protocols, the high rate of hormone receptor positivity and the important role of genetic mutations, especially BRCA2, highlight the necessity for male-specific clinical trials and personalized treatment strategies. Early intervention, especially for those with a genetic predisposition, remains the most effective way to improve survival outcomes in this patient group. [12-18]

## References

1. Siegel, R. L., Miller, K. D., & Jemal, A. (2024). Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians*, 74(1), 12-49.
2. Giordano, S. H. (2025). Breast Cancer in Men. *New England Journal of Medicine*, 392(4), 345-356.
3. Korde, L. A., et al. (2020). Management of Male Breast Cancer: ASCO Guideline. *Journal of Clinical Oncology*, 38(16), 1883-1898.
4. Gucalp, A., et al. (2024). NCCN Guidelines® Insights: Breast Cancer, Version 4.2024. *Journal of the National Comprehensive Cancer Network*, 22(5), 312-322.
5. Yadav, S., et al. (2020). Male Breast Cancer: An Updated Review of Epidemiology, Risk Factors, and Management. *The Oncologist*, 25(12), e1840-e1850.
6. White, J., et al. (2021). Stigma and delayed presentation in male breast cancer: A qualitative study. *Journal of Psychosocial Oncology*, 39(3), 412-425.
7. Cardoso, F., et al. (2018). Characterization of male breast cancer: Results of the EORTC 10085/TBCRC/BIG/NABCG International Program. *Annals of Oncology*, 29(2), 405-417.
8. Evans, G. W., et al. (2023). Imaging of the Male Breast: A Pictorial Review. *Radiographics*, 43(2), e220085.
9. Leon-Ferre, R. A., et al. (2022). Modern management of male breast cancer. *The Lancet Oncology*, 23(11), e491-e501.
10. Zagouri, F., et al. (2025). Aromatase inhibitors in male breast cancer: A systematic review and meta-analysis. *Breast Cancer Research and Treatment*, 201(1), 15-28.
11. National Comprehensive Cancer Network (NCCN). (2024). Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic (Version 1.2024).
12. Yadav, S., et al. (2021). Germline pathogenic variants in men with breast cancer: Results from a large commercial laboratory. *Journal of Clinical Oncology*, 39(15\_suppl), 10515-10515.
13. Ruddy, K. J., & Winer, E. P. (2013). Male breast cancer: Risk factors, biology, diagnosis and treatment. *CA: A Cancer Journal for Clinicians*, 63(2), 125-139.
14. Fentiman, I. S. (2018). The management of male breast cancer. *Expert Review of Anticancer Therapy*, 18(11), 1103-1110.
15. Gucalp, A., et al. (2019). Male breast cancer: A comprehensive review of the literature. *The Breast*, 44, 42-49.
16. Johnson, M. E., et al. (2023). Psychosocial support needs of men with breast cancer: A systematic review. *Psycho-Oncology*, 32(4), 512-524.
17. Kiluk, J. V., et al. (2011). Breast-conserving therapy for male breast cancer: Is it an option? *The American Surgeon*, 77(10), 1401-1405.

18. Massarweh, S. A., et al. (2022). Endocrine therapy for male breast cancer: Current standards and future directions. *Clinical Breast Cancer*, 22(3), 215-224.

# CHAPTER 8

---

## THE ROLE OF GUT MICROBIOTA IN BRAIN HEALTH AND PSYCHOLOGICAL - NEUROLOGICAL DISEASES

*Esra TAŞ<sup>1</sup>*

## 1. INTRODUCTION

Each individual harbors distinct microbial communities across different anatomical sites, varying in composition and function. The human microbiota is a complex ecosystem of bacteria, viruses, protozoa, and fungi that colonize the body and exhibits a dynamic, individualized structure. Its development is influenced by prenatal and early-life factors, including mode of delivery, breastfeeding, and maternal nutrition [1]. Although relatively stable over time, the gut microbiota varies among individuals depending on host genetics, diet, and lifestyle. The term gut microbiota refers to the community of bacteria, archaea, viruses, and eukaryotic microorganisms residing in the gastrointestinal tract [2]. Beyond its metabolic and immunological roles, increasing evidence indicates that the gut microbiota contributes to brain development and neurogenesis. Communication between the gut and the brain occurs through a bidirectional network involving the central and enteric nervous systems, the autonomic nervous system, the hypothalamic-pituitary-adrenal axis (HPA axis), and immune and endocrine pathways, collectively termed the gut-brain axis. The more specific interaction between microbiota and brain is defined as the microbiota-gut-brain axis. Although its mechanisms are not fully elucidated, animal and human studies suggest that the gut microbiota influences behavior and cognitive function [3].

### 1.1. BEHAVIOR AND THE MICROBIOTA-GUT-BRAIN AXIS

#### 1.1.1. Social Behavior

Sociability refers to interactions among individuals and represents a fundamental behavior that supports learning, cooperation, protection, and reproduction. Emerging evidence suggests that social behavior is strongly influenced by the gut microbiota. Germ-free (GF) mice exhibit reduced social interaction compared with conventionally colonized controls and fail to distinguish between familiar and unfamiliar conspecifics, indicating cognitive deficits in social recognition [4]. Similar impairments have been observed following antibiotic-induced depletion of gut microbial diversity in rodents, further supporting the role of microbiota in regulating social behavior [5].

Although the precise mechanisms remain unclear, multiple biological pathways are likely involved. The amygdala has been identified as a key brain region mediating microbiota-related effects on sociability. GF mice show altered neuronal activation patterns in the amygdala compared with conventional controls, suggesting that this region represents a critical interface between the microbiota and neural circuits underlying social behavior. Moreover, modulation of microbiota through diet or probiotic supplementation may influence sociability. For instance, administration of *Lactobacillus reuteri* has been shown to increase oxytocin levels and enhance social behavior in mice [4].

Diet-induced alterations in the microbiome may also affect social neurodevelopment. Experimental omega-3 polyunsaturated fatty acid (PUFA) deficiency has been associated with social behavior deficits in adult rats, whereas omega-3 supplementation prevented allergy-induced social impairments in mice. In addition to direct effects on brain membrane composition, PUFA-rich diets may exert pro-social effects by modulating gut microbiota

composition [6]. Future research should aim to translate these findings into clinical contexts, particularly in neuropsychiatric conditions characterized by social dysfunction, such as autism spectrum disorder, schizophrenia, and social anxiety disorder.

### **1.1.2. Cognition**

There is increasing evidence to support the idea that changes in the composition of the gut microbiota can affect cognitive function at multiple levels.

#### **1.1.2.1. Rodent studies**

The absence of gut microbiota is associated with significant cognitive impairments. GF mice exhibit deficits in object recognition and working memory, including impaired performance in spontaneous alternation tasks. These alterations are accompanied by changes in hippocampal BDNF expression, a key regulator of synaptic plasticity and cognition, suggesting a critical role for microbiota in hippocampus-dependent memory function [7].

Antibiotic-induced disruption of gut microbiota similarly affects cognitive performance. Early-life antibiotic exposure leads to persistent microbiota alterations, object recognition deficits, and long-term changes in hippocampal BDNF expression detectable in adulthood. Comparable impairments have also been reported following antibiotic administration in adult rodents, including altered expression of cognition-related signaling molecules such as BDNF and 5-HTT in memory-associated brain regions [8]. Chronic antibiotic treatment has further been linked to reduced hippocampal neurogenesis and memory deficits, although findings in other hippocampus-dependent tasks remain inconsistent, likely due to variations in experimental design, behavioral paradigms, and treatment duration. These discrepancies indicate that additional research is needed to clarify the impact of microbiota depletion on spatial memory.

Conversely, probiotic and prebiotic interventions have demonstrated beneficial cognitive effects. Various *Lactobacillus* strains prevent or reverse object recognition and spatial memory deficits in GF, colitis, stress, immunocompromised, and antibiotic-treated models, and also ameliorate deficits induced by dietary imbalance, aging, and hyperammonemia [9]. Select *Bifidobacterium* strains improve object recognition, reduce spatial memory errors, and enhance long-term learning in fear conditioning paradigms. The prebiotic B-GOS enhances cognitive flexibility and increases cortical NMDAR function [10]. Multi-strain formulations, including VSL#3, also reverse memory impairments associated with antibiotics, metabolic disturbances, and dietary interventions [11].

Overall, these findings underscore the pivotal role of gut microbiota in cognitive regulation and highlight the therapeutic potential of microbiota-targeted strategies in mitigating memory deficits.

### 1.1.2.2. Human studies

Although substantial animal evidence supports the role of gut microbiota in cognitive regulation, human studies remain limited. Nevertheless, emerging findings are promising. One study reported that differences in gut microbiota composition between obese and non-obese individuals were associated with performance in attention, processing speed, and cognitive flexibility, as measured by the Trail Making Test, as well as altered neural activity in the thalamus, hypothalamus, and amygdala. These findings suggest that obesity-related microbial alterations may influence cognitive function [12].

In infants, microbiota composition at one year of age has been linked to cognitive development assessed by the Mullen Scales of Early Learning and to differences in brain volume. Infants were categorized into three microbial profiles characterized by higher levels of *Faecalibacterium*, *Bacteroides*, or *Ruminococcaceae*, with superior cognitive performance observed in those with higher *Bacteroides* abundance. Notably, this group was less frequently delivered by cesarean section, underscoring the importance of early microbial colonization in neurodevelopment and supporting previous evidence linking delivery mode to cognitive outcomes [13].

Probiotic and prebiotic interventions have also shown cognitive benefits in humans. In healthy elderly individuals, *Lactobacillus* supplementation improved cognitive test performance compared with placebo [14]. In healthy women, probiotic-enriched fermented dairy products modulated brain activity in regions associated with cognitive processing during emotional attention tasks [15]. Prebiotic supplementation with inulin-type fructans improved memory performance and mood in healthy adults [16]. Moreover, a probiotic mixture containing *Bifidobacterium longum* and *Lactobacillus* strains enhanced cognitive and metabolic parameters in patients with Alzheimer's disease [17]. Cognitive improvements have also been reported in individuals with microbiota alterations and in patients with fibromyalgia following probiotic intervention.

Collectively, these findings suggest that microbiota-targeted interventions may enhance cognitive function in both healthy individuals and clinical populations. However, further well-designed studies are required to clarify the mechanisms underlying these effects and to determine which specific strains or interventions are most effective. Integrating neuroimaging approaches with neuropsychological assessments will be essential to advance understanding of the microbiota-gut-brain axis in cognitive regulation across diverse populations.

### 1.1.3. Fear

Fear regulation represents an important yet understudied aspect of cognition. Exaggerated fear is a core feature of clinical anxiety and is closely linked to stress, a key component of gut-brain axis function. The expression and extinction of learned fear are primarily mediated by the amygdala, hippocampus, and PFC in both rodents and humans—regions known to be sensitive to microbiota-related changes.

Human fMRI studies provide emerging evidence linking gut microbiota to neural responses during threat processing. In healthy women, consumption of multi-strain probiotic

fermented dairy products reduced neural reactivity to negative emotional faces (fear and anger), particularly within the PFC and parahippocampal gyrus [18]. Subsequent findings from the same group demonstrated that interindividual differences in microbiota composition were associated with altered right hippocampal responses to negative stimuli [19]. In children, variations in *Bacteroides* and *Lachnospiraceae* abundance were linked to differential brain reactivity to fearful faces, especially in the PFC [20].

Preclinical studies further support microbial modulation of learned fear, although effects appear strain-specific. For example, *B. longum* 1714 enhanced fear learning and short-term memory without affecting extinction, whereas *B. breve* 1205 showed no significant effects [20]. *L. rhamnosus* (JB-1) delayed extinction in mice, while heat-killed *Mycobacterium vaccae* accelerated extinction learning in rats. Additionally, a probiotic combination of *L. rhamnosus* R0011 and *L. helveticus* R0052 prevented the long-term effects of early-life stress on fear behavior and its supporting neural circuitry [21].

Early-life stress in rodents is associated with persistent fear memory and elevated relapse following extinction, with transgenerational transmission observed in male offspring. Probiotic treatment restored normal developmental trajectories of fear memory, promoting age-appropriate rapid forgetting and reducing relapse rates in both treated animals and their progeny [22].

#### 1.1.4. Stress-Related Behaviors

Preclinical studies using various animal models have demonstrated that stress can alter gut microbiota composition. This interaction is bidirectional: just as stress reshapes microbial communities, microbiota can modulate stress-induced changes in anxiety, memory, cognition, and neuroinflammation. Research in GF mice has been particularly instrumental in establishing this link and has prompted investigations into the therapeutic potential of microbiota manipulation under stress conditions.

Growing evidence indicates that targeted modulation of microbiota can attenuate stress-related behavioral alterations and HPA axis activation. Most studies have focused on probiotic interventions, particularly *Bifidobacterium* and *Lactobacillus* strains, which show beneficial effects on stress, anxiety, and depression in both preclinical and human research. For example, *L. rhamnosus* (JB-1) reduced corticosterone responses and anxiety- and depression-like behaviors in stressed mice [23], while *C. butyricum* prevented depressive-like behaviors induced by chronic unpredictable stress [24]. Importantly, probiotic treatments have also demonstrated efficacy during vulnerable developmental periods, including early-life stress.

In maternal separation models, several strains have shown protective effects. Combined *L. rhamnosus* and *L. helveticus* normalized fear behavior in stressed offspring and even in subsequent generations [21]. *B. infantis* reversed behavioral deficits in separated rats but had no effect in non-stressed animals [25,26]. Other strains, including *B. animalis*, *Propionibacterium jensenii*, and *B. bifidum* G9-1, mitigated stress-induced behavioral and microbial alterations in similar paradigms [27,28]. Human studies further support these findings: *L. plantarum* 299v reduced cortisol levels during exam stress [29], and *L. plantarum*

DR7 alleviated stress and anxiety in adults [30]. Although translation from animal models to humans remains challenging, current evidence supports continued investigation of probiotics as stress-modulating interventions.

In contrast, the effects of prebiotics on stress are less clearly defined, despite their capacity to modify microbiota composition. Preliminary findings suggest potential modulation of HPA axis activity, warranting further study.

Beyond probiotic and prebiotic approaches, other microbiota-modifying strategies also influence stress responses. Antibiotic-induced microbiota depletion has produced complex and sometimes conflicting outcomes. Prenatal exposure to non-absorbable antibiotics alters maternal microbiota and may increase anxiety-like behavior in offspring. Conversely, perinatal penicillin exposure has been reported to reduce anxiety-like behavior in a sex-specific manner, whereas later-life antibiotic exposure can reduce anxiety but impair cognition [31]. These findings highlight the timing-dependent effects of microbiota disruption on stress-related outcomes.

## 1.2. PSYCHOLOGICAL - NEUROLOGICAL DISEASES AND PROCESSES

As research on the microbiota-gut-brain axis advances, gut microbiota alterations have been implicated in an expanding range of neuropsychiatric and neurological disorders. Dysbiosis has been associated with conditions such as autism spectrum disorder, Parkinson's disease, Alzheimer's disease, multiple sclerosis, ischemic stroke, anxiety, depression, and amyotrophic lateral sclerosis. Although these associations are compelling, the underlying mechanisms remain insufficiently understood.

### 1.2.1. Autism Spectrum Disorder (ASD)

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by deficits in social interaction and communication, repetitive behaviors, anxiety, and cognitive impairments [32]. Both genetic and environmental factors contribute to its pathogenesis. Among environmental factors, gut microbiota alterations have gained attention, as gastrointestinal symptoms are common in individuals with ASD and correlate with symptom severity.

Individuals with ASD frequently report constipation, diarrhea, bloating, and indigestion, and these symptoms have been associated with greater social impairment, stereotypic behaviors, hyperactivity, and aggression [33]. Several studies indicate differences in gut microbiota composition between ASD and healthy controls [34].

At the phylum level, alterations in *Bacteroidetes* and *Firmicutes* have been reported, including changes in the *Bacteroidetes/Firmicutes* ratio [35]. At the genus level, increased *Lactobacillus* spp. and *Clostridium* spp. have been observed in some studies [36,37]. Differences in *Actinobacteria* and *Proteobacteria* have also been noted, along with reduced *Bifidobacterium* spp. and elevated *Desulfovibrio* spp. in certain cohorts [38]. Increased

abundance of *Sutterella* spp., including *Parasutterella excrementihominis*, has been reported in children with ASD and GI symptoms [40].

Probiotic interventions containing *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* strains have shown partial normalization of microbial composition, including restoration of the *Bacteroidetes/Firmicutes* ratio in some studies [39]. However, findings remain inconsistent, and some studies reported no significant microbiota differences in siblings of individuals with ASD [41].

Overall, although evidence suggests an association between gut dysbiosis and ASD, most studies are limited by small sample sizes and insufficiently controlled designs. Therefore, current data cannot yet be generalized, and larger, well-controlled studies are required to clarify the role of microbiota in ASD pathophysiology.

### 1.2.2. Major Depressive Disorder (MDD)

Major depressive disorder (MDD) is characterized by depressed mood and/or loss of interest, accompanied by at least five symptoms - including recurrent thoughts of death or suicide, sleep or appetite disturbances, psychomotor changes, low energy, feelings of worthlessness or guilt, and impaired concentration - persisting for a minimum of two weeks. It affects approximately 4.4% of the global population and represents a significant public health burden due to its detrimental effects on quality of life and functional capacity [42].

Although its precise etiology remains unclear, several neurobiological mechanisms have been implicated, including reduced levels of monoamine neurotransmitters (serotonin, noradrenaline, dopamine), dysregulation of neuroendocrine pathways, and increased pro-inflammatory cytokines such as IL-6 [43]. Recent human studies have reported alterations in stool microbiota composition in patients with MDD compared to healthy controls; however, findings remain inconsistent.

Given the limited treatment response and adherence challenges observed in depressive and anxiety disorders, microbiota-targeted interventions have gained attention as potential adjunctive therapies. Alterations in gut microbiota may influence depressive symptoms through neuroendocrine, autonomic, and immune pathways. Animal studies indicate that probiotic and prebiotic interventions can reduce depressive-like behaviors and modulate inflammatory responses.

In maternal separation models, increased HPA axis activity, altered immune responses, and changes in gut microbiota composition have been demonstrated [44]. Desbonnet et al. reported that maternally separated rats exhibited increased immobility in the forced swim test, decreased brain noradrenaline levels, and elevated peripheral IL-6 levels. Administration of *Bifidobacterium infantis* improved behavioral outcomes, reduced immune activation, and normalized basal noradrenaline levels [45]. Similar behavioral improvements were observed following long-term administration of *Lactobacillus plantarum* in mice exposed to early-life stress [46]. In maternally separated infant monkeys, reduced *Lactobacillus* abundance was associated with stress-related behaviors, impaired immune function, and increased susceptibility to infections [47].

In human studies, multi-strain probiotics containing *Lactobacillus* and *Bifidobacterium* species have been associated with reductions in negative cognitive patterns in patients with depression. In animal models, *Bifidobacterium longum* 1714 and *Bifidobacterium breve* 1205 reduced anxiety-like behaviors, and *Bifidobacterium longum* 1714 also demonstrated antidepressant-like effects [48].

Overall, systematic reviews suggest that probiotics may represent a promising adjunctive therapy in MDD; however, current evidence remains limited. Diet quality is increasingly recognized as both a risk factor and a therapeutic target in depression, potentially through increased intake of prebiotic-rich foods. Further well-designed studies are required to clarify how probiotic and prebiotic interventions modify gut microbiota composition and the mechanisms underlying their role in MDD pathophysiology.

### 1.2.3. Anxiety

Anxiety is both a physiological response to perceived threat and a symptom of various psychiatric disorders. It frequently co-occurs with depression, and most studies investigating the microbiota-gut-brain axis in anxiety also assess depressive symptoms, and vice versa.

Current evidence linking anxiety to the microbiota-gut-brain axis is largely derived from preclinical studies. In animal models, anxiety-like phenotypes are commonly evaluated through behavioral paradigms such as the open field test.

Recent research has identified *Lactobacillus plantarum* DR7 as a potential psychobiotic. In a randomized, double-blind, placebo-controlled trial, this strain was shown to alleviate anxiety and stress-related symptoms [49]. Nevertheless, further well-designed studies are required to clarify the specific effects of psychobiotics on anxiety.

### 1.2.4. Schizophrenia

Schizophrenia is a complex and heterogeneous neuropsychiatric disorder characterized by psychosis, cognitive impairment, delusions, apathy, and social withdrawal [50]. Although its etiology remains unclear, it is considered multifactorial, involving genetic susceptibility, stress, childhood trauma, prenatal and perinatal factors, environmental influences, inflammation, and immune dysregulation [51].

Given the role of immune dysfunction in schizophrenia and the central role of microbiota in immune regulation, recent studies have explored potential microbiota-related mechanisms. In patients experiencing a first psychotic episode, symptom severity was associated with the abundance of *Lactobacillus* in the gut microbiota. Moreover, an inverse relationship was reported between the extent of microbiota alterations and treatment response rates [52]. Castro-Nallar et al. investigated the oropharyngeal microbiota in patients with schizophrenia and reported increased *Candida* and *Eubacterium* species, as well as lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium*, compared with controls [53]. Alterations in bacteriophage composition, including *Lactobacillus* phage phiadh, were also observed [54]. In a randomized controlled trial evaluating probiotic supplementation in schizophrenia,

gastrointestinal symptoms improved; however, no significant changes were observed in positive or negative psychotic symptoms [55]. Overall, further large-scale and longitudinal studies are required to better elucidate the contribution of the microbiota-gut-brain axis to schizophrenia pathophysiology.

### 1.2.5. Bipolar Disorder

Bipolar disorder affects approximately 1-2% of the population and typically manifests between the ages of 15 and 35, with similar prevalence in men and women. It is characterized by recurrent and unpredictable mood episodes alternating between mania and depression. During manic episodes, individuals may exhibit hyperactivity, elevated mood, and increased energy, whereas depressive episodes are marked by social withdrawal, low mood, and, in severe cases, suicidal ideation [56]. Emerging evidence suggests a potential association between bipolar disorder and gut microbiota alterations. Stool microbiome analyses have demonstrated a reduced abundance of *Firmicutes*, particularly *Faecalibacterium*, in patients with bipolar disorder, and this reduction has been correlated with symptom severity. These findings indicate a possible therapeutic target. Notably, a recent study reported that adjunctive probiotic therapy reduced rehospitalization rates in patients recently discharged following hospitalization for mania [57]. Given the growing prevalence and clinical burden of bipolar disorder, further well-designed studies are required to clarify the role of the microbiota in its pathophysiology and treatment.

### 1.2.6. Addiction

Addiction is a chronic, relapsing disorder characterized by persistent engagement in maladaptive behaviors despite adverse consequences [58]. Recent research has increasingly focused on the microbiota-gut-brain axis in the context of addiction, particularly the impact of substance use on gut microbiota composition. In addition to the direct effects of addictive substances on the microbiota, addiction is frequently accompanied by stress, anxiety, depression, and chronic inflammation - conditions previously associated with microbiota-gut-brain interactions.

Alcohol use has been the most extensively studied substance in this context. Alterations in gut microbiota during chronic alcohol consumption have been associated with increased intestinal permeability and hepatic inflammation. However, some evidence suggests that reduced microbial diversity may also modulate alcohol-induced neuroinflammation and gut-brain inflammatory signaling [59]. Preclinical studies indicate that supplementation with *Lactobacillus rhamnosus* GG can attenuate the severity of alcoholic hepatitis [60]. Notably, alcohol-induced microbiota alterations have been observed not only with oral intake but also following vapor exposure, suggesting that non-oral substances may likewise influence gut microbial composition. In animal models, compulsive alcohol-seeking behavior has been associated with specific gut bacteria and altered striatal dopamine expression [61].

Clinical studies have linked alcohol-related adverse outcomes with increased intestinal permeability, systemic inflammation, and microbiota alterations. Emerging evidence suggests that other substances exert similar effects. In smokers, both active smoking and cessation have

been associated with shifts in gut microbiota composition and inflammatory markers, while chronic tobacco exposure in mice alters microbial composition and mucin gene expression [62]. Murine models of opioid exposure indicate a role for gut microbiota in morphine dependence, gastrointestinal dysfunction, increased intestinal permeability, inflammation, and metabolic disturbances [63]. Additionally, chronic administration of  $\Delta^9$ -tetrahydrocannabinol (THC), the primary psychoactive component of cannabis, has been shown to modify gut microbiota composition in mice [64]. Collectively, these findings highlight a growing body of evidence supporting a bidirectional relationship between gut microbiota alterations and addictive behaviors.

### 1.2.7. Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is a neurodevelopmental condition characterized by inattention (e.g., difficulties with organization and sustained focus), hyperactivity, impulsivity, or a combination of these features [65]. Although research on the role of the gut microbiome in ADHD remains limited, dietary influences on ADHD symptoms have long been investigated. A Western-style diet has been suggested as a potential risk factor, whereas elimination diets may alleviate symptoms in some individuals. Given the substantial impact of diet on gut microbiota composition, these findings indirectly support a possible role of the microbiota in ADHD pathophysiology.

A small clinical study involving adolescents and adults with ADHD reported an increased abundance of *Bifidobacterium* compared with healthy controls [66]. Additionally, perinatal probiotic interventions have been associated with a reduced risk of later ADHD diagnosis, highlighting the potential of early microbiota-targeted strategies [67]. Overall, current evidence is preliminary, and further large-scale, longitudinal studies are needed to clarify the contribution of the microbiota-gut-brain axis to ADHD.

### 1.2.8. Parkinson's Disease (PD)

Parkinson's disease (PD) is a slowly progressive neurodegenerative disorder primarily characterized by motor dysfunction. Clinical features include hypomimia, monotonous and dysarthric speech, a stooped posture, bradykinesia, tremor, rigidity, pain, and reduced mobility. These symptoms result from the degeneration and loss of dopaminergic neurons in the substantia nigra, a key structure involved in motor control [68].

Recent studies suggest an association between gut microbiota composition and PD. The first investigations comparing PD patients with healthy controls reported significant alterations in microbial diversity and composition, prompting further research in this field. In a longitudinal study following patients for five years from the onset of motor symptoms, *Prevotellaceae* abundance was significantly reduced in PD patients, whereas *Enterobacteriaceae* levels were increased and positively correlated with postural instability and gait disturbance [69].

Other studies have similarly demonstrated differences in gut microbiota composition in untreated PD patients. At the phylum level, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia* were found to be increased in PD stool samples. At the genus level, a higher abundance of putative pro-inflammatory bacteria was observed [70]. Additionally, increased

*Lactobacillus* and reduced *Clostridium coccoides* and *Bacteroides fragilis* groups have been reported in PD patients compared with controls. These alterations were further validated using PCR-based quantification methods [71].

It should be noted that many studies involved small sample sizes, underscoring the need for larger, prospective investigations to determine whether microbiota alterations persist or evolve during disease progression. Preclinical studies have also suggested therapeutic potential: supplementation with prebiotic fibers such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS) improved motor, cognitive, and gastrointestinal symptoms in a mouse model of PD [72].

Overall, further research is required to clarify whether modulation of the gut microbiota can ameliorate both motor and non-motor symptoms of PD and influence disease progression.

### 1.2.9. Alzheimer's Disease and Dementia

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of dementia. A potential microbial contribution to AD pathogenesis has long been debated, and accumulating evidence suggests an association between gut microbiota alterations and the disease.

Studies comparing patients with AD to age-matched controls have reported reduced richness and diversity of gut microbiota in individuals with AD. In one study involving 25 patients with very mild to mild dementia and 25 matched controls, significant reductions in microbial diversity were observed in the AD group [73]. A subsequent study similarly identified compositional alterations at multiple taxonomic levels, although specific findings varied between studies [74].

Preclinical evidence further supports a possible microbiota-related mechanism. In the triple-transgenic (3xTg) mouse model of AD, administration of the probiotic mixture SLAB51 (containing lactic acid bacteria and bifidobacteria) reduced oxidative stress [75]. Another probiotic formulation including *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Bifidobacterium lactis*, and *Bifidobacterium longum* improved memory and learning performance in a hippocampal amyloid- $\beta$  (A $\beta$ ) model of AD [76]. Additionally, *Lactobacillus plantarum* has been reported to attenuate cognitive decline in experimental models [77].

Although current findings provide preliminary support for psychobiotic interventions as potential adjunctive strategies in AD, evidence remains limited. Altered gut microbiota profiles may serve as early biomarkers of disease; however, larger longitudinal studies are necessary to clarify the role of microbiota-gut-brain axis signaling in AD pathophysiology and progression.

### 1.2.10. Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system characterized by motor impairment, visual disturbances, and sensory deficits that often occur unpredictably [78].

Given its immune-mediated pathogenesis, recent research has investigated the potential role of the gut microbiota in MS and its therapeutic modulation. Experimental studies have demonstrated that transplantation of fecal microbiota from patients with MS into mice can induce autoimmune encephalomyelitis, a key pathological feature of MS. In contrast, germ-free (GF) mice exhibit resistance to developing experimental autoimmune encephalomyelitis, highlighting the contribution of gut microbiota - particularly gram-positive segmented filamentous bacteria - to disease development [79].

Although these findings suggest a potential mechanistic link between gut microbiota and MS, further well-designed studies are required to clarify causality and therapeutic implications.

## **2. CONCLUSION**

Understanding how the gut microbiota communicates with distant organs such as the brain remains an emerging area of research. Within this bidirectional network, the brain regulates intestinal motility as well as sensory and secretory functions, while gut-derived signals influence brain activity. This complex gut-brain axis is tightly regulated by the endocrine, immune, autonomic, and enteric nervous systems. A comprehensive understanding of this axis may provide novel strategies for the prevention and treatment of neurological and psychiatric disorders.

Recent preclinical and clinical evidence suggests that modulation of the gut microbiota through dietary, prebiotic, or probiotic interventions may represent a promising psychobiotic approach for mood disorders, neurodevelopmental conditions, and neurodegenerative diseases. Such interventions may partially restore dysbiosis associated with these disorders. Given the association between altered gut microbiota composition and various CNS-related conditions, microbiota-targeted modulation constitutes a potential therapeutic avenue.

Although animal studies support a therapeutic role for specific probiotics, robust, well-controlled clinical trials are required to establish their efficacy in humans. Ongoing research aims to identify disease-specific microbial signatures and microbial-derived signaling molecules that could enable personalized interventions, including tailored dietary strategies and microbiota-based therapies. These advances may facilitate early diagnosis, preventive strategies, and adjunctive treatments for CNS and psychiatric disorders.

### 3. REFERENCES

- Christian Milani, Sabrina Duranti, Francesca Bottacini, Eoghan Casey, Francesca Turrone, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiology and Molecular Biology Reviews* 81:e00036-17, 2017.
- Sai Manasa Jandhyala, Rupjyoti Talukdar, Chivkula Subramanyam, Harish Vuyyuru, Mitnala Sasikala, D Nageshwar Reddy. Role of the normal gut microbiota. *World J Gastroenterol*; 21(29): 8787-8803, 2015.
- P.J. Kennedy, J.F. Cryan, T.G. Dinan, G. Clarke. Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology* 112 399-412, 2017.
- Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. *Cell* 165: 1762-1775, 2016.
- Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. *Mol Psychiatry* 19: 146 -148, 2014.
- Robertson RC, Kaliannan K, Strain CR, Ross RP, Stanton C, Kang JX. Maternal omega-3 fatty acids regulate offspring obesity through persistent modulation of gut microbiota. *Microbiome* 6: 95, 2018.
- Gareau MG, Wine E, Rodrigues DM, Cho JH, Whary MT, et al. Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* 60: 307-317, 2011.
- Fröhlich EE, Farzi A, Mayerhofer R, Reichmann F, Jac'an A, et al. Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. *Brain Behav Immun* 56: 140 -155, 2016.
- Wang T, Hu X, Liang S, Li W, Wu X, Wang L, Jin F. *Lactobacillus fermentum* NS9 restores the antibiotic induced physiological and psychological abnormalities in rats. *Benef Microbes* 6: 707-717, 2015.
- Gronier B, Savignac HM, Di Miceli M, Idriss SM, Tzortzis G, et al. Increased cortical neuronal responses to NMDA and improved attentional set-shifting performance in rats following prebiotic (B-GOS®) ingestion. *Eur Neuropsychopharmacol* 28: 211-224, 2018.
- Davari S, Talaei SA, Alaei H, Salami M. Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis. *Neuroscience* 240: 287-296, 2013.
- Fernandez-Real JM, Serino M, Blasco G, Puig J, Ricart W, et al. Gut Microbiota Interacts With Brain Microstructure and Function. *J Clin Endocrinol Metab* 100: 4505- 4513, 2015.
- Carlson AL, Xia K, Goldman BD, Ahn M, Styner MA, et al. Infant Gut Microbiome Associated With Cognitive Development. *Biol Psychiatry* 83: 148 -159, 2018.
- Chung YC, Jin HM, Cui Y, Kim DS, Jung JM, et al. Fermented milk of *Lactobacillus helveticus* IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults. *J Funct Foods* 10: 465- 474, 2014.
- Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, et al. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 144: 1394 -1401.e4, 2013.

- Smith AP, Sutherland D, Hewlett P. An Investigation of the Acute Effects of Oligofructose-Enriched Inulin on Subjective Wellbeing, Mood and Cognitive Performance. *Nutrients* 7: 8887- 8896, 2015.
- Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, et al. Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial. *Front Aging Neurosci* 8: 256, 2016.
- Tillisch K, Mayer EA, Gupta A, Gill Z, Brazeilles Ret al. Brain Structure and Response to Emotional Stimuli as Related to Gut Microbial Profiles in Healthy Women. *Psychosom Med* 79: 905-913, 2017.
- Callaghan BL, Fields A, Gee DG, Gabard-Durnam L, Caldera C, et al. Mind and gut: associations between mood and gastrointestinal distress in children exposed to adversity. *Dev Psychopathol* 28: 1-20, 2019.
- Savignac HM, Tramullas M, Kiely B, Dinan TG, Cryan JF. *Bifidobacteria* modulate cognitive processes in an anxious mouse strain. *Behav Brain Res* 287: 59 -72, 2015.
- Callaghan BL, Cowan CSM, Richardson R. Treating generational stress: effect of paternal stress on offspring memory and extinction development is rescued by probiotic treatment. *Psychol Sci* 27: 1171-1180, 2016.
- Callaghan BL, Richardson R. Early experiences and the development of emotional learning systems in rats. *Biol Mood Anxiety Disord* 3: 8, 2013.
- Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, et al. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci USA* 108: 16050 -16055, 2011.
- Sun J, Wang F, Hu X, Yang C, Xu H, et al. *Clostridium butyricum* Attenuates Chronic Unpredictable Mild Stress-Induced Depressive-Like Behavior in Mice via the Gut-Brain Axis. *J Agric Food Chem* 66: 8415- 8421, 2018.
- Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience* 170: 1179 -1188, 2010.
- Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG. The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *J Psychiatr Res* 43: 164 -174, 2008.
- Barouei J, Moussavi M, Hodgson DM. Effect of maternal probiotic intervention on HPA axis, immunity and gut microbiota in a rat model of irritable bowel syndrome. *PLoS One* 7: e46051, 2012.
- Fukui H, Oshima T, Tanaka Y, Oikawa Y, Makizaki Y, et al. Effect of probiotic *Bifidobacterium bifidum* G9-1 on the relationship between gut microbiota profile and stress sensitivity in maternally separated rats. *Sci Rep* 8: 12384, 2018.
- Andersson H, Tullberg C, Ahrné S, Hamberg K, Lazou Ahrén I, et al. Oral Administration of *Lactobacillus plantarum* 299v Reduces Cortisol Levels in Human Saliva during Examination Induced Stress: A Randomized, Double-Blind Controlled Trial. *Int J Microbiol* 2016: 8469018, 2016.
- Chong HX, Yusoff NAA, Hor YY, Lew LC, Jaafar MH, et al. *Lactobacillus plantarum* DR7 alleviates stress and anxiety in adults: a randomised, double-blind, placebo controlled study. *Benef Microbes* 10: 355-373, 2019.

- Desbonnet L, Clarke G, Traplin A, O'Sullivan O, Crispie F, et al. Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav Immun* 48: 165-173, 2015.
- Thomas C Fung, Christine A Olson, Elaine Y Hsiao. Interactions between the microbiota, immune and nervous systems in health and disease. *Nature Neuroscience*, 20(2), 145-155, 2017.
- Santocchi E, Guiducci L, Fulceri F, Billeci L, Buzzigoli E, et al. Gut to brain interaction in Autism Spectrum Disorders: a randomized controlled trial on the role of probiotics on clinical, biochemical and neurophysiological parameters. *BMC psychiatry*. 16(1):183, 2016.
- Mayer EA, Padua D, Tillisch K. Altered brain-gut axis in autism: Comorbidity or causative mechanisms? *Bioessays*. 36(10):933-9, 2014.
- Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, et al. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe*. 16(4):444-53, 2010.
- Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism-comparisons to typical children and correlation with autism severity. *BMC gastroenterology*. 11(1):22, 2011.
- Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Journal of medical microbiology*. 54(10):987-91, 2005.
- Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. *Applied and environmental microbiology*. 70(11):6459-65, 2004.
- Tomova A, Husarova V, Lakatosova S, Bakos J, Vlkova B, et al. Gastrointestinal microbiota in children with autism in Slovakia. *Physiol Behav*. 138:179-87, 2015.
- Williams BL, Hornig M, Parekh T, Lipkin WI. Application of novel PCR based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBio*. 3(1):e00261-11, 2012.
- Heberling CA, Dhurjati PS, Sasser M. Hypothesis for a systems connectivity model of autism spectrum disorder pathogenesis: Links to gut bacteria, oxidative stress, and intestinal permeability. *Medical hypotheses*. 80(3):264-70, 2013.
- Stephanie G. Cheung, Ariel R. Goldenthal, Anne-Catrin Uhlemann, J. John Mann, Jeffrey M. Miller, M. Elizabeth Sublette. Systematic Review of Gut Microbiota and Major Depression. *Front. Psychiatry* 2019.
- Rieder R, Wisniewski PJ, Alderman BL, Campbell SC. Microbes and mental health: A review. *Brain Behav Immun*. 66:9-17, 2017.
- Chopra K, Kumar B, Kuhad A. Pathobiological targets of depression. *Expert Opin Ther Targets* 15: 379- 400, 2011.
- Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. *Neuroscience*. 170(4):1179-88, 2010.
- Liu Y-W, Liu W-H, Wu C-C, Juan Y-C, Wu Y-C, et al. Psychotropic effects of *Lactobacillus plantarum* PS128 in early life-stressed and naïve adult mice. *Brain research*. 1631:112, 2016.

- Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*. 35(2):146-55, 1999.
- Savignac H, Kiely B, Dinan T, Cryan J. B ifidobacteria exert strain-specific effects on stress-related behavior and physiology in BALB/c mice. *Neurogastroenterology & Motility*. 26(11):1615-27, 2014.
- Jane A. Foster, Karen-Anne McVey Neufeld. Gut-brain axis: how the microbiome influences anxiety and depression. Volume 36, Issue 5, P305-312, May 01, 2013.
- Rowland AS, Lesesne CA, Abramowitz AJ. The epidemiology of attention-deficit/hyperactivity disorder (ADHD): a public health view. *Mental retardation and developmental disabilities research reviews*. 8(3):162-70, 2002.
- Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *The Lancet Psychiatry*. 2(3):258-70, 2015.
- Schwarz E, Maukonen J, Hyytiäinen T, Kieseppä T, Orešič M, et al. Analysis of microbiota in first episode psychosis identifies preliminary associations with symptom severity and treatment response. *Schizophrenia research*. 192:398-403, 2018.
- Castro-Nallar E, Bendall ML, Pérez-Losada M, Sabuncyan S, Severance EG, et al. Composition, taxonomy and functional diversity of the oropharynx microbiome in individuals with schizophrenia and controls. *PeerJ*. 3:e1140, 2015.
- Yolken RH, Severance EG, Sabuncyan S, Gressitt KL, Chen O, et al. Metagenomic sequencing indicates that the oropharyngeal phageome of individuals with schizophrenia differs from that of controls. *Schizophrenia bulletin*. 41(5):1153-61, 2015.
- Jørgensen BP, Krych L, Pedersen TB, Plath N, Redrobe JP, et al. Investigating the long-term effect of subchronic phencyclidine-treatment on novel object recognition and the association between the gut microbiota and behavior in the animal model of schizophrenia. *Physiology & behavior*. 141:32-9, 2015.
- Berit Kerner. Genetics of bipolar disorder. *Appl Clin Genet*. 7: 33-42, 2014.
- Dickerson F, Adamos M, Katsafanas E, Khushalani S, Origoni A, et al. Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: a randomized controlled trial. *Bipolar Disord* 20: 614 - 621, 2018.
- Koob GF. The dark side of emotion: the addiction perspective. *Eur J Pharmacol* 753: 73- 87, 2015.
- Lowe PP, Gyongyosi B, Satishchandran A, Iracheta-Vellve A, Cho Y, et al. Reduced gut microbiome protects from alcohol-induced neuroinflammation and alters intestinal and brain inflammasome expression. *J Neuroinflammation* 15: 298, 2018.
- Bull-Otterson L, Feng W, Kirpich I, Wang Y, Qin X, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment. *PLoS One* 8: e53028, 2013.
- Jadhav KS, Peterson VL, Halfon O, Ahern G, Fouhy F, et al. Gut microbiome correlates with altered striatal dopamine receptor expression in a model of compulsive alcohol seeking. *Neuropharmacology* 141: 249 - 259, 2018.

- Allais L, Kerckhof FM, Verschuere S, Bracke KR, De Smet R, et al. Chronic cigarette smoke exposure induces microbial and inflammatory shifts and mucin changes in the murine gut. *Environ Microbiol* 18: 1352-1363, 2016.
- Lee K, Vuong HE, Nusbaum DJ, Hsiao EY, Evans CJ, Taylor AMW. The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence. *Neuropsychopharmacology* 43: 2606 -2614, 2018.
- Cluny NL, Keenan CM, Reimer RA, Le Foll B, Sharkey KA. Prevention of Diet Induced Obesity Effects on Body Weight and Gut Microbiota in Mice Treated Chronically with  $\Delta$ 9-Tetrahydrocannabinol. *PLoS One* 10: e0144270, 2015.
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Philadelphia, PA: American Psy.
- Aarts E, Ederveen THA, Naaijen J, Zwieters MP, Boekhorst J, et al. Gut microbiome in ADHD and its relation to neural reward anticipation. *PLoS One* 12: e0183509, 2017.
- Pärty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. *Pediatr Res* 77: 823- 828, 2015.
- Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurology of axons. *Ann Neurol* 67: 715-725, 2010.
- Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord* 30: 350 - 358, 2015.
- Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord* 30: 1351-1360, 2015.
- Hasegawa S, Goto S, Tsuji H, Okuno T, Asahara T, et al. Intestinal Dysbiosis and Lowered Serum Lipopolysaccharide-Binding Protein in Parkinson's Disease. *PLoS One* 10: e0142164, 2015.
- Perez-Pardo P, Kliet T, Dodiya HB, Broersen LM, Garssen J, et al. The gut-brain axis in Parkinson's disease: possibilities for food-based therapies. *Eur J Pharmacol* 817: 86 - 95, 2017.
- Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, et al. Gut microbiome alterations in Alzheimer's disease. *Sci Rep* 7: 13537, 2017.
- Zhuang ZQ, Shen LL, Li WW, Fu X, Zeng F, et al. Gut Microbiota Is Altered in Patients with Alzheimer's Disease. *J Alzheimers Dis* 63: 1337-1346, 2018.
- Bonfili L, Cecarini V, Cuccioloni M, Angeletti M, Berardi S, et al. SLAB51 Probiotic Formulation Activates SIRT1 Pathway Promoting Antioxidant and Neuroprotective Effects in an AD Mouse Model. *Mol Neurobiol* 55: 7987- 8000, 2018.
- Athari Nik Azm S, Djazayeri A, Safa M, Azami K, Ahmadvand B, et al. *Lactobacilli* and *bifidobacteria* ameliorate memory and learning deficits and oxidative stress in  $\beta$ -amyloid (1-42) injected rats. *Appl Physiol Nutr Metab* 43: 718 -726, 2018.
- Nimgampalle M, Kuna Y. Anti-Alzheimer Properties of Probiotic, *Lactobacillus plantarum* MTCC 1325 in Alzheimer's Disease induced Albino Rats. *J Clin Diagn Res* 11: KC01-KC05, 2017.
- Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nat Rev Immunol* 15: 545-558, 2015.

Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc Natl Acad Sci USA 114: 10719 -10724, 2017.