e-ISSN: 2149-3189

European Research Journal

Volume 12 Issue 1 January 2026

Available at https://dergipark.org.tr/en/pub/eurj

Published by Prusa Medical Publishing

The European Research Journal

Aim and Scope

The European Research Journal (EuRJ) is an international, independent, double-blind peer reviewed, Open Access and online publishing journal, which aims to publish papers on all the related areas of basic and clinical medicine.

Editorial Board of the European Research Journal complies with the criteria of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), and Committee on Publication Ethics (COPE).

The journal publishes a variety of manuscripts including original research, case reports, invited review articles, technical reports, how-to-do it, interesting images and letters to the editor. The European Research Journal has signed the declaration of the Budapest Open Access Initiative. All articles are detected for similarity or plagiarism. Publication language is English.

EuRJ recommends that all of our authors obtain their own ORCID identifier which will be included on their article.

The journal is published monthly (12 issues per year)

Abstracting and Indexing

The journal is abstracted and indexed with the following: ULAKBIM TR Index (ULAKBİM TR DİZİN), NLM Catalog (NLM ID: 101685727), Google Scholar (h-index: 15), EMBASE, ProQuest Central, EBSCO Academic Search Ultimate, J-Gate, EZB, TURK MEDLINE, Turkish Citation Index, ResearhGate, SOBIAD, ScienceGate, Publons, (Clarivate Web of Science)

Publisher

The European Research Journal (EuRJ)
Prusa Medical Publishing
Konak Mh. Kudret Sk. Şenyurt İş Mrk. Blok No:6 İç kapı no: 3
Nilüfer/Bursa-Türkiye
info@prusamp.com

https://dergipark.org.tr/en/pub/eurj
https://www.prusamp.com

e-ISSN: 2149-3189

The European Research Journal, hosted by DergiPark ACADEMIC, is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

EDITORIAL BOARD

EDITOR-IN-CHIEF

Senol YAVUZ, MD.,
Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Cardiovascular Surgery,
Bursa, Türkiye

EDITORS

Soner CANDER, MD.,
Professor,
Uludag University Medical School,
Department of Endocrinology and Metabolism,
Bursa, Türkiye

Mesut ENGİN, MD.,
Associate Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Cardiovascular Surgery,
Bursa, Türkiye

OWNER ON BEHALF OF THE PRUSA MEDİCAL PUBLISHING

Rustem ASKIN, MD.,
Professor of Psychiatry,
İstanbul Ticaret University, Department of Psychology,
İstanbul, Türkiye

ASSISTANT EDITOR

Ugur BOLUKBAS,
Ministry Of Health Bursa Oral And Dental Health Training And Research Hospital
Bursa, Türkiye

SECTION EDITORS

Omer SENORMANCI, MD.,
Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Psychiatry,
Bursa, Türkiye

Mahmut KALEM, MD.,
Associate Professor,
Ankara University Medical School,
Department of Orthopedics and Traumatology,
Ankara, Türkiye

Meliha KASAPOGLU AKSOY, MD.,
Associate Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Physical Therapy and Rehabilitation,
Bursa, Türkiye

Arda ISIK, MD.,
Associate Professor,
Medeniyet University School of Medicine,
Department of General Surgery,
Istanbul, Türkiye

Kadir Kaan OZSIN, MD.,
Associate Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Cardiovascular Surgery,
Bursa, Türkiye

Cihan AYDIN, MD.,
Associate Professor,
Tekirdağ Namık Kemal University, Faculty of Medicine,
Department of Cardiology,
Tekirdağ, Türkiye

Sayad KOCAHAN, PhD.,
Professor,
University of Health Sciences, Gülhane Medical Faculty,
Department of Physiology,
Ankara, Türkiye

Gokhan OCAKOGLU, PhD.,
Associate Professor,
Uludag University School of Medicine,
Department of Biostatistics,
Bursa, Türkiye

Nurullah DOGAN, MD.,
Associate Professor,
Doruk Nilüfer Hospital,
Department of Radiology,
Bursa, Türkiye

Ömer Faruk KARATAS, PhD.,
Professor,
Erzurum Technical University,
Department of Molecular Biology and Genetics,
Erzurum, Türkiye

Serhat YALÇINKAYA, PhD.,
Associate Professor,
Priavate Bursa NEV Health Group,
Department of Thoracic Surgery,
Bursa, Türkiye

Gülten ÖZGEN,
Associate Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Gynecology and Obstetrics,
Bursa, Türkiye

Tuğba ONUR,
Associate Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Anesthesiology,
Bursa, Türkiye

Furkan SARIDAŞ,
Associate Professor,
Uludag University Medical School,
Department of Neurology and Neuromuscular Diseases,
Bursa, Türkiye

LANGUAGE EDITOR

İsmail SİVRİ,
Research Assistant,
Kocaeli University School of Medicine,
Department of Anatomy,
Kocaeli, Türkiye

ETHICAL EDITOR

Metin GÜÇLÜ, MD.,
Professor,
Düzce University School of Medicine,
Department of Endocrinology,
Düzce, Türkiye

SCIENTIFIC ADVISORY BOARD

Melih CEKINMEZ, MD.,
Professor,
University of Health Sciences, Adana City Training & Research Hospital,
Department of Neurosurgery,
Adana, Türkiye

Evren DİLEKTAŞLI, MD.,
Professor,
VM Medical Park Bursa Hospital
Department of General Surgery,
Bursa, Türkiye

Nurcan ÖZYAZICIOĞLU,
Professor,
Department Nursing and Health Sciences
Bursa Uludağ Unibersity
Bursa, Türkiye

Burcu DİNÇGEZ, MD.,
Professor,
University of Health Sciences, Bursa Yuksek Ihtisas Training & Research Hospital,
Department of Gynecology and Obstetrics,
Bursa, Türkiye

Yenal DUNDAR, MD.,
Consultant Psychiatrist
University of Liverpool,
Liverpool, UK

Başar CANDER, MD.,
Professor,
Bezmialem Vakif University,
Department of Emergency Medicine
İstanbul, Türkiye

Aylin COLPAN, MD.,
Associate Professor,
Jefferson University-Lehigh Valley Hospital
Department of Infectious Diseases
Allentown, ABD

Table of Contents

Original Articles

The European Research Journal 2026;12(1):1-12 Original Article

DOI: https://doi.org/10.18621/eurj.1792973 *Histology and Embryology*

Mitigating High-Fat Diet-Induced Testicular Oxidative Stress and Fibrosis with Bromelain

Burcu Gültekin[1], Raviye Özen Koca[2], Halime Tuba Canbaz[3], Hasan Basri Savaş[4], Mustafa Berk Başaran[2], Z. Işık Solak Görmüş[2]

[1]Department of Histology and Embryology, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye; [2]Department of Physiology, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye; [3]Department of Histology and Embryology, Hamidiye Faculty of Medicine, University of Health Sciences, Istanbul, Türkiye; [4]Department of Medical Biochemistry, Mardin Artuklu University, Faculty of Medicine, Mardin, Türkiye

ABSTRACT

Objectives: Obesity induced by a high-fat diet (HFD) is closely associated with impaired male fertility, primarily through oxidative stress, inflammation, and testicular dysfunction. Natural compounds with antioxidant and anti-inflammatory properties have gained increasing attention for their potential therapeutic effects. Bromelain, a proteolytic enzyme complex derived from Ananas comosus, exhibits antioxidant, anti-inflammatory, and antifibrotic activities, suggesting potential protective effects against obesity-related reproductive impairments.

Methods: Male Wistar rats were randomly assigned to four groups (n=9 per group). Animals were fed an HFD for 12 weeks to induce obesity, followed by one month of bromelain supplementation. Testicular tissues were histologically assessed using hematoxylin & eosin (H&E) and Masson's trichrome staining. Serum and testicular samples were analyzed for antioxidant and oxidative stress markers, including paraoxonase-1 (PON1), arylesterase (ARE), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI).

Results: The HFD group showed significant testicular alterations, including thickening of the tunica albuginea, perivascular collagen accumulation, germ cell loss, and disrupted seminiferous tubule architecture ($P<0.0001$). Biochemically, TAS ($P<0.0001$) and PON1 ($P=0.0041$) levels were significantly decreased, whereas TOS ($P<0.0001$) and OSI ($P<0.0001$) levels were elevated. Bromelain supplementation mitigated histopathological changes, reducing collagen deposition and tunica albuginea thickness. Moreover, Bromelain significantly restored TAS and PON1 levels while decreasing TOS and OSI. No significant differences were observed in ARE ($P=0.0002$) activity between the groups.

Conclusions: Bromelain supplementation attenuated HFD-induced oxidative stress and fibrotic alterations in testicular tissue, improving both histological and biochemical parameters. These findings suggest that Br may be a potentially beneficial natural supplement for alleviating obesity-induced impairments in male reproductive functions and associated metabolic dysfunctions.

Keywords: Bromelain, Fibrosis, Oxidative Stress, Testis, High-Fat Diet

Received: September 29, 2025 **Accepted:** November 19, 2025 **Available Online:** November 28, 2025 **Published:** January 4, 2026

How to cite this article: Gültekin B, Özen Koca R, Canbaz HT, Savaş HB, Başaran MB, Solak Görmüş ZI. Mitigating High-Fat Diet-Induced Testicular Oxidative Stress and Fibrosis with Bromelain. Eur Res J. 2026;12(1):1-12. doi: 10.18621/eurj.1792973

Corresponding author: Burcu Gültekin, PhD., Phone: +90 332 221 05 00, E-mail: bgultekin@erbakan.edu.tr

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

Long-term consumption of a high-fat diet (HFD) leads to obesity, increased body weight, and disturbances in glucose and lipid metabolism, contributing to the development of various metabolic disorders [1, 2]. Accumulating evidence indicates that diet-induced obesity negatively impacts male fertility [3, 4]. High-calorie diets and obesity can directly or indirectly impair sperm quality through multiple mechanisms, including inflammatory responses, oxidative stress, alterations in gut microbiota, and sperm DNA damage and remodeling. The cumulative effects of these mechanisms may result in irreversible structural and functional alterations in sperm cells [5]. Moreover, obesity adversely affects spermatogenesis via hormonal imbalances, elevated leptin and estrogen levels, increased testicular temperature, and oxidative stress [6]. Oxidative stress, resulting from the oxidation of various cellular components, increases reactive oxygen species (ROS) levels, which can cause testicular atrophy, structural disruption of the seminiferous epithelium, germ cell loss, apoptosis in Sertoli and Leydig cells, reduced steroidogenesis, and impaired sperm production and motility [7]. Excess adipose tissue in obesity triggers systemic inflammatory responses and promotes oxidative stress through adipose-derived proinflammatory mediators, further compromising semen parameters [8]. Spermatozoa are particularly vulnerable to oxidative stress due to limited antioxidant defenses and insufficient DNA damage detection and repair capacity [9]. Additionally, studies have reported that HFD-induced endoplasmic reticulum (ER) stress contributes to reduced sperm motility and male reproductive dysfunction through mitochondrial signaling pathways [10, 11]. Nutritional supplements and plant-derived (phytochemical) preparations with antioxidant capacity have shown beneficial effects by modulating apoptosis and inflammatory processes. Therefore, these natural compounds are considered promising strategies for managing male infertility, particularly obesity-associated infertility, as antioxidant therapies and lifestyle modifications effectively mitigate oxidative stress-related reproductive impairments [12]. Bromelain (Br), a crude extract derived from the stem and fruit of Ananas comosus, consists of a group of thiol endopeptidases and other bioactive components, exhibiting diverse biological functions as a natural anti-inflammatory enzyme [13]. Its medicinal properties are primarily attributed to proteolytic enzymes (proteases) and sulfur-containing protein-digesting enzymes, along with minor components such as peroxidases, acid phosphatases, protease inhibitors, and calcium ions. The biological activity of bromelain relies on the synergistic effects of these enzymes and proteins in modulating biochemical reactions within the organism [14]. Due to its anti-inflammatory, antioxidant, antiangiogenic, and analgesic properties, Br is considered a potential therapeutic and protective agent against inflammatory and oxidative stress-related pathologies, including testicular and nephrotoxicity [15-17]. Previous studies have demonstrated the protective potential of Br against various testicular impairments induced by different stressors. For instance, Jebur *et al*. [16] reported that aluminum chloride exposure causes oxidative stress, hormonal imbalance, and deterioration of sperm quality, whereas pretreatment with bromelain alleviated these harmful effects by enhancing antioxidant defense, suppressing ROS production, and improving testicular function. Similarly, Hosseinpour *et al*. [18] showed that bromelain exerts protective effects against Rapid Eye Movement (REM) sleep deprivation–induced testicular dysfunction through its strong antioxidant and anti-inflammatory properties. These findings collectively suggest that bromelain may play a beneficial role in preserving testicular integrity under conditions of oxidative and inflammatory stress.

HFD is a widely used model for inducing obesity. Obesity can negatively affect testicular function, causing decreased testosterone levels, increased oxidative stress, and impaired sperm production. Therefore, the HFD model provides a suitable experimental approach for studying obesity-related male reproductive disorders. In this study, we aimed to evaluate the protective and therapeutic effects of Br on testicular structure and biochemical changes in rats with HFD-induced obesity, and to clarify its possible role in reducing obesity-related reproductive disorders.

METHODS

Animals and Experimental Design

A total of 36 adult male Wistar Albino rats weighing 250-300 g were used in this study. All experimental procedures were conducted in accordance with the ARRIVE guidelines and approved by the Local Ethics

Committee of Necmettin Erbakan University (decision no: 2024-117, dated 28 November 2024). Animal care and use complied with the Guide for the Care and Use of Laboratory Animals. Rats were obtained from the Experimental Medicine Application and Research Center (KONÜDAM) of Necmettin Erbakan University. Animals were randomly assigned to four groups (n=9 per group): (1) standard diet control (SD), (2) standard diet + bromelain (SD+Br), (3) high-fat diet (HFD), and (4) high-fat diet + bromelain (HFD+Br). Animals were housed under controlled conditions at 21±2 °C with a 12 h light/12 h dark photoperiod. SD and SD+Br groups received standard chow and water ad libitum for 12 weeks, whereas HFD and HFD+Br groups were fed a high-fat diet ad libitum for the same period. Body weights were monitored regularly.

Obesity Induction and Bromelain Administration

Obesity was induced in the HFD and HFD+Br groups over a 3-month period using a high-fat feed (Arden Research & Experiment Company, Ankara, Turkey). According to the manufacturer's nutritional analysis, this diet consisted of 24% protein, 30% carbohydrate, and 35% fat (primarily from vegetable oils), providing a total energy content of 5.2 kcal/g, approximately 45% of which was derived from fat. Rats in the SD and SD+Br groups received standard rat chow. During the establishment of the obesity model, body weights of all rats were monitored regularly [7]. Baseline weights were recorded, and subsequent measurements were taken approximately every 10 days. After the obesity model was established, rats in the SD+Br and HFD+Br groups were administered bromelain via oral gavage at a dose of 200 mg/kg/day for 30 days [19]. The bromelain preparation, with a digestive activity of 2400 GDU/g (Meteoric Biopharmaceutical, batch number: BM2403332), was freshly dissolved in physiological saline for daily administration. Each rat received 1 mL/100 g body weight (200 mg/kg) using a 16-gauge flexible feeding needle. Dosing was performed each morning (09:00–10:00) after absorption. A single researcher gently restrained each rat, inserted the gavage needle, and visually confirmed swallowing to ensure full administration of the dose. Any regurgitation events were immediately addressed with repeated dosing, and all incidents were recorded. Rats in the SD and HFD groups were similarly handled and administered 1 mL/100 g body weight of physiological saline via oral gavage to control for handling and vehicle effects.

Histological Analyses

Testicular tissues were surgically excised and fixed in 10% formalin, dehydrated in graded alcohol series (70-100%), cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were cut and stained with hematoxylin–eosin (H&E) and Masson's trichrome. All histological evaluations were performed in a blinded manner by two independent observers to minimize observer bias. For each specimen, systematic random sampling was employed to select microscopic fields, ensuring representative and unbiased assessment of the tissue sections.

Hematoxylin & Eosin (H&E) Staining

Tissue sections obtained from paraffin-embedded blocks were deparaffinized by immersion in xylene for three consecutive 20-minute intervals. The sections were then sequentially rehydrated through a graded ethanol series (100%, 90%, 80%, 70%, and 50%) and rinsed with distilled water to remove residual alcohol. For nuclear staining, sections were immersed in hematoxylin solution, followed by thorough washing under running tap water. Differentiation was performed using acid alcohol to remove excess stain, and sections were subsequently blued in alkaline water. Eosin staining was then applied to visualize cytoplasmic components. The sections were dehydrated through an ascending ethanol series (70%, 80%, 90%, and 100%), cleared in xylene, and mounted with a coverslip using an appropriate mounting medium for microscopic examination.

In H&E stained testicular sections, the histological structure of the seminiferous tubules and interstitial areas was evaluated in detail. For each experimental group, ten randomly selected seminiferous tubule areas were examined under ×40 objective magnification using a light microscope (Axiocam Erc 5s, Carl Zeiss AG, Germany). Histopathological changes in the seminiferous tubules and disruptions in spermatogenesis were scored according to Johnsen's spermatogenesis evaluation criteria. Scoring was performed in a blinded manner, and the collected data were subjected to statistical analysis (Table 1) [20].

TABLE 1. Johnsen Scoring System for the Evaluation of Spermatogenesis.

Score	Evaluation of spermatogenesis
1	No cells visualized in tubular cross section
2	Sertoli cells only
3	Only spermatogonia present
4	No sperm cells or spermatids, few spermatocytes (<5)
5	No sperm cells or spermatids, presence of spermatocytes
6	No sperm cells, few spermatids (<5 to 10)
7	No sperm cells, presence of spermatids
8	Presence of few sperm cells (<5 to 10)
9	Some sperm cells, with a disorganized epithelium
10	Compete spermatogenesis with mature sperm cells

Masson's Trichrome Staining

Sections of 5 µm thickness were obtained from the testis blocks of experimental groups to assess fibrosis. Deparaffinization was conducted by passing the sections through xylene and a descending alcohol series (90%, 80%, 70%, and 50%). Masson's Trichrome Stain Kit (ChemBio, CB6095.0200, İstanbul, Türkiye) was then applied. The sections were covered with Entellan® after final rinsing with the alcohol series and xylene.

Biochemical Analyses

At the end of the experimental period, 10 mL of blood was collected from all animals into gel-coated biochemical tubes. The collected blood samples were centrifuged at 1500 × g for 10 minutes to obtain the serum fraction. The serum was aliquoted into labeled Eppendorf tubes and stored at −80 °C until analysis. On the day of analysis, all serum samples were thawed simultaneously to room temperature and vortexed to ensure homogeneity before biochemical measurements. Tissue samples obtained post-anesthesia were homogenized, sonicated, centrifuged, and the resulting supernatants were used for biochemical assays. Oxidative stress parameters analyzed in serum included paraoxonase-1 (PON1) and arylesterase (ARE). Serum PON1 (Rel Assay Diagnostics Kits, Cat. no: RL0031, Mega Tıp San., Gaziantep, Turkey) and ARE (Rel Assay Diagnostics Kits, Cat. no: RL0055, Mega Tıp San., Gaziantep, Turkey) activities were measured according to the manufacturer's protocols. In tissue samples, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were determined.

Measurement of PON1 Activity

Serum PON1 activity was assessed using a fully automated method developed by Rel Assay Diagnostics (Cat. no: RL0031). The assay evaluates paraoxonase activity based on the hydrolysis of paraoxon (diethyl p-nitrophenyl phosphate) in the presence of NaCl (basal and salt-stimulated activity). The formation of p-nitrophenol was monitored by absorbance increase at 412 nm at 37 °C. The amount of p-nitrophenol was calculated using a molar extinction coefficient of 17,000 M-1 cm-1 at pH 8. Net enzymatic activity was calculated by subtracting basal activity from salt-stimulated activity and expressed in units per liter, where 1 unit corresponds to the hydrolysis of 1 µmol of substrate per minute per liter [21].

Measurement of ARE Activity

Serum ARE activity was determined using a fully automated method (Rel Assay Diagnostics, Cat. no: RL0055). Phenyl acetate was used as a substrate and hydrolyzed to produce phenol and acetic acid. The resulting phenol reacted with 4-aminoantipyrine and potassium ferricyanide to form a colored complex, measured spectrophotometrically. ARE activity was calculated using the molar absorption coefficient of the colored complex and expressed as units per liter, with 1 unit defined as the hydrolysis of 1 µmol of phenyl acetate per minute per liter [22].

Measurement of TAS

TAS was measured using commercially available kits (Rel Assay Diagnostics Kits, Cat. no: RL0017). The fully automated method is based on the suppression of the characteristic color of the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants in the sample. The assay has an ideal deviation of less than 3%, and results were expressed in mmol Trolox equivalents per liter [23].

Measurement of TOS

TOS was measured using commercially available kits (Rel Assay Diagnostics Kits, Cat. no: RL0024). In this method, oxidants in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion, which in an acidic medium reacts with xylenol orange to form a colored complex. The color intensity, measured spectrophotometrically, is proportional to the total oxidant molecules present. Results were expressed in μmol hydrogen peroxide equivalents per liter (μmol H_2O_2 equiv/L) [24].

Calculation of OSI

The oxidative stress index (OSI) was calculated as the ratio of TOS to TAS. TAS values were converted to μmol/L, and OSI was determined using the following formula:

$$\text{OSI (arbitrary unit)} = \frac{TOS\ (\mu mol\ H_2O_2\ equivalent/L)}{TAS\ (mmol\ Trolox\ equivalent/L)} \times 100$$ [25, 26].

Statistical Analysis

Normality was assessed using the Shapiro–Wilk test. Intergroup differences were analyzed using one-way ANOVA followed by Tukey's post hoc test when appropriate. Data are presented as mean ± standard deviation (SD), and $P<0.05$ was considered statistically significant. Statistical analyses were performed using Microsoft Office 365 and GraphPad Prism 8.

RESULTS

Hematoxylin-Eosin Staining Findings

Microscopic examination of H&E-stained testicular sections revealed that the SD and SD+Br groups exhibited normal seminiferous tubule architecture, characterized by an intact basal membrane with spermatogonia, dispersed Sertoli cells, and well-organized germinal cells, including spermatids and spermatozoa.

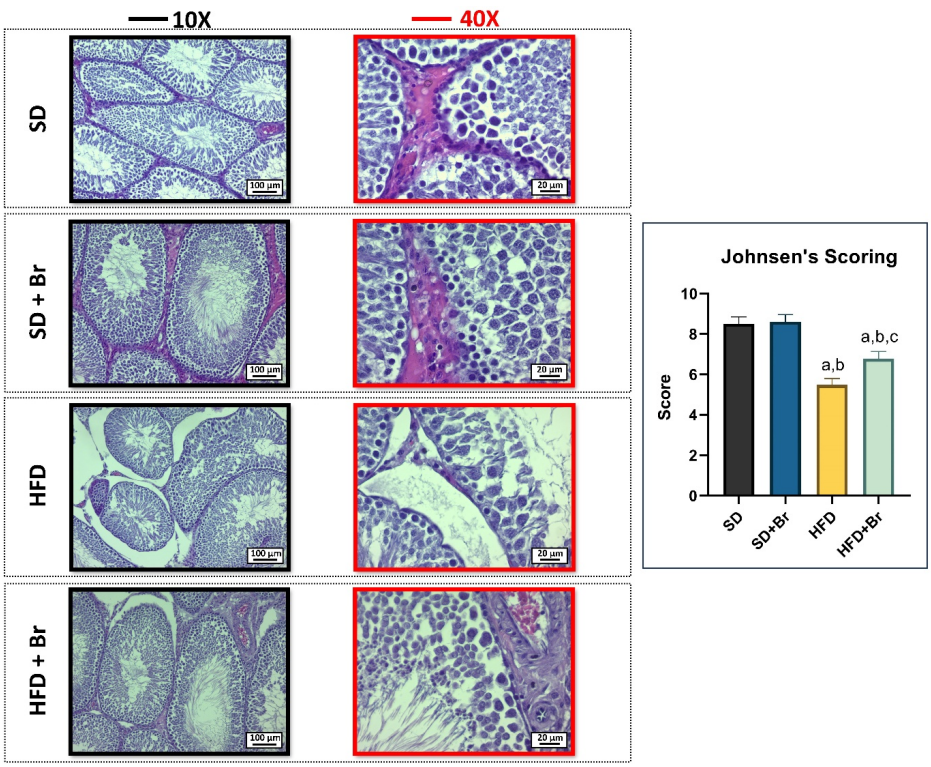

FIGURE 1. Hematoxylin–Eosin (H&E) staining of testicular tissue sections. Each experimental group consisted of 9 rats. In the standard diet (SD) and standard diet+Bromelain (SD+Br) groups, seminiferous tubules exhibited normal architecture with spermatogonia in the basal membrane, dispersed Sertoli cells, well-organized germinal cells, and morphologically normal Leydig cells. In the high-fat diet (HFD) group, seminiferous tubules displayed germ cell degeneration, vacuolization, atrophy, disorganization, and a reduction in the interstitial area. In the high-fat diet+Bromelain (HFD+Br) group, focal atrophy was observed; however, the overall seminiferous tubular structure was largely preserved, and the interstitial area appeared normal. Statistical analysis of Johnsen's scores is presented as mean±SD. [a](P<0.05 vs. SD), [b](P<0.05 vs. SD+Br), [c](P<0.05 vs. HFD).

The interstitial connective tissue and Leydig cells appeared structurally intact. In contrast, testicular sections from the HFD group demonstrated marked histopathological alterations in germ cells, including degeneration, vacuolization, atrophy, and disorganization of the seminiferous epithelium. The interstitial space was also reduced in this group. In the HFD+Br group, focal atrophy of seminiferous tubules was observed; however, the overall tubular architecture was largely preserved, and the interstitial area maintained normal morphology. Mean Johnsen scores were calculated for 10 randomly selected seminiferous tubules per animal according to the criteria outlined in Table 1. Statistical analysis revealed that the HFD and HFD+Br groups differed significantly from the SD and SD+Br groups. While the HFD+Br group had significantly lower scores than the SD and SD+Br groups, it showed significantly higher scores compared to the HFD group ($P<0.0001$) (Figure 1).

Masson's Trichrome Staining of Testicular Tissue

Masson's Trichrome staining was performed to visualize collagen fibers and fibrosis in the connective tissue. In the SD group, the tunica albuginea layers of the testes exhibited normal thickness, whereas in the SD+Br group, the tunica albuginea was slightly thinner. In the HFD group, the tunica albuginea layers were observed to be thicker compared to the SD and SD+Br groups. In the HFD+Br group, the tunica albuginea thickness resembled that of the SD group. Examination of collagen fibers in the perivascular area revealed an increase in the HFD group relative to the other groups (Figure 2).

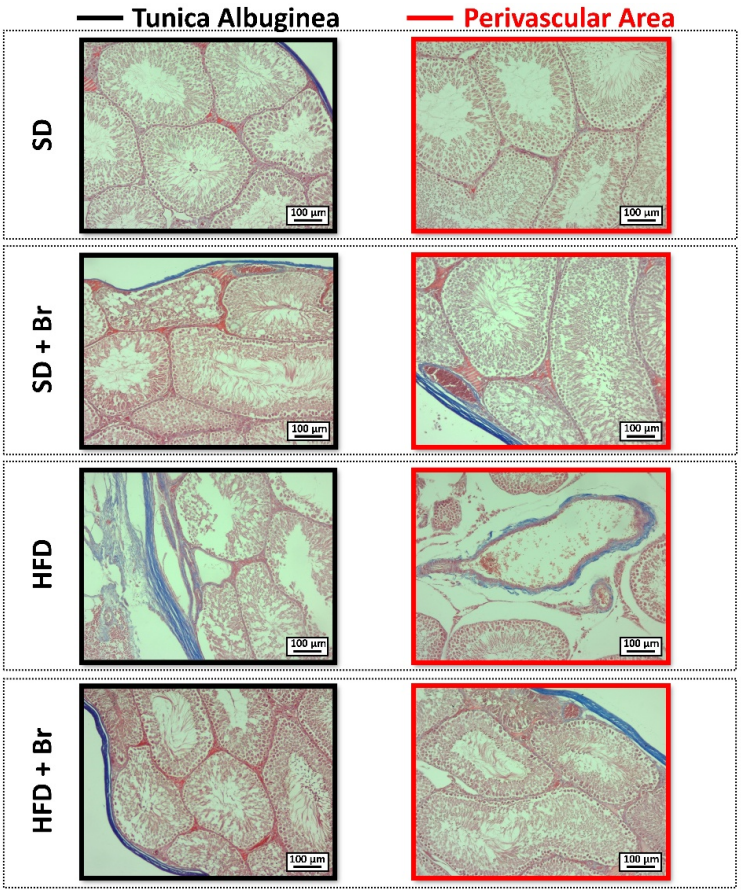

FIGURE 2. Masson's Trichrome staining of testicular tissue. Each experimental group consisted of 9 rats. In the standard diet (SD) group, the tunica albuginea exhibited normal thickness, while in the standard diet + Bromelain (SD+Br) group, slight thinning was observed. In the high-fat diet (HFD) group, the tunica albuginea was markedly thickened, and an increased amount of collagen fibers was observed in the perivascular area. In the high-fat diet+Bromelain (HFD+Br) group, the tunica albuginea thickness was similar to that of the SD group. Areas stained blue indicate the density of collagen fibers.

Biochemical Findings

Serum PON1 and ARE Results

PON1 enzyme activity, which decreases under conditions of elevated oxidative stress and organ damage, was found to be high and comparable in the SD and SD+Br groups, whereas it was lower in HFD group. In the HFD+Br group, PON1 activity was lower than in the SD and SD+Br groups but slightly higher than in the HFD group. Statistical analysis revealed that PON1 levels in the HFD group were significantly different from those in the SD, SD+Br, and HFD+Br groups (P=0.0041). When the ARE enzyme activities were examined across all groups, the activity in the HFD group was found to be significantly decreased compared to the SD+Br group (P=0.0002).

Tissue TAS, TOS, and OSI Results

TAS, an important biochemical parameter reflect-

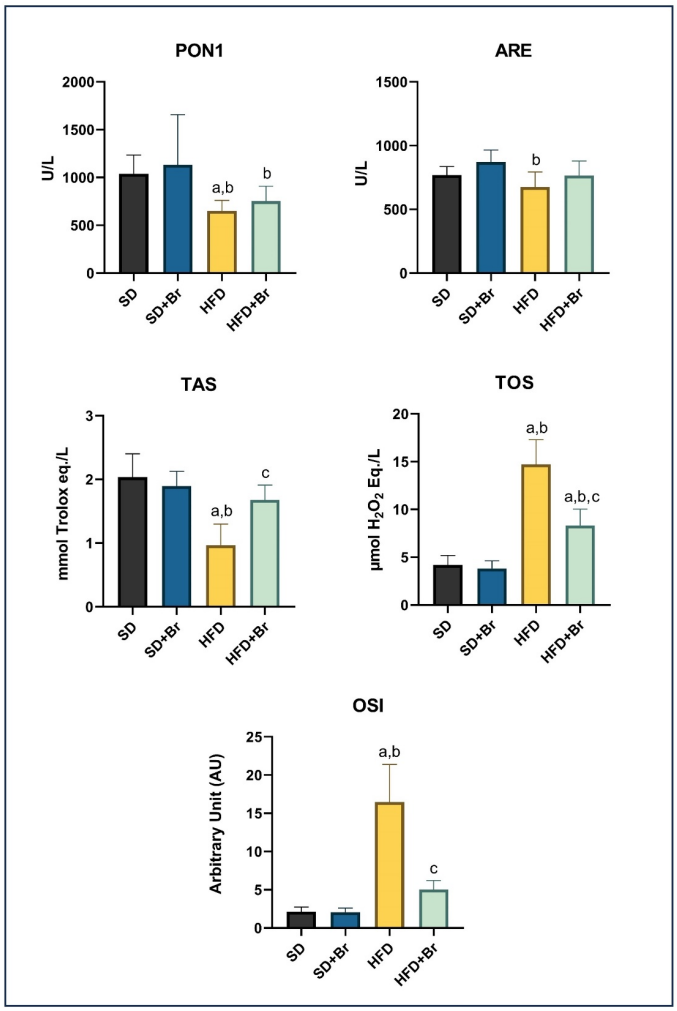

FIGURE 3. Biochemical parameters in serum and testicular tissues across all experimental groups. Each experimental group consisted of 9 rats. Serum paraoxonase-1 (PON1) and arylesterase (ARE) enzyme activities are shown. PON1 activity was high in the standard diet (SD) and standard diet + Bromelain (SD+Br) groups, whereas a significant decrease was observed in the high-fat diet (HFD) group. In the high-fat diet+Bromelain (HFD+Br) group, PON1 activity was partially higher than in the HFD group but remained lower than in the SD and SD+Br groups. There was a statistically significant difference in ARE activity between SD+Br and HFD groups. In testicular tissue, total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) levels are presented. TAS levels were high in the SD and SD+Br groups, significantly decreased in the HFD group, and increased in the HFD+Br group. TOS levels were low in the SD and SD+Br groups, elevated in the HFD group, and reduced in the HFD+Br group. OSI values were low in the SD and SD+Br groups, high in the HFD group, and significantly decreased in the HFD+Br group. Data are presented as mean±SD. Statistical significance is indicated by [a](P<0.05 vs. SD), [b](P<0.05 vs. SD+Br), [c](P<0.05 vs. HFD).

ing the overall antioxidant capacity of the organism, was evaluated in testicular tissue. TAS levels were high and similar in the SD and SD+Br groups, while a decrease was observed in the HFD group. In the HFD+Br group, TAS levels increased. Statistical analysis indicated that the HFD group differed significantly from the SD, SD+Br, and HFD+Br groups ($P<0.0001$).

TOS was measured to assess the intensity of oxidative stress and monitor the efficacy of antioxidant interventions. TOS levels were low in the SD and SD+Br groups, whereas a significant increase was observed in the HFD group. In the HFD+Br group, TOS levels decreased but remained higher than in the SD group ($P<0.0001$).

OSI, reflecting the balance between oxidant and antioxidant systems, was low in the SD and SD+Br groups. Conversely, OSI values were markedly elevated in the HFD group. In the HFD+Br group, a significant reduction and improvement in OSI were observed ($P<0.0001$) (Figure 3).

DISCUSSION

Approximately 20% of reproductive problems in couples worldwide are increasingly attributed solely to male infertility, which can arise from multiple factors, including metabolic disorders such as obesity [27]. Obesity is a systemic metabolic disorder affecting numerous major organs, with the testes being among the most severely impacted [7]. In this context, the present study employed a HFD for 12 weeks to establish an obesity model in rats. Following confirmation of obesity, animals received Br treatment for 30 days to investigate whether Br could mitigate obesity-induced testicular impairments through histopathological assessment, oxidative stress parameters, and fibrosis evaluation.

The present findings are consistent with previous studies indicating that prolonged exposure to HFD leads to reproductive dysfunction. For instance, Demirci and Şahin [28] demonstrated that HFD altered testicular histology and negatively affected spermatogenesis, as assessed using Johnsen scores. Similarly, Mohammadi Roushandeh *et al*. [29] reported that oxidative stress induced by HFD and obesity adversely affected sperm motility, morphology, and viability, directly impacting spermatogenesis. In line with our findings, they observed histological alterations in testicular tubules, including vacuolization, reduced mature sperm counts, depletion of sperm from the epididymal lumen, and basal membrane disruption. The role of oxidative stress in testicular fibrosis and collagen synthesis is also supported by the literature. Cultured human fibroblasts exposed to oxidative stress have been shown to exhibit increased collagen synthesis [30]. Masson-Trichrome staining allows for the quantitative evaluation of collagen fibers in interstitial edema and peritubular fibrosis [31]. In our study, HFD exposure led to increased collagen fiber content in both the tunica albuginea and perivascular regions of the testicular tissue, confirming prior reports of collagen upregulation in mammalian testes [32].

Antioxidant dietary supplements and phytochemicals may provide beneficial effects in the treatment of obesity-related reproductive disorders by reducing apoptosis and inflammation, thereby preserving testicular function [12]. In particular, Br, a proteolytic enzyme derived from Ananas comosus, enhances antioxidant capacity by reducing lipid peroxidation and other oxidative stress markers. Bromelain exhibits antioxidant properties by scavenging free radicals and ROS [33–37]. Supplementation with Br has been reported to improve sperm count and morphology, elevate testosterone levels, and enhance antioxidant enzyme activities [18]. Additionally, ethanolic extracts of Ananas comosus and Br protect cellular components against oxidative damage via ROS detoxification and the glutathione redox cycle [38]. Previous studies demonstrated that Br activates the Nrf2 antioxidant pathway and suppresses NF−κB−mediated inflammatory responses in non−reproductive tissues (e.g., liver, lung) and cell models [39, 40]. In light of these data, the protective effects observed in our HFD−induced testicular dysfunction model may be mediated, at least in part, via similar modulation of Nrf2 and NF−κB signalling. Consistent with these findings, our study demonstrated that Br treatment histologically ameliorated HFD-induced testicular damage.

PON1 protects low-density lipoprotein (LDL) from oxidation and neutralizes hydrogen peroxide and peroxidized phospholipids in oxidized LDL [41]. Due to structural similarities between LDL and cellular membranes, some authors have suggested that HDL-associated PON1 antioxidant activity provides general

protection against membrane lipid peroxidation [42]. Serum PON1 activity has been inversely correlated with oxidative stress in serum and macrophages, and PON1 deficiency has been reported to exacerbate oxidative stress. Elkiran *et al*. [43] found that serum PON1 and ARE activities were significantly lower in smoking lung cancer patients compared to smoking healthy controls. Similarly, in our study, the partial restoration of PON1 activity following Br supplementation suggests that Br not only suppresses oxidative load but may also positively modulate lipid metabolism and enzymatic antioxidant defense mechanisms. The absence of significant changes in ARE activity indicates that this enzyme may be more resistant to diet-induced oxidative challenges relative to other parameters [44]. Regarding ARE activity, the absence of significant changes may be due to cell type-specific or substrate-specific responses, meaning that the measured antioxidant response might not fully reflect the actual intracellular changes [45, 46]. Additionally, some enzymes may be influenced by adaptive regulatory mechanisms; under prolonged oxidative stress or elevated antioxidant load, certain enzymes can be downregulated or maintained at a steady activity via feedback mechanisms [47]. Therefore, unchanged ARE activity does not necessarily indicate ineffective antioxidant defense but may reflect substrate specificity or adaptive enzyme regulation.

our study, the HFD-induced decreases in PON1 and TAS levels, together with increases in TOS and OSI, indicate that a high-fat diet suppresses endogenous antioxidant defenses while increasing oxidant load. Br supplementation exerted strong antioxidant effects, evidenced by increased TAS and significant reductions in TOS and OSI. Moreover, the strong negative correlation between TOS and TAS suggests that elevated oxidant burden reduces antioxidant capacity, shifting the oxidative/antioxidative balance toward oxidants [23]. Similarly, Akkoca *at al*. [48] demonstrated that ischemia-reperfusion (IR) decreases TAS, increases TOS, and that MitoTEMPO exerts protective effects against these changes. Bromelain has also been shown to reduce AGE receptor (RAGE)-mediated inflammation and tissue damage by disrupting advanced glycation end products (AGE)-associated pathways [49–51].

Several studies have demonstrated that HFD induces oxidative stress and testicular dysfunction in rat models. For instance, micronutrient-based antioxidant interventions have been shown to ameliorate HFD-induced sperm and testicular oxidative damage [52], while quercetin supplementation reduced oxidative stress and improved spermatogenesis in HFD-fed Wistar rats [53]. These studies provide relevant context and support the role of oxidative mechanisms in HFD-induced reproductive impairment, reinforcing the rationale for investigating Br's protective effects.

Overall, our results indicate that Br supplementation can partially restore testicular structure and oxidative balance in HFD-induced obese rats, highlighting its potential as a protective agent against obesity-associated reproductive dysfunction. Future studies should further investigate the protective effects of Br on HFD-induced testicular dysfunction at a detailed molecular level. Direct assessment of Nrf2 and NF−κB signalling pathways, evaluation of different doses and treatment durations, and long-term safety profiling are warranted. Additionally, the effects of Br on other reproductive parameters and hormone levels, as well as its clinical applicability, should be explored. Such investigations would provide a clearer understanding of the potential therapeutic role of bromelain in male reproductive health.

Strengths and Limitations

Strengths of the study include the use of a well-controlled experimental design, the incorporation of both biochemical and histological evaluations to provide comprehensive insight into tissue alterations, and the application of standardized methods for assessing oxidative stress and reproductive parameters. The study also contributes valuable preliminary data regarding the potential protective effects of Br against diet-induced testicular damage, thereby establishing a foundation for future mechanistic and translational research. However, it should be noted that this study was conducted in an experimental rat model, which may not fully replicate human pathophysiology. The sample size was relatively limited, and only short-term Br treatment was evaluated. In addition, molecular pathways underlying the protective effects of Br were not investigated in depth. Therefore, further studies involving larger cohorts, longer treatment periods, and

mechanistic analyses are required to confirm the translational relevance of these findings. Another limitation of this study is that, although sham gavage was performed in the SD group, the potential adverse effects of repeated gavage-induced stress on oxidative and reproductive parameters were not elucidated. Additionally, firstly no effect size calculations were reported, which limits the interpretation of the magnitude of the observed differences; secondly, a priori power analysis was not performed, and therefore the risk of type II errors cannot be excluded.

CONCLUSION

Our findings indicate that the increase in oxidative stress parameters induced by HFD can be alleviated by Br administration, leading to improvements in the biochemical and histological structure of rat testicular tissue. Consequently, Br treatment may be considered a protective and therapeutic agent against diet-induced testicular damage.

Ethics Approval and Consent to Participate

This study was approved by the Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Center Animal Experiments Local Ethics Committee (Decision No: 2024-117; date: 28.11.2024). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: BG, RÖK, MBB; Study Design: BG, HTC; Supervision: BG, RÖK; Funding: BG, HTC, MBB; Materials: MBB, RÖK; Data Collection and/or Processing: BG, HBS, ZISG; Statistical Analysis and/or Data Interpretation: ZISG, HBS; Literature Review: BG, HTC, RÖK; Manuscript Preparation: BG, MBB, HBS; and Critical Review: BG, HTC, ZSIG.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The entire content of this study was produced by the author(s) in full compliance with scientific research methodologies and academic ethical standards. The author(s) affirm that all scientific components of the manuscript were independently generated by themselves, and explicitly state that ChatGPT 4.0 AI was used solely for English language editing, without contributing to the scientific interpretation, analysis, or conclusions of the study.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. El-Sawy SA, Amin YA, El-Naggar SA, Abdelsadik A. Artemisia annua L. (Sweet wormwood) leaf extract attenuates high-fat diet-induced testicular dysfunctions and improves spermatogenesis in obese rats. J Ethnopharmacol. 2023;313:116528. doi: 10.1016/j.jep.2023.116528.
2. Jia YF, Feng Q, Ge ZY, et al. Obesity impairs male fertility through long-term effects on spermatogenesis. BMC Urol. 2018;18(1):42. doi: 10.1186/s12894-018-0360-5.
3. Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 2010;7(3):153-161. doi: 10.1038/nrurol.2010.6.
4. Macdonald AA, Stewart AW, Farquhar CM. Body mass index in relation to semen quality and reproductive hormones in New

Zealand men: a cross-sectional study in fertility clinics. Hum Reprod. 2013;28(12):3178-3187. doi: 10.1093/humrep/det379.
5. Liu X, Qi Y, Zhu T, Ding X, Zhou D, Han C. Butyrate improves testicular spermatogenic dysfunction induced by a high-fat diet. Transl Androl Urol. 2025;14(3):627-636. doi: 10.21037/tau-2024-660.
6. Komninos D, Ramos L, van der Heijden GW, et al. High fat diet-induced obesity prolongs critical stages of the spermatogenic cycle in a Ldlr-/-.Leiden mouse model. Sci Rep. 2022;12(1):430. doi: 10.1038/s41598-021-04069-y.
7. Rigi MH, Asghari SZ, Eshtad E, et al. Corrigendum to "Protective effect of hydrogen-rich water on spermatogenesis in high-fat diet obese rats" [Toxicology and Applied Pharmacology Volume 499, June 2025, 117334]. Toxicol Appl Pharmacol. 2025;501:117356. doi: 10.1016/j.taap.2025.117356.
8. Bellastella G, Menafra D, Puliani G, Colao A, Savastano S; Obesity Programs of nutrition, Education, Research and Assessment (OPERA) Group. How much does obesity affect the male reproductive function? Int J Obes Suppl. 2019;9(1):50-64. doi: 10.1038/s41367-019-0008-2.
9. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nat Rev Urol. 2017;14(8):470-485. doi: 10.1038/nrurol.2017.69.
10. Mu Y, Yin TL, Huang XX, Hu X, Yin L, Yang J. Sulforaphane ameliorates high-fat diet-induced spermatogenic deficiency in mice. Biol Reprod. 2019;101(1):223-234. doi: 10.1093/biolre/ioz067.
11. Ju J, Wen S, Zhao X, Cheng J, Yang H, Zhu G. Omega-3 polyunsaturated fatty acids ameliorate high-fat-diet-induced structural and functional impairments of testicular tissue via ER stress inhibition. Transgenic Res. 2025;34(1):32. doi: 10.1007/s11248-025-00448-7.
12. Santos HO, Cadegiani FA, Forbes SC. Nonpharmacological Interventions for the Management of Testosterone and Sperm Parameters: A Scoping Review. Clin Ther. 2022;44(8):1129-1149. doi: 10.1016/j.clinthera.2022.06.006.
13. Taussig SJ, Batkin S. Bromelain, the enzyme complex of pineapple (Ananas comosus) and its clinical application. An update. J Ethnopharmacol. 1988;22(2):191-203. doi: 10.1016/0378-8741(88)90127-4.
14. Chakraborty AJ, Mitra S, Tallei TE, et al. Bromelain a Potential Bioactive Compound: A Comprehensive Overview from a Pharmacological Perspective. Life (Basel). 2021;11(4):317. doi: 10.3390/life11040317.
15. El-Demerdash FM, Baghdadi HH, Ghanem NF, Mhanna ABA. Nephroprotective role of bromelain against oxidative injury induced by aluminium in rats. Environ Toxicol Pharmacol. 2020;80:103509. doi: 10.1016/j.etap.2020.103509.
16. Jebur AB, El-Demerdash FM, Kang W. Bromelain from Ananas comosus stem attenuates oxidative toxicity and testicular dysfunction caused by aluminum in rats. J Trace Elem Med Biol. 2020;62:126631. doi: 10.1016/j.jtemb.2020.126631.
17. Kansakar U, Trimarco V, Manzi MV, Cervi E, Mone P, Santulli G. Exploring the Therapeutic Potential of Bromelain: Applications, Benefits, and Mechanisms. Nutrients. 2024;16(13):2060. doi: 10.3390/nu16132060.
18. Hosseinpour M, Dalband AM, Lajevardi Z, et al. The protective effect of bromelain on testicular dysfunction and oxidative stress induced by REM sleep deprivation in adult male rats. Tissue Cell. 2025;95:102927. doi: 10.1016/j.tice.2025.102927.
19. Pavan R, Jain S, Shraddha, Kumar A. Properties and therapeutic application of bromelain: a review. Biotechnol Res Int. 2012;2012:976203. doi: 10.1155/2012/976203.
20. Sönmez MF, Karabulut D, Kilic E, et al. The effects of streptozotocin-induced diabetes on ghrelin expression in rat testis: biochemical and immunohistochemical study. Folia Histochem Cytobiol. 2015;53(1):26-34. doi: 10.5603/FHC.a2015.0006.
21. Ayar G, Atmaca YM, Alışık M, Erel Ö. Effects of paraoxonase, arylesterase, ceruloplasmin, catalase, and myeloperoxidase activities on prognosis in pediatric patients with sepsis. Clin Biochem. 2017;50(7-8):414-417. doi: 10.1016/j.clinbiochem.2016.12.013.
22. Kilinc F, Sener S, Akbaş A, et al. Oxidative stress parameters in localized scleroderma patients. Arch Dermatol Res. 2016;308(9):625-629. doi: 10.1007/s00403-016-1682-3.
23. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37(4):277-85. doi: 10.1016/j.clinbiochem.2003.11.015.
24. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11. doi: 10.1016/j.clinbiochem.2005.08.008.
25. Savran M, Aslankoc R, Ozmen O, et al. Agomelatine could prevent brain and cerebellum injury against LPS-induced neuroinflammation in rats. Cytokine. 2020;127:154957. doi: 10.1016/j.cyto.2019.154957.
26. Savran M, Asci H, Ozmen O, et al. Melatonin protects the heart and endothelium against high fructose corn syrup consumption-induced cardiovascular toxicity via SIRT-1 signaling. Hum Exp Toxicol. 2019;38(10):1212-1223. doi: 10.1177/0960327119860188.
27. Chaudhuri GR, Das A, Kesh SB, et al. Obesity and male infertility: multifaceted reproductive disruption. Middle East Fertil Soc J. 2022; 27(1):8. doi: 10.1186/s43043-022-00099-2.
28. Demirci T, Sahin E. The effect of chronic stress and obesity on sperm quality and testis histology in male rats; a morphometric and immunohistochemical study. Histol Histopathol. 2019;34(3):287-302. doi: 10.14670/HH-18-077.
29. Mohammadi Roushandeh A, Salehi I, Mortazavi M. Protective effects of restricted diet and antioxidants on testis tissue in rats fed with high-fat diet. Iran Biomed J. 2015;19(2):96-101. doi: 10.6091/ibj.1398.2015.
30. Rivera J, Sobey CG, Walduck AK, Drummond GR. Nox isoforms in vascular pathophysiology: insights from transgenic and knockout mouse models. Redox Rep. 2010;15(2):50-63. doi: 10.1179/174329210X12650506623401.
31. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180-3. doi: 10.1016/j.redox.2015.01.002.
32. Omirinde JO, Olukole SG, Oke BO. Age-Related Changes in the Testicular Morphophysiology of the Cane Rat (Thryonomys swinderianus). J Microsc Ultrastruct. 2021;10(3):118-126. doi: 10.4103/jmau.jmau_84_20.
33. Rasheedi S, Haq SK, Khan RH. Guanidine hydrochloride denaturation of glycosylated and deglycosylated stem bromelain. Biochemistry (Mosc). 2003;68(10):1097-100. doi:

10.1023/a:1026354527750.
34. Bakare AO, Owoyele BV. Antinociceptive and neuroprotective effects of bromelain in chronic constriction injury-induced neuropathic pain in Wistar rats. Korean J Pain. 2020;33(1):13-22. doi: 10.3344/kjp.2020.33.1.13.
35. Hu PA, Chen CH, Guo BC, Kou YR, Lee TS. Bromelain Confers Protection against the Non-Alcoholic Fatty Liver Disease in Male C57bl/6 Mice. Nutrients. 2020;12(5):1458. doi: 10.3390/nu12051458.
36. Hu R, Chen G, Li Y. Production and Characterization of Antioxidative Hydrolysates and Peptides from Corn Gluten Meal Using Papain, Ficin, and Bromelain. Molecules. 2020;25(18):4091. doi: 10.3390/molecules25184091.
37. López-Pedrouso M, Borrajo P, Pateiro M, Lorenzo JM, Franco D. Antioxidant activity and peptidomic analysis of porcine liver hydrolysates using alcalase, bromelain, flavourzyme and papain enzymes. Food Res Int. 2020;137:109389. doi: 10.1016/j.foodres.2020.109389.
38. Al-Otaibi WR, Virk P, Elobeid M. Ameliorative potential of stem bromelain on lead-induced toxicity in Wistar rats. Acta Biol Hung. 2015;66(2):149-60. doi: 10.1556/018.66.2015.2.2.
39. Didamoony MA, Atwa AM, Abd El-Haleim EA, Ahmed LA. Bromelain ameliorates D-galactosamine-induced acute liver injury: role of SIRT1/LKB1/AMPK, GSK3β/Nrf2 and NF-κB p65/TNF-α/caspase-8, -9 signalling pathways. J Pharm Pharmacol. 2022;74(12):1765-1775. doi: 10.1093/jpp/rgac071.
40. Majumder D, Debnath R, Nath P, et al. Bromelain and Olea europaea (L.) leaf extract mediated alleviation of benzo(a)pyrene induced lung cancer through Nrf2 and NFκB pathway. Environ Sci Pollut Res Int. 2021;28(34):47306-47326. doi: 10.1007/s11356-021-13803-y.
41. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest. 1998;101(8):1581-1590. doi: 10.1172/JCI1649.
42. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler Thromb Vasc Biol. 2001;21(4):473-480. doi: 10.1161/01.atv.21.4.473.
43. Elkiran ET, Mar N, Aygen B, Gursu F, Karaoglu A, Koca S. Serum paraoxonase and arylesterase activities in patients with lung cancer in a Turkish population. BMC Cancer. 2007;7:48. doi: 10.1186/1471-2407-7-48.
44. Otocka-Kmiecik A, Orłowska-Majdak M, Stawski R, et al. Effect of Exercise Repetitions on Arylesterase Activity of PON1 in Plasma of Average-Trained Men-The Dissociation between Activity and Concentration. Antioxidants (Basel). 2023;12(6):1296. doi: 10.3390/antiox12061296.
45. Boas SM, Joyce KL, Cowell RM. The NRF2-Dependent Transcriptional Regulation of Antioxidant Defense Pathways: Relevance for Cell Type-Specific Vulnerability to Neurodegeneration and Therapeutic Intervention. Antioxidants (Basel). 2021;11(1):8. doi: 10.3390/antiox11010008.
46. Raghunath A, Sundarraj K, Nagarajan R, et al. Antioxidant response elements: Discovery, classes, regulation and potential applications. Redox Biol. 2018;17:297-314. doi: 10.1016/j.redox.2018.05.002.
47. Lei XG, Zhu JH, Cheng WH, et al. Paradoxical Roles of Antioxidant Enzymes: Basic Mechanisms and Health Implications. Physiol Rev. 2016;96(1):307-364. doi: 10.1152/physrev.00010.2014.
48. Akkoca A, Büyükakıllı B, Ballı E, et al. Protective effect of MitoTEMPO against cardiac dysfunction caused by ischemia-reperfusion: MCAO stroke model study. Int J Neurosci. 2024;134(12):1582-1593. doi: 10.1080/00207454.2023.2273768.
49. Lu HC, Ng MY, Liao YW, Maekawa S, Lin T, Yu CC. Bromelain inhibits the inflammation and senescence effect in diabetic periodontitis: A preliminary in vitro study. J Dent Sci. 2023;18(2):659-665. doi: 10.1016/j.jds.2022.09.018.
50. Foroncewicz B, Mucha K, Heidland A, et al. Modulation of serum levels of sRAGE by bromelain in patients with chronic kidney disease: a pilot study. Pol Arch Med Wewn. 2012;122(10):514-516. doi: 10.20452/pamw.1427.
51. Gross P, Seelert H, Meiser P, Müller R. Characterization of bromelain indicates a molar excess of inhibitor vs. enzyme molecules, a Jacalin-like lectin and Maillard reaction products. J Pharm Biomed Anal. 2020;181:113075. doi: 10.1016/j.jpba.2019.113075.
52. Billah MM, Khatiwada S, Lecomte V, Morris MJ, Maloney CA. Ameliorating high-fat diet-induced sperm and testicular oxidative damage by micronutrient-based antioxidant intervention in rats. Eur J Nutr. 2022;61(7):3741-3753. doi: 10.1007/s00394-022-02917-9.
53. Hafez MH, Gad SB, El-Sayed YS. Quercetin-mediated restoration of high-fat diet-induced male reproductive dysfunction through modifying spermatogenesis and unraveling 3β-HSD, 17β-HSD, and StAR pathways. BMC Pharmacol Toxicol. 2025;26(1):90. doi: 10.1186/s40360-025-00918-y.

The European Research Journal 2026;12(1):13-21 Original Article

DOI: https://doi.org/10.18621/eurj.1795016 *Hematology and Oncology*

The Effect of Uric Acid to High Density Lipoprotein Cholesterol Ratio on Prognosis in Diffuse Large B Cell Lymphoma

Kadir Ilkkilic[1], Bayram Sen[2]

[1]Department of Hematology, School of Medicine, Recep Tayyip Erdogan University, Rize, Türkiye; [2]Department of Biochemistry, School of Medicine, Recep Tayyip Erdogan University, Rize, Türkiye

ABSTRACT

Objectives: International prognostic index (IPI) score, genetic mutation heterogeneity and inflammatory markers play an important role in predicting prognosis in diffuse large B-cell lymphoma (DLBCL). The uric acid to high-density lipoprotein cholesterol ratio (UHR) has been shown to be a new marker that allows better prediction of metabolic deterioration and inflammatory processes. The aim of the study is to evaluate the effect of UHR on prognosis in DLBCL patients.

Methods: In this retrospective cohort study, 120 patients were included. The effects of UHR on prognosis were evaluated.

Results: Of the total cohort, 79 patients survived, whereas 41 patients died. Mortality was higher in patients with higher UHR and IPI score ≥ 3 (P=0.047 and P=0.001, respectively). Univariate analysis revealed that age, Eastern Cooperative Oncology Group performance status (ECOG PS), disease stage, extranodal involvement, uric acid levels, IPI score, and UHR were significant predictors of mortality. Multivariate analysis revealed that ECOG PS, IPI score, and UHR were found to be independent predictors. Kaplan-Meier analysis showed that higher UHR (>0.1075) and IPI scores (≥3) were associated with decreased overall survival. (P=0.003 and P=0.005, respectively).

Conclusions: Elevated pre-treatment UHR independently predicts prognosis and is associated with reduced overall survival in patients with DLBCL. It may serve as a surrogate marker of systemic metabolic dysfunction and help identify high-risk patients alongside the IPI score.

Keywords: Diffuse Large B-Cell Lymphoma, Uric Acid to High Density Lipoprotein Cholesterol Ratio, Prognosis

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease in terms of clinical and treatment outcomes. International prognostic index (IPI) score and its derivatives, genetic mutation heterogeneity, and inflammatory markers have an important place in predicting disease progression and mortality risk [1, 2]. Hyperuricemia, which occurs as a result of a high rate of cellular destruction and renewal cycle, is frequently encountered in hematologic malignancies [3]. Elevated uric acid has been identified as a predictor of adverse long-term prognosis in DLBCL [4]. High-density lipoprotein cholesterol

Received: October 1, 2025 **Accepted:** November 2, 2025 **Available Online:** November 27, 2025 **Published:** January 4, 2026

How to cite this article: Ilkkilic K, Sen B. The Effect of Uric Acid to High Density Lipoprotein Cholesterol Ratio on Prognosis in Diffuse Large B Cell Lymphoma. Eur Res J. 2026;12(1):13-21. doi: 10.18621/eurj.1795016

Corresponding author: Kadir İlkkılıç, MD., Assist. Prof., Phone: +90 464 213 04 91, E-mail: kadir.ilkkilic@erdogan.edu.tr, kadir.ilkkilic@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

(HDL-C) is a component that plays an important role in the interplay between cancer biology and lipid metabolism [5].

Evidence indicates that its anti-inflammatory, immunomodulatory, and antioxidant effects enable it to modulate proliferative and inflammatory mechanisms during the progression of malignancies [6, 7]. Apolipoprotein A-1 (Apo A-1), one of the main components of HDL-C, was identified as an independent prognostic marker associated with poor overall survival and progression-free survival in DLBCL patients [8]. Although both uric acid levels and HDL-C have been shown to play an important role in lymphoma biology, the prognostic value of these parameters alone is limited [9, 10].

The use of ratios provides a more comprehensive approach in terms of reflecting inflammation and oxidative stress together. Uric acid to HDL-C ratio (UHR) has been shown to be a new inflammatory marker that allows better prediction of metabolic deterioration and inflammatory processes [11-13]. High inflammation burden and oxidative stress are unfavorable factors in the prognosis of DLBCL [14, 15]. Elevated UHR in such an aggressive lymphoma type may be associated with reduced response to treatment, along with increased tumor burden and systemic inflammation. Therefore, UHR may provide additional benefit to traditional prognostic scores in predicting survival in DLBCL patients.

This study aimed to assess the impact of UHR examined at the time of diagnosis on prognosis in DLBCL patients.

METHODS

Study Population

A total of 120 patients with DLBCL were included in this retrospective cohort study who were followed up at the Hematology Clinic of Recep Tayyip Erdogan University School of Medicine between January 2015 and January 2025.

Study Design and Data Collection

Age, gender, body mass index (BMI), comorbidities, Eastern Cooperative Oncology Group performance status (ECOG PS), Ann Arbor disease stage, B symptoms (fever, weight loss, night sweats), and the presence of bulky disease (lymphadenopathy larger than 7.5 cm in any region).

B symptoms (fever, unexplained weight loss, night sweats), and bulky disease (lymph node enlargement exceeding 7.5 cm in any area) and extranodal involvement were recorded. At the time of diagnosis, after fasting for at least 8-12 hours, lipid profile, uric acid, albumin, lactate dehydrogenase (LDH), uric acid to HDL-C ratio, and triglyceride to HDL-C ratio were recorded.

The IPI score was calculated and scored between 0-5. Patient age, Ann Arbor staging, serum LDH levels, extranodal involvement, and ECOG PS parameters were used in the calculation [2].

UHR was calculated as uric acid divided by HDL-C, and the triglyceride to HDL-C ratio as triglyceride divided by HDL-C.

Overall survival (OS) was calculated from the time of diagnosis to death from any cause or the date of last follow-up. Progression-free survival (PFS) was calculated from the time of diagnosis to disease progression, relapse, or death.

•Inclusion Criteria

Patients aged >18 years who received first-line rituximab-based chemoimmunotherapy regimens, including rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), or its variants (R-mini-CHOP or R-CVP), were included.

•Exclusion Criteria

Patients with active or chronic infection at the time of diagnosis, renal dysfunction, taking uric acid-lowering drugs (allopurinol or febuxostat), diagnosis of hyperlipidemia or taking statins, fenofibrates, niacin, patients with autoinflammatory diseases (rheumatoid arthritis, lupus), gout, and patients who use alcohol were excluded from the study. In addition, DLBCL patients with human immunodeficiency virus (HIV) or Epstein-Barr virus (EBV) co-infection, history of malignancy or prior chemo-radiotherapy, receiving treatment other than rituximab-based chemoimmunotherapy, and whose laboratory and clinical data were not available were excluded from the study.

Patients were divided into 2 groups: Survivors and Non-survivors.

Statistical Analysis

Statistical analysis was conducted by using IBM SPSS Statistics for Windows, version 23.0; IBM Corp., Armonk, NY. Descriptive characteristics of the groups were summarized using frequency and percentage (n, %) for each category. For continuous variables, the normality analysis was carried out by taking into account the number of participants in the groups (Kolmogorov-Smirnov (KS) and Shapiro-Wilk (SW) tests). Accordingly, variables with normal distribution were shown as mean ± standard deviation (SD), and variables that did not conform to normal distribution were shown as median (minimum- maximum). Differences in numerical variables between two groups were evaluated using the Student t-test for variables with normal distribution and the Mann-Whitney U test for variables without normal distribution. The relationship between mortality and categorical variables in patients with DLBCL was evaluated by Chi-Square analysis. Significance values were given considering the number of patients in the categories (Pearson's chi-square test or Fisher's exact test). The performance of uric acid to HDL-C ratio in the prediction of mortality in patients with DLBCL was examined by a receiver operating characteristic (ROC) analysis. Cut-off value, sensitivity, specificity, and area under the curve (AUC) values were identified. The predictive value of the parameters was evaluated by Cox regression analysis,

TABLE 1. Clinical and Demographic Characteristics of the Patients

		Survival status		
		Survivors	**Non-survivors**	**P-value**
Gender	Female	38 (48.1)	18 (43.9)	0.662
	Male	41 (51.9)	23 (56.1)	
ECOG PS	0-1	75 (94.9)	28 (68.3)	**<0.001**
	2-4	4 (5.1)	13 (31.7)	
Ann Arbor stage	1-2	36 (45.6)	6 (14.6)	**0.001**
	3-4	43 (54.4)	35 (85.4)	
B symptoms	No	59 (74.7)	26 (63.4)	0.198
	Yes	20 (25.3)	15 (36.6)	
Bulky disease	No	68 (86.1)	33 (80.5)	0.426
	Yes	11 (13.9)	8 (19.5)	
Extranodal involvement	No	36 (45.6)	8 (19.5)	**0.005**
	Yes	43 (54.4)	33 (80.5)	
LDH (U/L)	<240	44 (55.7)	13 (31.7)	**0.013**
	>240	35 (44.3)	28 (68.3)	
IPI Score	0-2	42 (53.2)	9 (22)	**0.001**
	3-5	37 (46.8)	32 (78)	
Age (years)		67 (27-88)	71 (43- 89)	**0.015**
Uric acid (mg/dL)		5 (2.4-9.5)	6.1 (2.5-10.5)	**0.018**
HDL-C (mg/dL)		44.6 (13.1)	41.8 (11)	0.237
Uric acid/HDL-C ratio		0.11 (0.05-0.39)	0.14 (0.05- 0.7)	**0.047**
Triglyceride/HDL-C ratio		3.3 (0.2-26.8)	3.2 (1.4-24.6)	0.565

Data are shown as mean±standard deviation or n (%) or median (minimum-maximum) where appropriate. ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; IPI, international prognostic index; HDL-C, high-density lipoprotein cholesterol.

Statistically significant P-values are shown in bold.

and the hazard ratio (HR) with 95% condidence interval (CI) was provided. Variables found to be significant in the univariate analysis were then included in the multivariate analysis. The effects of variables on mortality were evaluated by Kaplan-Meier survival analysis and log-rank test. P-value <0.05 was considered statistically significant.

RESULTS

Among the 120 patients included in the study, 56 (46.7%) were female and 64 (53.3%) were male. The mean age of the patients was 67±13 years, and the median follow-up period was 22 months (max 113 months). Seven-nine patients survived, whereas 41 patients died.

Among the patients, 34 (28.3%) had diabetes mellitus, 69 (57.5%) had hypertension and 23 (19.2%) had coronary artery disease. The mean BMI of the patients was 26.7±3.9 kg/m^2. Clinical and demographic data are given in Table 1.

Mortality was associated with ECOG PS, Ann Arbor stage, extranodal disease, LDH, IPI score, age, uric acid levels, and UHR. Higher mortality was observed in patients with ECOG performance status ≥2, Ann Arbor stage ≥3, presence of extranodal involvement, LDH level above 240, and IPI score ≥ 3 (P<0.001, P=0.001, P=0.005, P=0.013, and P=0.001, respectively). Patients who died were significantly older and had higher uric acid and UHR levels. (P=0.015, P=0.018, and P=0.047, respectively). In addition, no significant association was found between HDL-C levels and mortality (P=0.237). Correlation analysis revealed no significant relationship between HDL-C levels and ECOG PS or age (P=0.948 and P=0.313, respectively) (Table 1).

The optimal cut-off value for UHR was determined by ROC analysis. (AUC: 0.611 (95% CI 0.505-0.717), P=0.047, sensitivity: 80.5%, specificity: 45.6%). Mortality rate was higher in patients with UHR above 0.1075.

Independent predictors for mortality were determined by univariate analysis. Patient age, ECOG performance status, Ann Arbor stage, extranodal involvement, uric acid, IPI score, and UHR were found to predict mortality in univariate analysis. Age, ECOG performance status, Ann Arbor stage, extran-

TABLE 2. Univariate and Multivariate Analysis of Clinical and Laboratory Parameters

	Univariate Analysis		Multivariate Analysis	
	P-value	**95% CI for HR**	**P-value**	**95% CI for HR**
Gender	0.757	1.102 (0.595-2.043)		
Age (years)	**0.007**	1.04 (1.011-1.071)		
ECOG PS	**<0.001**	3.554 (1.77-7.134)	**0.011**	2.676 (1.257-5.697)
Ann arbor stage	**0.004**	3.583 (1.504-8.533)		
B symptoms	0.120	1.664 (0.876-3.163)		
Bulky disease	0.630	1.21 (0.558-2.622)		
Extranodal involvement	**0.029**	2.369 (1.093-5.137)		
LDH (U/L)	0.066	1.858 (0.961-3.594)		
Uric acid (mg/dL)	**0.003**	1.307 (1.097-1.558)		
HDL-C (mg/dL)	0.144	0.982 (0.958-1.006)		
IPI score	**<0.001**	1.787 (1.336-2.389)	**0.012**	1.471 (1.087-1.990)
Uric acid/HDL-C ratio	**0.006**	2.981 (1.376-6.461)	**0.01**	2.782 (1.273-6.078)
Triglyceride/HDL-C ratio	0.224	1.504 (0.778-2.907)		

ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; IPI, international prognostic index; HDL-C, high-density lipoprotein cholesterol; CI, confidence interval; HR, hazard ratio.
Statistically significant P-values are shown in bold.

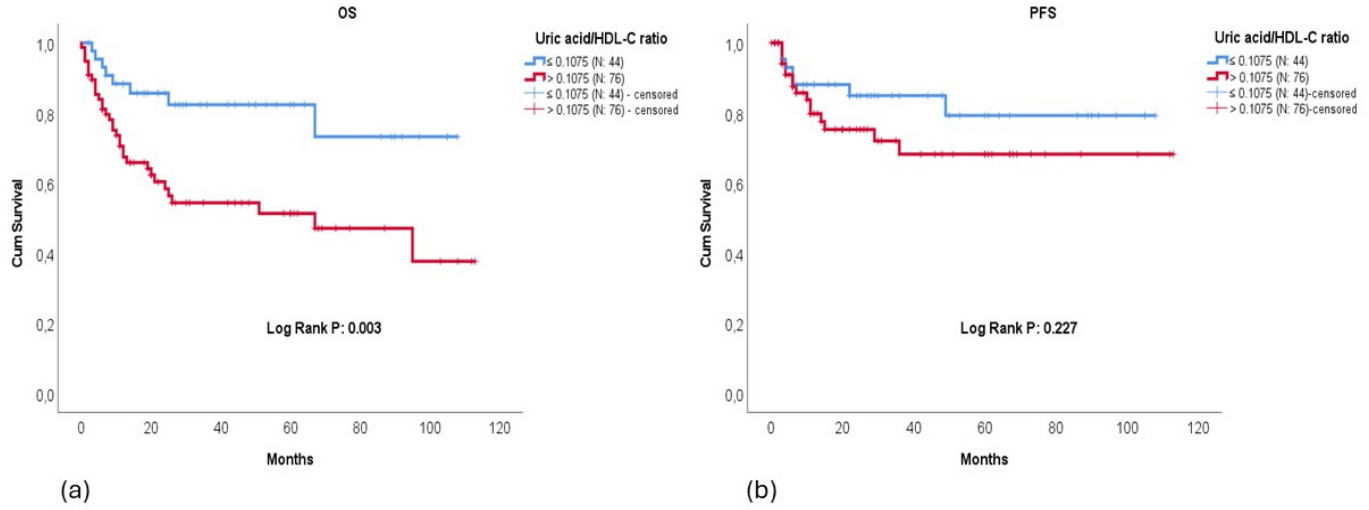

FIGURE 1. Kaplan–Meier analysis. (a) Overall survival (OS) and (b) Progression-free survival (PFS) stratified by uric acid/HDL-C ratio (UHR> 0.1075 [n=76] vs. ≤0.1075 [n=44]).

odal involvement, IPI score, and UHR were chosen as covariates. ECOG performance status, IPI score, and UHR were found to be independent predictors in multivariate analysis (Table 2).

In Kaplan-Meier analysis, the association of UHR and IPI score with PFS and OS was evaluated (Figures 1 and 2). UHR>0.1075 and IPI score ≥3 were significantly associated with decreased OS (P=0.003 and P=0.005, respectively) (Figure 1a and. Figure 2a). While shorter PFS was found in patients with IPI score ≥3, UHR was not associated with PFS (P=0.002 and P=0.227, respectively) (Figure 1b and Figure 2 b).

DISCUSSION

This study is the first to examine the prognostic value of UHR in DLBCL patients. UHR, age, uric acid, ECOG performance status, Ann Arbor stage, extranodal involvement, and IPI score were found to predict mortality. ECOG PS, IPI score, and UHR were found to be independent predictors. Patients with higher UHR and IPI scores≥ 3 had shorter OS.

The IPI score and its derivatives are one of the prognostic scores used in DLBCL. Previous studies have shown that OS and PFS decreased in patients

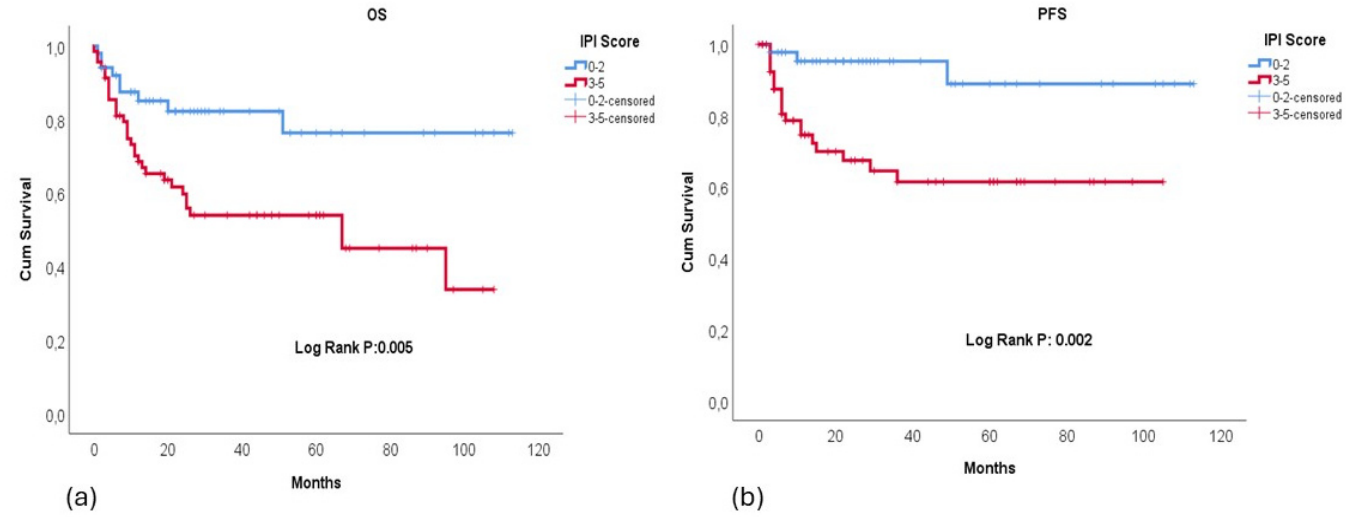

FIGURE 2. Kaplan–Meier analysis. (a) Overall survival (OS) and (b) progression-free survival (PFS) stratified by IPI score.

with high IPI scores [2, 16]. In our study, OS and PFS were shortened in patients with an IPI score of 3 and above and were consistent with the literature.

ECOG PS is widely recognized as a prognostic indicator in patients with DLBCL and has been associated with survival outcomes. Patients with an ECOG PS ≥2 generally present at a more advanced stage and have comorbidities, which result in lower PFS and OS. Therefore, ECOG PS is included as an independent predictor in commonly used prognostic models such as the IPI [16]. In our study, consistent with the literature, a high ECOG PS was found to be associated with mortality, once again highlighting its importance in prognosis and treatment planning.

Uric acid represents the final product of purine metabolism, and hyperuricemia resulting from high cellular turnover is a common complication of hematologic malignancies. In a study on hyperuricemia and risk factors in hematologic cancers, hyperuricemia was found in 20.9% of DLBCL and low-grade lymphoma patients [3]. A study by Prochazka *et al*. [4] showed that high uric acid levels were associated with adverse long-term outcomes in patients with DLBCL. In this study, while there was no association between uric acid levels and relapse, higher uric acid levels were detected in patients who died during survival assessment. This result suggests that increased uric acid levels may be associated with poor prognosis or increased mortality. Therefore, maintaining low uric acid levels at baseline and during treatment may improve survival.

In addition to regulating lipid traffic, HDL-C plays an important role in integrating innate and adaptive immunity and has anti-inflammatory and antioxidant capacity [17]. HDL-C has been reported to modulate the inflammatory response by inhibiting cytokine-induced expression of adhesion molecules in endothelial cells and suppressing chemotaxis of monocytes and lymphocytes [18]. In cancer cells, the balance between cholesterol synthesis, efflux, and influx, which promotes proliferation and invasion, results in intracellular cholesterol accumulation, thus creating a backdrop of impaired reverse cholesterol transport in cancer. Apo A-1 plays an active role in reverse cholesterol transport and has an inhibitory effect on the proliferation and growth of tumor cells that show a high demand for increased cholesterol [19]. Yu *et al.* [8] found that low Apo A-1 levels were associated with poor OS and PFS in DLBCL patients. In a cohort study, decreased levels of HDL-C or ApoA-1 have been associated with an increased risk of developing various cancers, including non-Hodgkin lymphoma and multiple myeloma [20]. Another study found that low HDL-C and low-density lipoprotein cholesterol (LDL-C) levels were associated with poor prognosis in DLBCL patients and that HDL-C or LDL-C elevations after chemotherapy were associated with better survival [21]. A recent study has shown that high HDL-C is associated with a reduced risk of progression in DLBCL patients [10]. Current evidence suggests that low HDL-C levels adversely impact cancer prognosis and demonstrate the paradoxical relationship between HDL-C, inflammatory response and malignancy. This study found no association between HDL-C levels and survival or relapse. The discrepancy between our results and previous studies suggesting a prognostic role of HDL-C may be attributed not only to the limited sample size of our cohort but also to differences in patient characteristics, comorbidity profiles, and treatment modalities among studies. Variations in chemotherapy protocols, supportive care, and metabolic background could influence HDL-C levels and their association with survival outcomes.

UHR has been recognized as a new inflammatory and metabolic marker. Recent studies indicate that UHR can function as an indicator of chronic, low-grade inflammation in multiple diseases. [11, 12, 22]. Inflammation plays an important role in the pathogenesis and progression of DLBCL [15, 23]. Jaeger *et al*. [14] found that inflammatory cytokines direct disease progression and disease symptoms in T-cell lymphoma. Another study reported that inflammatory cytokines may be strong markers in determining treatment response and predicting prognosis in newly diagnosed DLBCL patients [24].

In this study, we found that a high UHR in patients with DLBCL was associated with shorter OS, but not with PFS, and represented an independent adverse prognostic factor. The fact that UHR is associated with OS, but not with PFS, suggests that this biomarker reflects general systemic metabolic status and concomitant comorbidities rather than lymphoma-specific tumor progression. Elevated uric acid levels are an indicator of oxidative stress and inflammation and are closely associated with cardiovascular mortality and metabolic syndrome [25]. Similarly, low HDL levels

contribute to atherosclerotic events and increased mortality risk due to reduced antioxidant and anti-inflammatory effects [26]. A cohort study reported that UHR is a strong predictor of both all-cause mortality and cardiovascular mortality [27]. Therefore, high UHR may be a marker of systemic inflammation, endothelial dysfunction, and metabolic abnormalities that increase the risk of mortality due to non-lymphoma causes in DLBCL patients. This situation may explain why UHR is only effective on OS. Accordingly, the finding that UHR correlates with overall survival rather than progression-free survival should be considered a hypothesis-generating observation, which necessitates confirmation in well-designed prospective cohorts with comprehensive cause-specific mortality data. Our findings indicate that UHR is a meaningful biomarker in DLBCL, as its elevation is independently associated with shorter overall survival. Although it is not associated with progression-free survival, it can reflect patients' general systemic and metabolic status and accompanying comorbidities, which makes it valuable for risk classification and prognostic assessment. Although the specificity of UHR was relatively low (45.6%), its sensitivity was high (80.5%), suggesting that it may effectively identify patients at increased risk of poor outcomes. Given that UHR reflects systemic metabolic and inflammatory alterations rather than tumor biology alone, its clinical utility may be as a complementary prognostic indicator rather than a standalone biomarker. Nevertheless, the relatively modest AUC (0.611) and low specificity represent important limitations, and UHR should be interpreted together with established prognostic indices such as the IPI score.

Given that UHR is a combination of inflammatory response and lipid metabolism, this marker can serve as an indicator of systemic metabolic dysfunction and may contribute to identifying high-risk groups among DLBCL patients.

Strengths and Limitations

Our study is important in terms of showing the negative effect of UHR elevation on prognosis in DLBCL patients. To our knowledge, this is the first study showing the prognostic value of UHR in newly diagnosed DLBCL patients. Our limitations are the relatively small number of patients and the fact that it was performed in a single center. Also, information on the causes of death was not available, as survival status was obtained from hospital electronic records. Therefore, we could not distinguish between lymphoma-related and non-lymphoma-related deaths. The retrospective design of this study limits the ability to infer causal relationships. Additionally, the relatively low specificity of UHR observed in the ROC analysis may restrict its standalone clinical applicability. Another limitation is the inability to fully control metabolic factors that may affect HDL.

CONCLUSION

Our study demonstrated that elevated pre-treatment UHR independently predicts prognosis and is associated with reduced overall survival in patients with DLBCL. While these findings do not imply a direct causal relationship, they suggest that high UHR may serve as a surrogate marker of systemic metabolic dysfunction linked to poorer clinical outcomes. UHR may be considered a potential marker that could help identify high-risk patients in DLBCL patients in addition to the IPI score. Future prospective studies are warranted to investigate whether interventions targeting metabolic abnormalities might indirectly improve outcomes in patients with DLBCL.

Ethics Approval and Consent to Participate

This study was approved by the Recep Tayyip Erdoğan University Non-Interventional Clinical Research Ethics Committee (Decision No: 2025/201; date: 26.05.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. As this is a retrospective study involving patient data obtained from medical records, the requirement for obtaining written informed consent was waived by the ethics committee.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: KI, BS; Study Design: KI, BS; Supervision: KI, BS; Funding: KI, BS; Materials: KI; Data Collection and/or Processing: KI, BS; Statistical Analysis and/or Data Interpretation: BS; Literature Review: KI; Manuscript Preparation: KI, BS; and Critical Review: KI, BS.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. Nat Med. 2018;24(5):679-690. doi: 10.1038/s41591-018-0016-8.
2. Ruppert AS, Dixon JG, Salles G, et al. International prognostic indices in diffuse large B-cell lymphoma: a comparison of IPI, R-IPI, and NCCN-IPI. Blood. 2020;135(23):2041-2048. doi: 10.1182/blood.2019002729.
3. Kunlayawutipong T, Rattanathammethee T, Punnachet T, et al. Prevalence and risk factors for hyperuricemia and hyperuricosuria in patients with hematologic malignancies. Front Med (Lausanne). 2024;11:1343000. doi: 10.3389/fmed.2024.1343000.
4. Prochazka KT, Melchardt T, Posch F, et al. NCCN-IPI score-independent prognostic potential of pretreatment uric acid levels for clinical outcome of diffuse large B-cell lymphoma patients. Br J Cancer. 2016;115(10):1264-1272. doi: 10.1038/bjc.2016.325.
5. Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. Oncogenesis. 2016;5(1):e189. doi: 10.1038/oncsis.2015.49.
6. Yvan-Charvet L, Pagler T, Gautier EL, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. Science. 2010;328(5986):1689-1693. doi: 10.1126/science.1189731.
7. Pirro M, Ricciuti B, Rader DJ, Catapano AL, Sahebkar A, Banach M. High density lipoprotein cholesterol and cancer: Marker or causative? Prog Lipid Res. 2018;71:54-69. doi: 10.1016/j.plipres.2018.06.001.
8. Yu T, Luo D, Luo C, Xu-Monette ZY, Yu L. Prognostic and therapeutic value of serum lipids and a new IPI score system based on apolipoprotein A-I in diffuse large B-cell lymphoma. Am J Cancer Res. 2023;13(2):475-484.
9. Yiu A, Van Hemelrijck M, Garmo H, et al. Circulating uric acid levels and subsequent development of cancer in 493,281 individuals: findings from the AMORIS Study. Oncotarget. 2017;8(26):42332-42342. doi: 10.18632/oncotarget.16198.
10. Zhao D, Xu H, Tang F, Cui T, Sun X, Song G. Association of dynamic changes in metabolic syndrome components with clinical outcomes in diffuse large B-cell lymphoma. Front Oncol. 2025;15:1524498. doi: 10.3389/fonc.2025.1524498.
11. Bazmandegan G, Dehghani MH, Karimifard M, et al. Uric acid to HDL ratio: A marker for predicting incidence of metabolic syndrome in patients with type II diabetes. Nutr Metab Cardiovasc Dis. 2024;34(4):1014-1020. doi: 10.1016/j.numecd.2023.12.022.
12. Cheng Y, Zhang H, Zheng H, et al. Association between serum uric acid/HDL-cholesterol ratio and chronic kidney disease: a cross-sectional study based on a health check-up population. BMJ Open. 2022;12(12):e066243. doi: 10.1136/bmjopen-2022-066243.
13. Li Y, Jin-Si-Han EE, Feng C, et al. An evaluation model of hepatic steatosis based on CT value and serum uric acid/HDL cholesterol ratio can predict intrahepatic recurrence of colorectal cancer liver metastasis. Int J Clin Oncol. 2024;29(9):1263-1273. doi: 10.1007/s10147-024-02550-y.
14. Jaeger A, Gambheer SMM, Sun X, et al. Activated granulocytes and inflammatory cytokine signaling drive T-cell lymphoma progression and disease symptoms. Blood. 2023;141(23):2824-2840. doi: 10.1182/blood.2022015653.
15. Carbone A, Tripodo C, Carlo-Stella C, Santoro A, Gloghini A. The role of inflammation in lymphoma. Adv Exp Med Biol. 2014;816:315-333. doi: 10.1007/978-3-0348-0837-8_12.
16. Zhou Z, Sehn LH, Rademaker AW, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood. 2014;123(6):837-842. doi: 10.1182/blood-2013-09-524108.
17. Catapano AL, Pirillo A, Bonacina F, Norata GD. HDL in innate and adaptive immunity. Cardiovasc Res. 2014;103(3):372-383. doi: 10.1093/cvr/cvu150.
18. Cockerill GW, Huehns TY, Weerasinghe A, et al. Elevation of plasma high-density lipoprotein concentration reduces inter-

leukin-1-induced expression of E-selectin in an in vivo model of acute inflammation. Circulation. 2001;103(1):108-112. doi: 10.1161/01.cir.103.1.108.
19. Cruz PM, Mo H, McConathy WJ, Sabnis N, Lacko AG. The role of cholesterol metabolism and cholesterol transport in carcinogenesis: a review of scientific findings, relevant to future cancer therapeutics. Front Pharmacol. 2013;4:119. doi: 10.3389/fphar.2013.00119.
20. Pedersen KM, Çolak Y, Bojesen SE, Nordestgaard BG. Low high-density lipoprotein and increased risk of several cancers: 2 population-based cohort studies including 116,728 individuals. J Hematol Oncol. 2020;13(1):129. doi: 10.1186/s13045-020-00963-6.
21. Gao R, Liang JH, Wang L, et al. Low serum cholesterol levels predict inferior prognosis and improve NCCN-IPI scoring in diffuse large B cell lymphoma. Int J Cancer. 2018;143(8):1884-1895. doi: 10.1002/ijc.31590.
22. Park B, Jung DH, Lee YJ. Predictive Value of Serum Uric Acid to HDL Cholesterol Ratio for Incident Ischemic Heart Disease in Non-Diabetic Koreans. Biomedicines. 2022;10(6):1422. doi: 10.3390/biomedicines10061422.
23. Diakos CI, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. Lancet Oncol. 2014;15(11):e493-503. doi: 10.1016/S1470-2045(14)70263-3.
24. Bao C, Gu J, Huang X, You L, Zhou Z, Jin J. Cytokine profiles in patients with newly diagnosed diffuse large B-cell lymphoma: IL-6 and IL-10 levels are associated with adverse clinical features and poor outcomes. Cytokine. 2023;169:156289. doi: 10.1016/j.cyto.2023.156289.
25. Ndrepepa G. Uric acid and cardiovascular disease. Clin Chim Acta. 2018;484:150-163. doi: 10.1016/j.cca.2018.05.046.
26. Barter P, Gotto AM, LaRosa JC, et al; Treating to New Targets Investigators. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med. 2007;357(13):1301-10. doi: 10.1056/NEJMoa064278.
27. Li Z, Liu Q, Yao Z. The serum uric acid-to-high-density lipoprotein cholesterol ratio is a predictor for all-cause and cardiovascular disease mortality: a cross-sectional study. Front Endocrinol (Lausanne). 2024;15:1417485. doi: 10.3389/fendo.2024.1417485.

The European Research Journal 2026;12(1):22-31 Original Article

DOI: https://doi.org/10.18621/eurj.1759091 *Cardiology*

The Role of Systemic Immune-Inflammation Index in Severe Bicuspid Aortic Stenosis

Hakan Süygün[1]

[1]*Department of Cardiology, Faculty of Medicine, Karamanoğlu Mehmetbey University, Karaman, Türkiye*

ABSTRACT

Objectives: Bicuspid aortic valve (BAV) is the most common congenital cardiac abnormality and often leads to severe aortic stenosis (AS) at a younger age compared to tricuspid valves. Inflammation plays a key role in the pathogenesis of AS. This study aimed to investigate the association between the Systemic Immune-Inflammation Index (SII) and disease severity in patients with severe BAV-AS.

Methods: In this retrospective observational study, 76 patients with severe BAV-AS and 76 age- and sex-matched controls without AS were included. Routine laboratory data and transthoracic echocardiographic parameters were recorded. SII was calculated as platelet count × neutrophil count / lymphocyte count. The severity of AS was determined by aortic valve area (AVA) and mean transvalvular gradient. Correlation analyses, Receiver Operating Characteristic (ROC) curve, and logistic regression were used to assess the relationship between SII and AS severity.

Results: The BAV-AS group had significantly higher SII values compared to controls (median: 931.7 vs. 534; P<0.001). SII showed a moderate inverse correlation with AVA (r = –0.547) and a positive correlation with mean gradient (r=0.535). The optimal SII cut-off for predicting severe BAV-AS was 691 (AUC=0.790), with sensitivity of 71.1% and specificity of 77.6%. Multivariate analysis identified SII as an independent predictor of severe BAV-AS (OR: 3.841, 95% CI: 1.395-10.576; P=0.009).

Conclusions: SII is significantly elevated in patients with severe BAV-AS and may serve as a useful inflammatory biomarker for disease burden. Further studies are needed to confirm its utility in clinical decision-making.

Keywords: Bicuspid Aortic Valve, Systemic Immune-Inflammation Index, Aortic Stenosis, Inflammation, Echocardiography, Biomarkers

Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation, affecting approximately 1-2% of the general population, and represents a significant risk factor for the early development of aortic stenosis (AS) [1]. Unlike tricuspid aortic valves, BAV is associated with accelerated calcification and structural degeneration, often resulting in severe AS at a younger age [2, 3]. While tricuspid AS tends to develop in the elderly, Severe aortic stenosis in BAV (BAV-AS) patients is particularly challenging due to its progressive nature and the need for timely clinical evaluation and intervention [4]. Despite advances in imaging, early biomarkers to predict disease severity remain an unmet clinical need.

Aortic stenosis is a progressive disease characterized by chronic inflammation, lipid accumulation, my-

Received: August 5, 2025 **Accepted:** October 10, 2025 **Available Online:** Novemver 24, 2025 **Published:** January 4, 2026

How to cite this article: Süygün H. The Role of Systemic Immune-Inflammation Index in Severe Bicuspid Aortic Stenosis. Eur Res J. 2026;12(1):22-31. doi: 10.18621/eurj.1759091

Corresponding author: Hakan Süygün, MD., Assist. Prof., Phone: +90 338 226 20 00, E-mail: drhakansuygun1@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

ofibroblast activation, and ultimately valve calcification [5, 6]. Emerging evidence supports that AS shares common pathophysiological mechanisms with atherosclerosis, including endothelial dysfunction, oxidative stress, and immune system activation [7, 8]. Accordingly, the interplay between inflammatory and immune responses has gained increasing interest in recent years, particularly regarding their utility in assessing disease severity and prognosis.

Several hematological markers derived from peripheral blood counts have been studied in this context, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR), all of which have demonstrated associations with valvular disease severity and adverse cardiovascular outcomes [9-11]. The systemic immune-inflammation index (SII), which integrates neutrophil, lymphocyte and platelet counts [(platelet × neutrophil)/lymphocyte], was first described by Hu *et al*. [12], as a robust biomarker of inflammatory conditions in oncology patients [13-15]. SII has since been shown to be associated with severity in numerous cardiovascular diseases, including coronary artery disease (CAD) [16], heart failure (HF) [17] and acute myocardial infarction (AMI) [18], but its role in BAV-AS is unexplored.

Given the unique clinical and anatomical features of BAV, it is important to investigate whether SII retains its diagnostic value in this population. The present study aims to evaluate the diagnostic utility of SII in patients with BAV-AS and to examine its correlation with the echocardiographic severity of stenosis. Our findings could establish SII as a practical, cost-effective tool for risk stratification in this high-risk population. This investigation represents one of the first attempts to apply inflammation-based biomarkers specifically to BAV-AS.

METHODS

Study Design and Population

This single-center, retrospective, observational study included patients diagnosed with severe AS due to BAV who were evaluated at the cardiology outpatient clinic of our institution between 2014 and 2022. The diagnosis of BAV was confirmed by transthoracic echocardiography (TTE), based on visualization of a two-leaflet aortic valve in the parasternal short-axis view during systole. Patients with confirmed BAV and echocardiographically severe AS (defined as aortic valve area [AVA] <1.0 cm² or mean transvalvular gradient ≥40 mmHg) were enrolled in the study.

A control group consisting of age- and sex-matched individuals without structural heart disease or valvular pathology was selected from patients who underwent routine check-ups during the same period. Individuals with known chronic inflammatory diseases, hematological disorders, malignancy, recent infection, autoimmune conditions or chronic use of anti−inflammatory medications (including NSAIDs or corticosteroids) were excluded from both groups to minimize confounding effects on inflammatory markers. Patients with moderate-to-severe aortic regurgitation, more than mild involvement of other valves (e.g., mitral or tricuspid), congenital heart diseases other than BAV, and any previous valve surgery were excluded from the analysis. Our study complied with the Declaration of Helsinki and was approved by the local Ethical Committee of the Karamanoglu Mehmetbey University, Karaman, Turkey. (Approval date: 04/06/2025, Approval number: 21-2025/04).

Data Collection

Demographic characteristics (age, sex), cardiovascular risk factors (hypertension, diabetes mellitus, hyperlipidemia, smoking status), and comorbidities (atrial fibrillation, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease) were recorded from electronic medical records.

Venous blood samples obtained from patients on the day of their outpatient clinic admission were retrospectively retrieved from the institutional database. Routine laboratory analyses included complete blood count, renal and inflammatory markers such as C-reactive protein (CRP, mg/dL). Hematologic parameters—including neutrophil, lymphocyte, and platelet counts - were measured using an automated hematology analyzer (Mindray BC-6000, Mindray Bio-Medical Electronics Co., Shenzhen, China). Serum creatinine, electrolytes, liver enzymes, and lipid profiles were assessed using a Beckman Coulter AU5800 modular chemistry analyzer (Beckman Coulter Inc., Brea, CA, USA).

Based on complete blood count results, the Sys-

temic Immune-Inflammation Index (SII) was calculated using the following formula: SII= (Platelet count×Neutrophil count) / Lymphocyte count. All blood samples were processed within one hour of collection to ensure analytical accuracy. Laboratory assessments were performed within 24 hours of the echocardiographic evaluation.

Echocardiographic Evaluation

All patients underwent comprehensive two-dimensional, M-mode, and Doppler TTE examinations using a Philips iE33 xMatrix system (Philips Healthcare, Andover, MA, USA) equipped with 2.5- and 3.5-MHz transducers. Echocardiographic assessments were performed by experienced cardiologists blinded to the patients' laboratory and clinical data.

The diagnosis of severe AS was based on standard echocardiographic criteria. AVA was calculated using the continuity equation, while the maximum transvalvular pressure gradient was derived from peak aortic jet velocity using continuous-wave Doppler. The mean aortic pressure gradient was calculated based on the time-velocity integral of the aortic jet signal. In all participants, left ventricular ejection fraction (LVEF) was also measured using the modified Simpson's biplane method.

Statistical Analysis

Data analysis was carried out using IBM SPSS Statistics version 25.0 (IBM Corporation, Armonk, NY, USA). As there were more than 50 data points, skewness and kurtosis values were examined, and the distribution was considered normal when these values were within ±2 (19). Categorical variables were presented as frequencies and percentages, while continuous variables were expressed as mean±standard deviation (SD) for normally distributed data and as median with interquartile range (25th-75th percentile) for non-normally distributed data. Group comparisons for continuous variables were performed using the independent samples t-test or Mann–Whitney U test, depending on distribution characteristics. Categorical variables were compared using Pearson's chi-square test. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of the SII and to identify an optimal cut-off value for predicting the presence of severe AS. The optimal threshold was calculated based on Youden's index, incorporating both sensitivity and specificity for SII. Pearson correlation test was conducted in order to investigate the correlation between mean aortic transvalvular pressure gradient, AVA, and SII. To determine independent predictors associated with the presence of severe aortic stenosis, multivariate logistic regression analysis was performed. Variables with a P-value <0.15 in univariate analysis were included in the multivariate models. The models demonstrated acceptable goodness of fit for model 1 (Hosmer–Lemeshow χ^2=43.503; P>.05) and model 2 (Hosmer–Lemeshow χ^2=45.801; P>.05). For each variable, odds ratios (ORs) and 95% confidence intervals (CIs) were reported. The statistical significance of the results was determined by a P-value of 0.05.

RESULTS

A total of 152 individuals were included in the study, comprising 76 patients with severe BAV-AS and 76 age- and sex-matched control subjects. The baseline demographic and clinical characteristics of both groups are presented in Table 1. The groups were comparable in terms of age, sex distribution, and most comorbidities. However, hypertension was significantly more prevalent in the BAV-AS group (82.8% vs. 67.1%, P=0.025). The BAV-AS group also exhibited significantly lower hemoglobin and albumin levels, and reduced LVEF compared to controls (P=0.041, P=0.040, and P=0.002, respectively).

With respect to inflammatory parameters, the BAV-AS group demonstrated significantly higher neutrophil counts, platelet counts, and SII values, while lymphocyte counts were not significantly different. Median SII values were markedly elevated in the BAV-AS group compared to controls (931.7 vs. 534; P<0.001). Additionally, SII >691 was significantly more common among BAV-AS patients (72.3% vs. 22.3%; P<0.001).

Echocardiographic evaluation revealed a mean aortic pressure gradient of 54.2±11.2 mmHg and an average AVA of 0.64±0.15 cm² in the BAV-AS group. Pearson correlation analysis showed a significant inverse correlation between SII and AVA (r = –0.547, P<0.001) and a positive correlation between SII and

TABLE 1. Baseline Demographic and Laboratory Characteristics of the Study Population

Variables	Overall	BAV group (n=76)	Control group (n=76)	P-value
Demographics				
Age (year)	66.5±8.7	66.9±8.3	66±9.2	0.537[A]
Sex (male)	101 (66.4)	52 (68.4)	49 (64.5)	0.721[B]
BMI (kg/m^2)	27.3 (24.4-30)	27.9 (26.4-30)	27.1 (25.3-29.7)	0.071[C]
Smoking	92 (60.5)	45 (59.2)	47 (61.8)	0.741[B]
Comorbidities and drugs, n (%)				
Diabetes mellitus	69 (45.3)	32 (42.1)	37 (48.6)	0.415[B]
Hypertension	114 (75)	63 (82.8)	51 (67.1)	**0.025[B]**
Hyperlipidemia	81 (53.2)	43 (56.5)	38 (50)	0.416[B]
Atrial fibrillation	18 (11.8)	10 (13.1)	8 (10.5)	0.616[B]
CAD	101 (66.4)	52 (68.4)	49 (64.5)	0.621[B]
COPD	40 (26.3)	18 (23.6)	22 (28.9)	0.461[B]
GFR < 60mL/min	20 (13.1)	9 (11.8)	11 (14.4)	0.631[B]
ACE inhibitors	53 (34.8)	30 (39.4)	23 (30.2)	0.233[B]
ARB	41 (26.9)	18 (26.6)	23 (30.2)	0.361[B]
Statins	56 (36.8)	26 (34.2)	30 (39.4)	0.501[B]
Laboratory parameters				
Neutrophil (×10^3/µL)	4.26 (3.6-5.27)	5 (4–6.1)	3.89 (3.5–4.4)	**<0.001[C]**
Lymphocyte (×10^3/µL)	1.64±0.42	1.61±0.45	1.66±0.38	0.420[A]
Platelet (×10^3/µL)	260.4±74.4	287.6±77.2	233.3±60.7	**<0.001[A]**
SII	659.1 (493.7-1036)	931.7 (627.9-1320)	534 (420.6-681.4)	**<0.001[C]**
SII > 691, n (%)	72 (47.3)	55 (72.3)	17 (22.3)	**<0.001[B]**
Hemoglobin (g/dL)	13±1.59	12.7±1.46	13.3±1.69	**0.041[A]**
Albumin (g/L)	4.18±0.33	4.13±0.35	4.24±0.3	**0.04[A]**
CRP (mg/L)	1.93±1	1.96±1.1	1.9±0.99	0.112[A]
LVEF (%)	53.7±6.93	52.1±7.29	55.4±6.14	**0.002[C]**
Mean aortic gradient (mmHg)	-	54.2±11.2	-	-
AVA (cm^2)	-	0.64±0.15	-	-

Continuous variables were shown as mean±standard deviation or median (25th - 75th percentiles) where appropriate. ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; AVA, aortic valve area; BMI, body mass index; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; GFR, glomerular filtration rate; LVEF, left ventricular ejection fraction; SII, systemic immune-inflammation index.

[A]Student's t test, [B]Pearson's χ^2 test, [C]Mann Whitney U test. Statistically significant P-values are shown in bold.

mean aortic gradient (r=0.535, P<0.001), as illustrated in Figures 1 and 2, respectively.

In univariate logistic regression analysis, neutrophil count, platelet count, hemoglobin, albumin, LVEF, and both continuous and categorical SII values were significantly associated with BAV-AS. In multivariate models, SII remained an independent predictor of severe BAV-AS, whether evaluated as a continuous (OR: 1.002, 95% CI: 1.001–1.003; P=0.05) or categorical variable (SII >691: OR: 3.841, 95% CI: 1.395-10.576; P=0.009), after adjusting for potential confounders (Table 2).

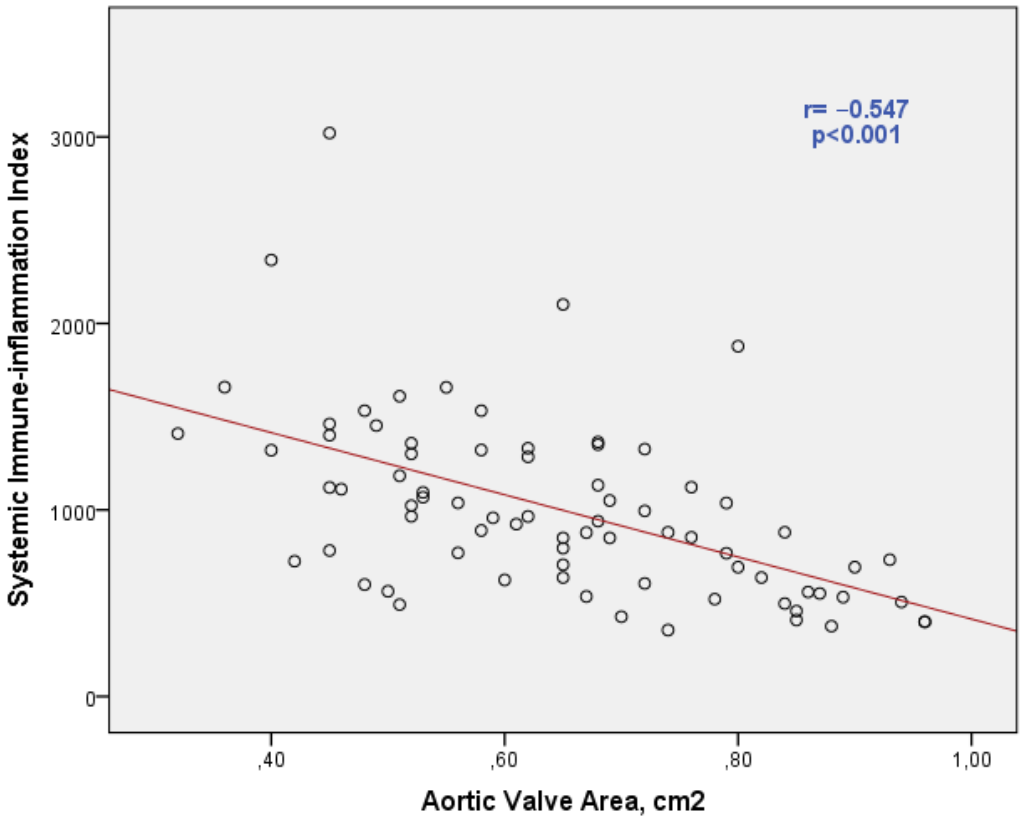

FIGURE 1. **Correlation between systemic immune-inflammation index and aortic valve area.**

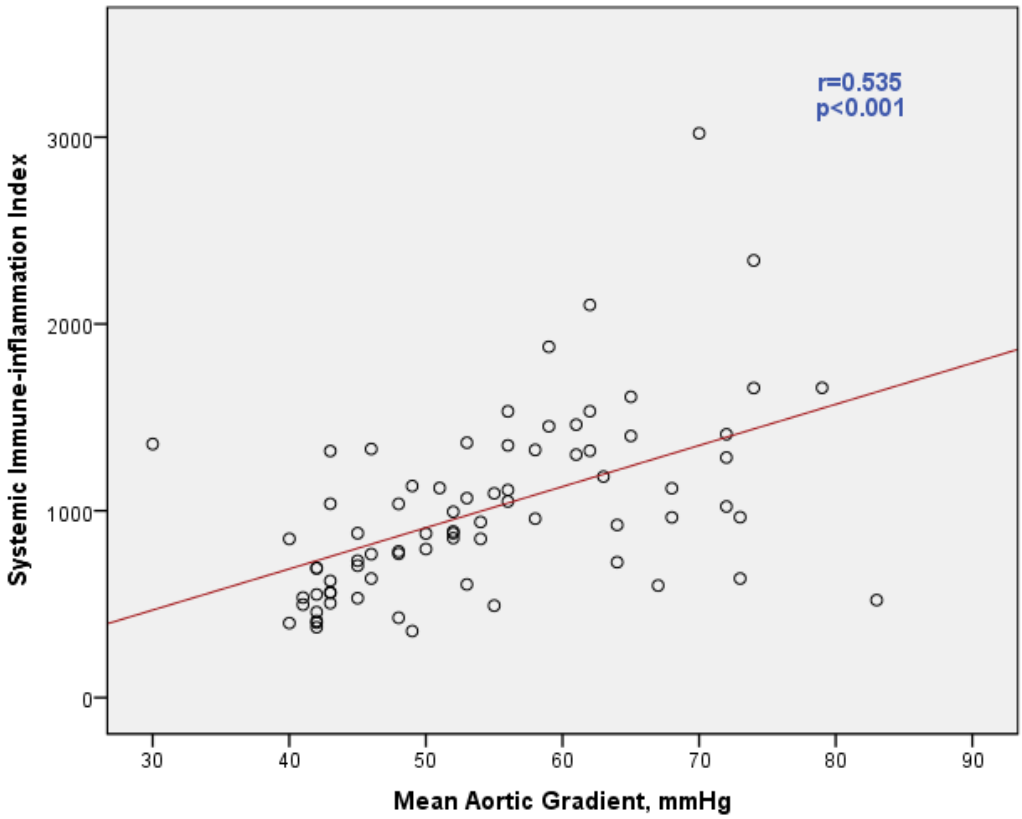

FIGURE 2. **Correlation between systemic immune-inflammation index and mean aortic transvalvular pressure gradient.**

TABLE 2. Independent Predictors of Severe Bicuspid Aortic Stenosis by Logistic Regression Analysis

Variables	Univariate analysis		Multivariate analysis–Model 1[a]		Multivariate analysis–Model 2[b]	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
HT	2.37 (1.1-5.1)	**0.027**	1.720 (0.657-4.504)	0.270	1.821 (0.687-4.826)	0.228
Albumin	0.356 (0.131-0.965)	**0.042**	0.424 (0.121-1.485)	0.180	0.382 (0.107-1.366)	0.139
Hemoglobin	0.808 (0.657-0.994)	**0.044**	0.822 (0.640-1.055)	0.124	0.814 (0.628-1.055)	0.120
CRP	1.057 (0.77-1.45)	0.734	-	–	-	-
Neutrophil	2.564 (1.772-3.712)	**<0.001**	1.765 (1.11-2.809)	**0.016**	1.854 (1.212-2.836)	**0.004**
Platelet	1.011 (1.006-1.017)	**<0.001**	1.005 (0.997-1.012)		1.003 (0.996-1.011)	0.338
Lymphocyte	0.729 (0.340-1.565)	0.417	-	–	-	-
SII	1.004 (1.002-1.005)	**<0.001**	1.002 (1.001-1.003)	0.05	-	-
SII >691	9.09 (4.34-19)	**<0.001**	-	–	3.841 (1.395-10.576)	**0.009**
LVEF (%)	0.926 (0.880-0.975)	**0.004**	0.961 (0.904-1.020)	0.192	0.955 (0.898-1.015)	0.140

CI, confidence interval; CRP, C-reactive protein; HT, hypertension; LVEF, left ventricular ejection fraction; OR, odds ratio; SII, systemic immune-inflammation index. [a]SII presented as a continuous variable, [b]SII presented as a categorical variable. Statistically significant P-values are shown in bold.

To assess the discriminative performance of inflammatory markers in identifying severe BAV-AS, ROC curve analysis was performed for SII, NLR, and PLR. The area under the curve (AUC) for SII was 0.790 (95% CI: 0.718-0.861, P<0.001), indicating good diagnostic accuracy. In comparison, NLR and PLR yielded lower AUC values of 0.734 (95% CI: 0.663-0.819) and 0.690 (95% CI: 0.606-0.774), respectively (Figure 3, Table 3). An SII cut-off value of 691 was associated with a sensitivity of 71.1% and specificity of 77.6%. These findings suggest that SII outperforms other commonly used inflammatory markers in discriminating patients with severe BAV-AS.

DISCUSSION

This study is the first, to our knowledge, to investigate the relationship between the SII and disease severity in patients with severe BAV-AS, a relatively underrepresented subgroup within the spectrum of valvular heart disease. While inflammation has long been implicated in the pathogenesis of calcific AS, the application of composite inflammatory biomarkers such as SII specifically to the BAV population has not been previously explored. Unlike tricuspid AS, BAV disease is a congenital condition characterized by abnormal cusp morphology, altered shear stress, and accelerated calcification, resulting in severe aortic stenosis at a younger age. These distinct pathophysiological mechanisms may also lead to different patterns of inflammatory activation, making systemic inflammatory biomarkers such as the SII particularly relevant in this subgroup [2, 3]. Our findings demonstrate that higher SII values are moderate yet significant correlations with reduced AVA and increased transvalvular gradients in patients with BAV-AS, suggesting a potential pathophysiologic link between systemic immune-inflammatory activation and disease progression in this distinct clinical entity.

Previous studies have established that AS shares numerous features with atherosclerosis, including endothelial dysfunction, lipid accumulation, and inflammatory cell infiltration [5, 7]. Inflammatory activity is known to contribute to valvular calcification and fibrosis, processes that are central to the evolution of stenotic lesions. In this regard, various inflammation-based indices such as NLR, PLR, and LMR have been

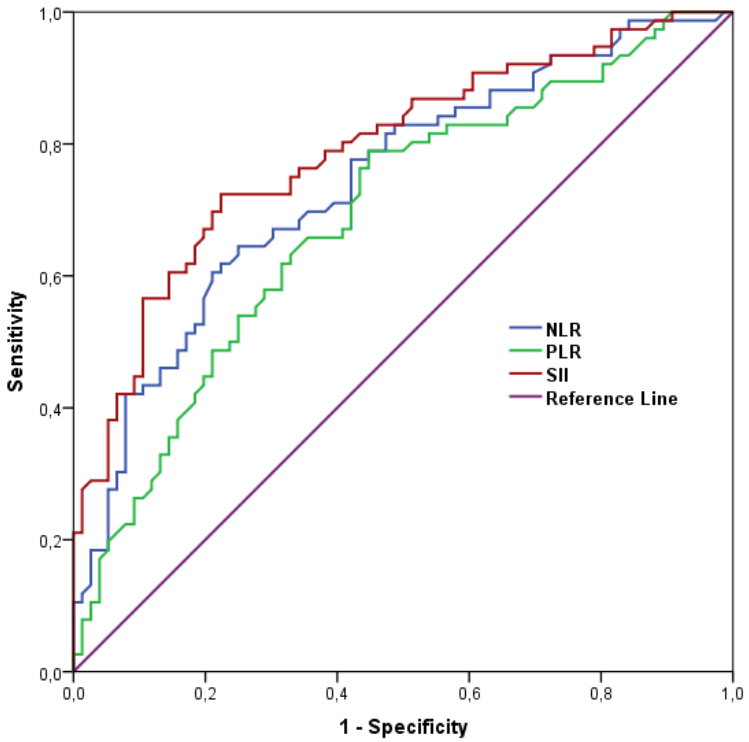

FIGURE 3. The ROC curves of NLR, PLR and SII for bicuspid severe aortics stenosis. NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

explored in the context of valvular disease severity and outcomes [9, 11, 20]. However, SII - by integrating neutrophil, lymphocyte, and platelet counts - provides a broader representation of the interplay between innate immunity, lymphocyte suppression, and thrombopoietic activity. In our cohort, SII demonstrated superior discriminative ability compared to traditional inflammatory markers such as NLR and PLR, supporting its potential role as a more comprehensive biomarker that integrates neutrophil-mediated inflammation, lymphocyte-related immune regulation, and thrombocytic activity in BAV-AS pathophysiology.

In a previous study by Erdoğan *et al*. [21] involving patients with tricuspid aortic valve calcific AS, SII was significantly elevated in patients with severe AS and showed moderate correlations with mean aortic pressure gradient and AVA. Our findings are in alignment with that study and extend its implications to the bicuspid valve population, thereby confirming the potential of SII as a non-invasive, blood-based marker of hemodynamic severity across different anatomical subtypes of AS. Notably, the correlation coefficients between SII and both AVA and mean gradient in our study (r = –0.547 and r=0.535, respectively) are comparable to those previously reported in tricuspid AS cohorts, reinforcing the consistency of this association. These findings suggest that SII may not only reflect systemic inflammation but also serve as a surrogate marker of

TABLE 3. ROC Curve Analysis of SII for Bicuspid Severe Aortic Stenosis

Variable	AUC (%95)	P-value	Cut-off	Sensitivity (%)	Specificity (%)
SII	0.790 (0.718–0.861)	**0.001**	691	0.711	0.776
NLR	0.734 (0.663–0.819)	**0.001**	2.78	0.647	0.745
PLR	0.690 (0.606–0.774)	**0.001**	159.1	0.658	0.645

AUC, area under curve; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ROC, receiver operating characteristic; SII, systemic immune-inflammation index. Statistically significant P-values are shown in bold.

valvular disease severity in patients with BAV-AS.

Furthermore, ROC curve analysis in our study demonstrated good diagnostic accuracy for SII in identifying BAV-AS, with an AUC of 0.790 and an optimal cut-off value of 691, providing a sensitivity of 71.1% and specificity of 77.6%. These metrics are consistent with prior cardiovascular studies in which SII was found to be a reliable marker for disease prediction, including in CAD, HF, acute coronary sydromes, myocardial ischemia on myocardial perfusion scintigraphy [16-18, 22-24]. Interestingly, the optimal SII cut-off identified in our cohort was lower than the value reported by Erdoğan *et al*. [21] in patients with tricuspid aortic stenosis. This difference may be attributed to the younger age profile typically observed in the BAV population [2, 3], as systemic inflammatory activity and related indices such as SII tend to increase with advancing age. Thus, lower SII thresholds may be more appropriate for younger patients with congenital valve morphology such as BAV. Importantly, our logistic regression analysis showed that SII remained an independent predictor of severe BAV-AS even after adjustment for other hematologic and clinical variables, underscoring its potential utility in risk stratification. Although the SII was identified as an independent predictor of severe BAV-AS, the odds ratio (OR = 1.002) may appear modest at first glance. However, as a continuous variable with a wide range, even small per-unit increases in SII can translate into clinically meaningful risk differences when elevated levels are considered. Similar effect sizes have been reported for SII in various cardiovascular conditions, especially when modeling it as a continuous biomarker [16, 21].

Our study also revealed that BAV-AS patients had significantly lower LVEF values than controls, and this reduction in systolic function was paralleled by elevated inflammatory indices. This is in agreement with the findings of Kasapkara *et al*. [25], who demonstrated that increased NLR was associated with decreased EF in patients with severe AS. The present study expands on these findings by showing that SII, a more comprehensive inflammatory marker, may also reflect early myocardial impairment in BAV-AS, even before overt clinical deterioration.

From a clinical perspective, the identification of elevated SII levels in patients with BAV-AS may offer practical utility in patient risk stratification. As a readily accessible and inexpensive biomarker derived from routine blood tests, SII could be incorporated into clinical workflows to identify patients at higher inflammatory risk who may require more frequent surveillance or earlier consideration for intervention such as aortic valve replacement or TAVI. Particularly in patients with equivocal symptoms or borderline echocardiographic findings, elevated SII may prompt clinicians to adopt a more proactive management approach. Additionally, SII might help to anticipate perioperative risk or guide multidisciplinary heart team decisions in the context of surgical planning. Prospective studies are needed to validate whether serial measurements or threshold-based approaches can be integrated into clinical algorithms for BAV-AS management.

Strengths and Limitations

To our knowledge, this is the first study to investigate the clinical relevance of the SII specifically in patients with severe BAV-AS, a relatively underrepresented but clinically distinct subgroup. By focusing on this unique population, SII provide novel insights into the role of inflammatory biomarkers in a congenital valvular condition with earlier disease onset. Furthermore, the study employed comprehensive echocardiographic assessment and evaluated the correlation of SII with both anatomical AVA and hemodynamic (mean gradient) severity indices. The inclusion of ROC analyses comparing SII with other commonly used inflammatory markers, such as NLR and PLR, further strengthens the discriminatory value of SII in this context.

Nevertheless, several limitations should be acknowledged. The retrospective and single-center design limits generalizability, and causality cannot be established. Moreover, while SII reflects systemic inflammation, it may be influenced by transient conditions such as infections or other inflammatory states not fully accounted for. Despite these limitations, our study provides novel insights into the inflammatory profile of BAV-AS and highlights the need for prospective, multi-center investigations.

CONCLUSION

In this study, we demonstrated for the first time that the SII is significantly associated with disease severity in patients with severe BAV-AS. Elevated SII values

were independently correlated with lower AVA and higher aortic transvalvular gradients, suggesting that systemic inflammatory burden may play a contributory role in the progression of valvular obstruction in this population. Given its simplicity, cost-effectiveness, and availability from routine blood tests, SII may serve as a useful non-invasive marker for identifying patients with advanced BAV-AS. Future prospective, multicenter studies are warranted to validate these findings and to explore the potential utility of SII in risk stratification and clinical decision-making for patients with bicuspid valve disease.

Ethics Approval and Consent to Participate

This study was approved by the the local Ethical Committee of the Karamanoglu Mehmetbey University, Karaman, Türkiye (Decision No: 2025/04-21; date: 04.06.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: HS; Study Design: HS; Supervision: HS; Funding: HS; Materials: HS; Data Collection and/or Processing: HS; Statistical Analysis and/or Data Interpretation: HS; Literature Review: HS; Manuscript Preparation: HS; and Critical Review: HS.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles. During the preparation of this work, the authors used ChatGPT to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol. 2002;39(12):1890-900. doi: 10.1016/s0735-1097(02)01886-7.
2. Tzemos N, Therrien J, Yip J, et al. Outcomes in adults with bicuspid aortic valves. JAMA. 2008;300(11):1317-1325. doi: 10.1001/jama.300.11.1317.
3. Siu SC, Silversides CK. Bicuspid aortic valve disease. J Am Coll Cardiol. 2010;55(25):2789-2800. doi: 10.1016/j.jacc.2009.12.068.
4. Michelena HI, Desjardins VA, Avierinos JF, et al. Natural history of asymptomatic patients with normally functioning or minimally dysfunctional bicuspid aortic valve in the community. Circulation. 2008;117(21):2776-2784. doi: 10.1161/CIRCULATIONAHA.107.740878.
5. Pawade TA, Newby DE, Dweck MR. Calcification in Aortic Stenosis: The Skeleton Key. J Am Coll Cardiol. 2015;66(5):561-577. doi: 10.1016/j.jacc.2015.05.066.
6. Yutzey KE, Demer LL, Body SC, et al. Calcific aortic valve disease: a consensus summary from the Alliance of Investigators on Calcific Aortic Valve Disease. Arterioscler Thromb Vasc Biol. 2014;34(11):2387-2393. doi: 10.1161/ATVBAHA.114.302523.
7. Freeman RV, Otto CM. Spectrum of calcific aortic valve disease: pathogenesis, disease progression, and treatment strategies. Circulation. 2005;111(24):3316-3326. doi: 10.1161/CIRCULATIONAHA.104.486738.
8. Yetkin E, Waltenberger J. Molecular and cellular mechanisms of aortic stenosis. Int J Cardiol. 2009;135(1):4-13. doi: 10.1016/j.ijcard.2009.03.108.
9. Akdag S, Akyol A, Asker M, Duz R, Gumrukcuoglu HA. Platelet-to-Lymphocyte Ratio May Predict the Severity of Calcific Aortic Stenosis. Med Sci Monit. 2015;21:3395-400. doi: 10.12659/msm.894774.
10. Ma X, Ma H, Yun Y, et al. Lymphocyte-to-monocyte ratio in

predicting the calcific aortic valve stenosis in a Chinese case-control study. Biomark Med. 2020;14(14):1329-1339. doi: 10.2217/bmm-2020-0228.
11. Avci A, Elnur A, Göksel A, et al. The relationship between neutrophil/lymphocyte ratio and calcific aortic stenosis. Echocardiography. 2014;31(9):1031-1035. doi: 10.1111/echo.12534.
12. Hu B, Yang XR, Xu Y, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res. 2014;20(23):6212-6222. doi: 10.1158/1078-0432.CCR-14-0442.
13. Hong X, Cui B, Wang M, Yang Z, Wang L, Xu Q. Systemic Immune-inflammation Index, Based on Platelet Counts and Neutrophil-Lymphocyte Ratio, Is Useful for Predicting Prognosis in Small Cell Lung Cancer. Tohoku J Exp Med. 2015;236(4):297-304. doi: 10.1620/tjem.236.297.
14. Geng Y, Shao Y, Zhu D, et al. Systemic Immune-Inflammation Index Predicts Prognosis of Patients with Esophageal Squamous Cell Carcinoma: A Propensity Score-matched Analysis. Sci Rep. 2016;6:39482. doi: 10.1038/srep39482.
15. Gao Y, Guo W, Cai S, et al. Systemic immune-inflammation index (SII) is useful to predict survival outcomes in patients with surgically resected esophageal squamous cell carcinoma. J Cancer. 2019;10(14):3188-3196. doi: 10.7150/jca.30281.
16. Yang YL, Wu CH, Hsu PF, et al. Systemic immune-inflammation index (SII) predicted clinical outcome in patients with coronary artery disease. Eur J Clin Invest. 2020;50(5):e13230. doi: 10.1111/eci.13230.
17. Liu Z, Xv Y, Liu X, Zhou X. Associations of systemic inflammatory markers with the risks of chronic heart failure: A case-control study. Clinics (Sao Paulo). 2022;77:100056. doi: 10.1016/j.clinsp.2022.100056.
18. Wei X, Zhang Z, Wei J, Luo C. Association of systemic immune inflammation index and system inflammation response index with clinical risk of acute myocardial infarction. Front Cardiovasc Med. 2023;10:1248655. doi: 10.3389/fcvm.2023.1248655.
19. George D. SPSS for windows step by step: A simple study guide and reference, 17.0 update, 10/e: Pearson Education India; 2011.
20. Efe TH, Gayretli Yayla K, Yayla C, et al. Calcific aortic stenosis and its correlation with a novel inflammatory marker, the lymphocyte/monocyte ratio. Rev Port Cardiol. 2016;35(11):573-578. doi: 10.1016/j.repc.2016.06.008.
21. Erdoğan M, Öztürk S, Kardeşler B, et al. The relationship between calcific severe aortic stenosis and systemic immune-inflammation index. Echocardiography. 2021;38(5):737-744. doi: 10.1111/echo.15044.
22. Erdoğan M, Erdöl MA, Öztürk S, Durmaz T. Systemic immune-inflammation index is a novel marker to predict functionally significant coronary artery stenosis. Biomark Med. 2020;14(16):1553-1561. doi: 10.2217/bmm-2020-0274.
23. Özkan U, Gürdoğan M, Öztürk C, et al. Systemic Immune-Inflammation Index: A Novel Predictor of Coronary Thrombus Burden in Patients with Non-ST Acute Coronary Syndrome. Medicina (Kaunas). 2022;58(2):143. doi: 10.3390/medicina58020143.
24. Süygün H, Yalçınkaya Öner D, Karakulak UN. The Value of the Naples Prognostic Score and the Systemic Immune-Inflammation Index in Predicting Ischemia on Myocardial Perfusion Scintigraphy. Diagnostics (Basel). 2025;15(11):1372. doi: 10.3390/diagnostics15111372.
25. Kasapkara HA, Aslan AN, Ayhan H, et al. Higher neutrophil to lymphocyte ratio is related to a lower ejectionfraction in bicuspid aortic valve patients. Turk J Med Sci. 2016;46(4):1144-1150. doi: 10.3906/sag-1508-4.

The European Research Journal 2026;12(1):32-50 Original Article

DOI: https://doi.org/10.18621/eurj.1786405 *Infectious Diseases*

On the Trail of Foodborne Zoonoses: Bibliometric Map of Salmonellosis Publications in the European Union/European Economic Area Countries

Cemile Uyar[1], **Sevil Alkan**[2]

[1]Department of Infectious Diseases, Faculty of Medicine, Kütahya Health Sciences University, Kütahya, Türkiye; [2]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

ABSTRACT

Objectives: Salmonella is the second gastrointestinal infection in the European Union and the European Economic Area (EU/EEA). This study aims to identify the academic publications on salmonellosis in EU/EEA countries.

Methods: This study analyses 2,900 articles published in EU/EEA countries between 2000-2025 using Web of Science data. Publications were bibliometrically examined.

Results: Germany (1,390 articles) was the leading contributor, followed by France (996 articles). The thematic focus of the studies was antibiotic resistance (34.5%), molecular characterization of *Salmonella enterica* serotypes (28%) and outbreak epidemiology (22%). The annual number of publications peaked in 2021 with 144 articles. In terms of cooperation, the United Kingdom and Germany were the leaders. Thematic evolution analysis has shown a significant increase in the terms 'antimicrobial resistance' and 'whole genome sequencing' since 2015.

Conclusions: This study showed that research is dominated by antibiotic resistance and molecular characterization studies, with Germany leading the publication output.

Keywords: Bibliometric, Publications, Salmonellosis, *Salmonella*, Bibliometric Analysis, Antimicrobial Resistance, Whole Genome Sequencing, EU/EEA, Foodborne Zoonoses

Salmonellosis is an inclusive term for enteric infections due to non-typhoidal serotypes of Salmonella species, excluding typhoid (*Salmonella Typhi*) and paratyphoid (*Salmonella Paratyphi*) [1]. Salmonella is a foodborne pathogen mainly residing in the intestines of humans and animals. Infections produced by this bacterium are associated with several diseases ranging from gastroenteritis to bacteraemia, sepsis, and focal mucosal infections, which may result fatal, particularly in the most severe cases [2]. Salmonella has a broad host range and serves as reservoirs of infection a variety of animals, predominantly including chickens, pigs, cattle, and reptiles [1, 2]. In most cases, people get sick from eating raw or undercooked infected food. The incubation period and the symptoms may vary according to the dose of bacteria

Received: September 18, 2025 **Accepted:** November 19, 2025 **Available Online:** November 26, 2025 **Published:** January 4, 2026

How to cite this article: Uyar C, Alkan S. On the Trail of Foodborne Zoonoses: Bibliometric Map of Salmonellosis Publications in the European Union/European Economic Area Countries. Eur Res J. 2026;12(1):32-50. doi: 10.18621/eurj.1786405

Corresponding author: Cemile Uyar, MD, Assist. Prof., Phone: +90 274 265 22 86, E-mail: cemileuyar@hotmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

in food and the immune status of the individual [1]. Gram-negative *Salmonella* organisms are ingested in contaminated food or spread through the fecal-oral route [3]. The process by which Salmonella enters the host cell and disseminates throughout the host is not completely elucidated; thus, there are still many unresolved questions [2].

Salmonella is a bacterial genus having two species: *S. enterica* and *S. bongori*. *S. enterica* is subdivided into 6 subspecies and *S. bongori* has 22 serotypes [6]. Approximately 99% of human salmonellosis is caused by the enterica subspecies of *S. enterica*. Typhoidal serotypes cause typhoid fever, while non-typhoidal serotypes (NTS) generally cause self-limiting gastroenteritis [7]. Whole Genome Sequencing (WGS) technologies are of great importance for this bacterium, which has a high capacity for environmental adaptation. WGS has enabled the rapid detection of strains and the global study of transmission processes from the environment to hosts using more than 340,000 genome data, thereby strengthening diagnostic processes [8].

In 2022, according to the ECDC, salmonellosis was the second most reported gastrointestinal infection in the EU and European Economic Area (60), and a significant contributor to foodborne outbreaks. There were 65,967 laboratory-confirmed cases in the area in 2022, 81 of which were fatal (15.5 per 100,000 population). The number of cases, which fell in 2020 because of the COVID-19 pandemic, rose again in 2022, but was still low relative to pre-pandemic levels. The incidence rate is highest among children aged 0 to 4 years (81.5 cases per 100,000 population) - tenfold higher than in adults. Eggs and egg products remain the most common vehicle for the occurrence of salmonella outbreaks and the largest outbreak in 2022 was attributed to chocolate [1].

The increasing number of cases necessitates a comprehensive bibliometric mapping of the research environment. The present study conducted a bibliometric analysis using academic articles on salmonellosis, a foodborne zoonotic disease in EU/AA countries, to identify research trends, collaborations and major themes. Therefore, the objective of this study is to assess the present stage of scientific production in the combat against salmonellosis and to establish the perspectives for the future.

METHODS

Data Source and Search Strategy

In the study, the Web of Science (WoS) Core Collection database was searched on June 2, 2025. The search was limited to publications indexed in Science Citation Index Expanded (SCIE) and Emerging Sources Citation Index (ESCI). The initial search using the keyword TS=('Salmonella' OR "salmonellosis" OR 'non-typhoidal Salmonella') identified 51,961 publications.

Inclusion Criteria

•**Time Interval:** Articles published between January 1, 2000, and May 30, 2025, were included.

•**Document Type:** Only research articles (excluding reviews, conference proceedings, etc.) were selected, resulting in 29,343 publications.

•**Geographical Scope:** Restriction was made to EU/EEA countries, and 7,096 articles were identified.

•**Research Area:** Only publications in the fields of microbiology, infectious diseases, public health, immunology, internal medicine, and pediatrics were selected, resulting in a final sample of 2900 articles.

•**Definition of the European Union (EU) and the European Economic Area (EEA) countries:** The European Union (EU) is an economic and political union of 27 countries. The EU's single market ensures the free movement of goods, services, capital and people between member states. Germany, France, Italy, Spain, the Netherlands, Belgium, Luxembourg, Denmark, Ireland, Austria, Sweden, Finland, Portugal, Greece, Poland, Hungary, the Czech Republic, Slovakia, Slovenia, Estonia, Latvia, Lithuania, Malta, the Republic of Cyprus, Bulgaria, Romania, Croatia are the 27 full members of the EU. The AEA (European Economic Area) countries are Iceland, Liechtenstein and Norway [9].

Data Extraction, Bibliometric Tools and Metrics

All eligible publications were downloaded from WoS as plain text files in a single day to ensure time consistency.

This study explored various aspects such as annual publication trends, contributions from countries and institutions, attribute networks, keyword collocation, top published journals, and thematic evolution,etc.

We conducted a comprehensive analysis with

various advanced bibliometric tools:

(a) VOSviewer: We uncovered thematic trends in the field by visualizing author collaborations and keyword relationships with VOSviewer 1.6.19 [10].

(b) R Studio (Biblioshiny version 4.5.1): Due to its adaptable structure and statistical properties, we were able to analyze and demonstrate complex bibliometric data [11].

We used a similar methodology to that used by Jangid *et al*. [12]. To visualize thematic evolution in Salmonella research, we identified dominant themes, emerging trends and literature gaps by analyzing keyword clusters. In bibliometric analysis, we examined (1) research volume by number of publications, (2) influential studies by citation analysis, (3) global collaborations by co-authorship networks, (4) thematic shifts by keyword frequencies and collocations.

This systematic approach provides a robust dataset to assess the EU/EEA's 25-year profile in Salmonella research while minimizing selection bias.

(c) Academic evaluation metrics: The H-index combines the total number of publications and citations to measure the number and quality of an author's publications, but it may be insufficient. The G-index aims to complement the shortcomings of the H-index by considering the most cited articles. The G-index means that if the author's citations to at least g highly cited articles are more than g^2, the author's G-index is g. This better reflects the impact of highly cited articles. The M-index divides the author's H-index by the year of publication to remove bias due to publication time. These indices can also be applied for countries and journals [13, 14].

As this study is a bibliometric study using publicly available aggregate data, ethical approval is not required. No human or animal subjects were involved, and anonymity has been preserved.

Statistical Analysis

In this study, descriptive statistics (Frequency) were used to determine annual publication and contribution trends; Network Analysis (Co-authorship and Co-occurrence Networks) was used to model global collaborations and keyword relationships; Cluster Analysis to visualize themes, and Citation Analysis (H-, G-, and M-index) to measure the impact of publications. These analyses were performed using VOSviewer 1.6.19, particularly for author collaborations and keyword mapping, and R Studio's Biblioshiny 4.5.1 package for the analysis and presentation of complex bibliometric data.

RESULTS

General Information

For our sample, we analyzed data from the year 2000 to 2025, and we included in the analysis 2900 articles assembled from 204 sources (journals, books, etc.) based on inclusion criteria. The annual growth rate was -3.35%; the average age of the articles was 12.6 years; and the number of citations per article was 35.45. A total of 58,677 references were employed in the analysis, with 4,849 Keywords Plus (ID) and 3,851 author keywords (DE) i.e. articles. A total of 11,783 authors existed who contributed to the research, and 13 authors wrote singly authored articles. The analysis revealed that 19 articles were authored by one author; the average number of authors per article was 7.22, and the rate of international co-authorship was 48.38%.

A total of 2,900 articles, 2,800 (96.6%) in Science Citation Index Expanded (SCI-EXPANDED), 100 (3.4%) in Emerging Sources Citation Index (ESCI) journals.

In the analysis of the publication style of a total of 2,900 articles; 2,146 articles (74%) were made available in open access; with the most common access category being Gold Open Access for 920 articles (31.7%), followed by Free to Read for 579 articles (20%) and Gold-Hybrid for 103 articles (3.6%). Under the Green open access heading, the published version of the articles is recorded as 1,574 (54.3%), the submitted version as 282 (9.7%), and the accepted version as 155 (5.3%).

These results indicate that most of the documents are open (green open access, 69.3%), although the percentage of gold open access publications is limited (35.3%). In addition, it is interesting to note that 1 out of 5 articles (20%) can be accessed for free reading, however they do not allow or offer the option of downloading.

Publications and Citations Per Year

The number of articles fluctuated in the 2000–2009 period. After 115 articles in 2000, numbers

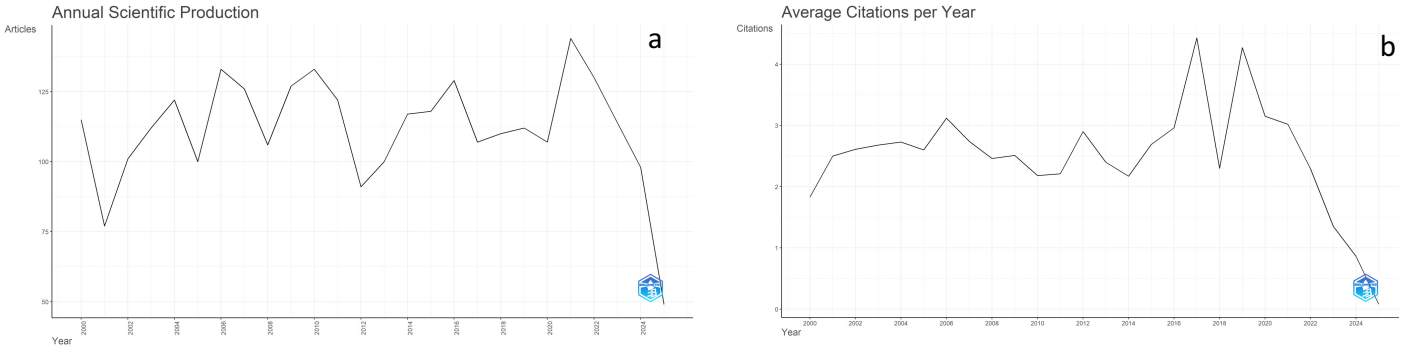

FIGURE 1. **(a) Annual scientific production and (b) Average citations per year.**

dropped to 77 in 2001, but then increased again to 101 in 2002, and 112 in 2003. The number of articles was 122 in 2004, 100 in 2005, 133 in 2006, 126 in 2007, 106 in 2008 and 127 in 2009.

The distribution of articles during the period 2010–2019 remained relatively stable. It began with 133 articles in 2010, declined to 122 in 2011, further dropped to 91 in 2012 and increased back to 100 in 2013. The number of publications in the last 6 years rose from 117 in 2014 and 118 in 2015 to 129 in 2016, 107 in 2017, 110 in 2018 and 112 in 2019.

As summarised in Figure 1a, publication numbers show overall stability during the 2000–2025 period, although the sudden peak in publication numbers in 2021 and the subsequent decline are noteworthy.

TABLE 1. **Citation Topics Micro**

Rank	Research topic	Citation count
1	Salmonella and Campylobacter	1,785
2	Antimicrobial Resistance	242
3	Bacterial Gene Regulation	100
4	Listeria Monocytogenes	100
5	Quorum Sensing	88
6	Lactic Acid Bacteria	50
7	Bacteriophage	50
8	Gut Microbiota	32
9	Outer Membrane	27
10	Yersinia Pathogenesis	19
11	E. coli Pathogenesis	18
12	Microbial Diversity	18
13	Essential Oil	15
14	Sepsis Immunology	13
15	Iron Metabolism	12
16	Toll-like Receptors	11
17	Bacterial Motility	10
18	Poultry Nutrition	10
19	Water Sanitation	9
20	RNA Translation Dynamics	9

Citation Distribution and Analysis by Research Topics

(a) Citation analysis of articles

The analysis of articles cited demonstrated a clear pattern of research focus on a global basis to food-borne pathogens (*Salmonella* and *Campylobacter* 1,785 citations, Listeria 100 citations), and antimicrobial resistance 242 citations), along with how bacteria and sicken or a-foodstuff - (palms 88 citations). The range of specialty areas covered is impressive and new niche topics like gut microbiota (32 citations), bacteriophages (50 citations) and Yersinia pathogenesis (19 citations) further diversify the research limits. Furthermore, isolated studies, such as artificial intelligence and health (1 citation) and the epidemiology of leptospirosis (1 citation), suggest interdisciplinary applications. Less frequently cited but emerging topics are novel areas such as biomedical Raman spectroscopy (5 citations), antimicrobial peptides (8 citations), synthetic biology (2 citations) and nanotoxicology (1 citation). Finally, unbalanced research on clinical and basic microbiology has been verified but the distribution in environmental microbiology, biotechnology and rare infections is an obvious index of research

TABLE 2. Annual Citation Metrics Analysis

Year	Mean total citations per article	Number of articles	Mean citations per year	Citable years
2000	47.57	115	1.83	26
2001	62.53	77	2.50	25
2002	62.61	101	2.61	24
2003	61.64	112	2.68	23
2004	60.02	122	2.73	22
2005	54.65	100	2.60	21
2006	62.5	133	3.12	20
2007	52.08	126	2.74	19
2008	44.28	106	2.46	18
2009	42.61	127	2.51	17
2010	34.85	133	2.18	16
2011	33.11	122	2.21	15
2012	40.63	91	2.90	14
2013	31.25	100	2.40	13
2014	26.08	117	2.17	12
2015	29.56	118	2.69	11
2016	29.58	129	2.96	10
2017	39.9	107	4.43	9
2018	18.36	110	2.30	8
2019	29.86	112	4.27	7
2020	18.93	107	3.15	6
2021	15.11	144	3.02	5
2022	9.15	130	2.29	4
2023	4.04	114	1.35	3
2024	1.73	98	0.86	2
2025	0.08	49	0.08	1

diversity. Citation counts embody the waves of science, especially the food safety and antimicrobial resistance issues, which are urgent worldwide (Table 1).

Table 2 shows the citation performance of reported articles from the year of publication 2000 to the maximal year 2025 of publication. The mean number of citations for articles published between 2000 and 2006 ranged from 47.57 to 62.61. In 2002 specifically, it set a 26-year peak at around an average 62.61 citations. The number of citations was directly related to the citable years (years since publication). On average, for the other years the number of citations received by articles published in year t-25 was 47.57, while for those published in year t (11 years accumulated), 29.56 citations were received. The 2020-2025 period is showing a strong decline. Instead, 2023 articles were cited at a mean of 4.04 times, which fell to 0.08 times by 2025. This indicates that the time to impact of the articles would be too short for any newly published articles to have been cited. Articles from 2017 have the highest average number of citations/year (n=4.43) for a given year; the citation curve peaks around 7-10 years after publication. The average number of citations per year is presented in Figure 1b.

TABLE 3. The Top Funding Agencies

Rank	Funding agency	Country/region	Record count
1	European Union (EU)	Multi-country	169
2	German Research Foundation (DFG)	Germany	132
3	Spanish Government	Spain	119
4	United States Department of Health & Human Services	USA	115
5	National Institutes of Health (NIH)	USA	105
6	UK Research and Innovation (UKRI)	United Kingdom	93
7	Wellcome Trust	United Kingdom	66
8	Biotechnology and Biological Sciences Research Council (BBSRC)	United Kingdom	54
9	Swedish Research Council	Sweden	48
10	Medical Research Council (MRC)	United Kingdom	46
11	Fundação para a Ciência e a Tecnologia (FCT)	Portugal	45
12	NIH National Institute of Allergy & Infectious Diseases (NIAID)	USA	44
13	Agence Nationale de la Recherche (ANR)	France	43
14	Federal Ministry of Education & Research (BMBF)	Germany	36
15	Bill & Melinda Gates Foundation	USA (International)	34
16	Consultative Group on International Agricultural Research (CGIAR)	International	30
17	Swiss National Science Foundation (SNSF)	Switzerland	27
18	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)	Brazil	24
19	Institut Pasteur	France	24
20	European Research Council (ERC)	European Union	23
21	FWO (Research Foundation - Flanders)	Belgium	22
22	Instituto de Salud Carlos III	Spain	22
23	Science Foundation Ireland (SFI)	Ireland	22
24	Bill & Melinda Gates Foundation Grand Challenges Explorations Initiative	USA (International)	21
25	Ministry of Health	Italy	21

Funding Agencies

Subsequently, with regards to the analysis of most funding institutions, the European Union is the leading performer, with 169 publications, followed by the German Research Foundation (DFG) with 132 and the Spanish Government with 119. Among US institutions, US department of Health & Human Services and National Institutes of Health are the key funders with 115 and 105 publications. The top contributing provider was UK Research and Innovation, with 93 publications, followed by Wellcome Trust (n=66), Biotechnology and Biological Sciences Research Council (n=54) and Medical Research Council (n=46). From the Nordic countries, the Swedish Research Council could be cited 48 times, whereas Agence Nationale de la Recherche and Institut Pasteur, France could be cited 43 and 24 times, respectively. The Bill & Melinda Gates Foundation shows the highest number of publications (n=34) and the Consultative Group on International Agricultural Research (CGIAR) follows with 30 publications. With respect to developing regions, Brazil's CAPES program was notable for 24

TABLE 4. Top Published Countries

Country	Number of Articles	Total Citations	Average Citations/Article	EU/EEA Member?
Germany	**1390**	**17,103**	**44.70**	**Yes**
France	**996**	**10,656**	**35.60**	**Yes**
Spain	**876**	**8,975**	**30.10**	**Yes**
United kingdom (uk)	662	4,86	40.20	No
Italy	**648**	**5,528**	**27.80**	**Yes**
Usa	643	6,621	51.30	No
Denmark	**634**	**8,297**	**40.90**	**Yes**
Belgium	**445**	**4,157**	**37.80**	**Yes**
Netherlands	**403**	**4,648**	**40.80**	**Yes**
Sweden	**403**	**4,241**	**37.50**	**Yes**
Ireland	**321**	**4,356**	**46.80**	**Yes**
Portugal	**232**	**1,832**	**30.00**	**Yes**
Poland	**203**	**742**	**11.60**	**Yes**
China	176	777	20.40	No
Switzerland	162	3,355	**90.70**	No
Greece	**152**	**984**	**19.70**	**Yes**
Norway	**136**	**1,369**	**38.00**	**Yes**
Canada	134	2,099	65.60	No
Finland	**116**	**894**	**27.10**	**Yes**
Brazil	115	597	20.60	No
Czech republic	**111**	**1,227**	**28.50**	**Yes**
Austria	**107**	**1,023**	**39.30**	**Yes**
Australia	75	574	33.80	No
Slovakia	**74**	**462**	**13.60**	**Yes**
Hungary	**66**	**436**	**18.20**	**Yes**
India	56	431	28.70	No
Israel	53	618	41.20	No
Iran	52	51	7.30	No
Bulgaria	**51**	**189**	**11.80**	**Yes**
Romania	**39**	-	-	**Yes**
Croatia	**25**	-	-	**Yes**
Estonia	**22**	-	-	**Yes**
Slovenia	**22**	-	-	**Yes**
Luxembourg	**19**	-	-	**Yes**
Iceland	**8**	-	-	**Yes**
Lithuania	**6**	-	-	**Yes**
Latvia	**5**	-	-	**Yes**
Cyprus	**3**	-	-	**Yes**
Malta	**3**	-	-	**Yes**

publications, whereas the Swiss National Science Foundation (n=27), Belgium's FWO (n=22), and the Irish Science Foundation (n=22) were standout among other European countries (Table 3).

Distribution of Publications by Region

Looking into multi-authored publications, Germany (n=1,390) was the most frequently occurring country considering the countries of all authors. France (n=996), Spain (n=876), Italy (n=648) and Denmark (n=634) are the other most productive countries among the EU/EEA countries. In addition, China (n=176) and Switzerland (n=162) show their active participation in the global research network outside the EU/EEA. These results reflect the core position of Europe in Salmonella research, indicating a significant proportion of international cooperative studies. When considering author affiliations only, Germany (n=1,390) leads significantly. With respect to EU/EEA, France (n=996), Spain (n=876), Italy (n=648) and Denmark (n=634) are likewise among the other most productive countries. Outside the EU/EEA, the UK (n=662) and the USA (n=643) are also well inputting countries, as well as China (n=176) and Switzerland (n=162), showing the strength of their connections to the global research network. These results indicate the importance of Europe in academic research, but they are also indicative of the common involvement of authors from around the world (Table 4).

Table 4 demonstrates the academic publication performance of selected countries in terms of publication output, total citations and average citations per publication. While Germany (1,390 publications, 17,103 publications) is the clear leader in terms of publications and citations, it has significantly lower citations per article (n=44.70) than countries like Switzerland (n=90.70), USA (51.30) and Ireland (46.80). Whereas Switzerland demonstrates the quality of its publications with it citations per article and the USA stands out with its high average citation (51.30) despite its modest number of publications (n=643). European nations are prominent in academic terms occupying 9 of top 10 (non-USA), however, the likes of Canada (n=65.6) and Malawi (n=65.70) rank high in average citations received, unexpectedly. On the other hand, Poland (n=11.60), Bulgaria (n=11.80) and Iran (n=7.30) are demonstrating low impact of research with low average of citations.

Analysis of the country−wise distribution of academic publications on Salmonella during 2000–2025 provided important insights into trends in the research activity and outcome (Figure 2). On the contrary, from 43 in 2000, Germany showed a constant rise and 1390

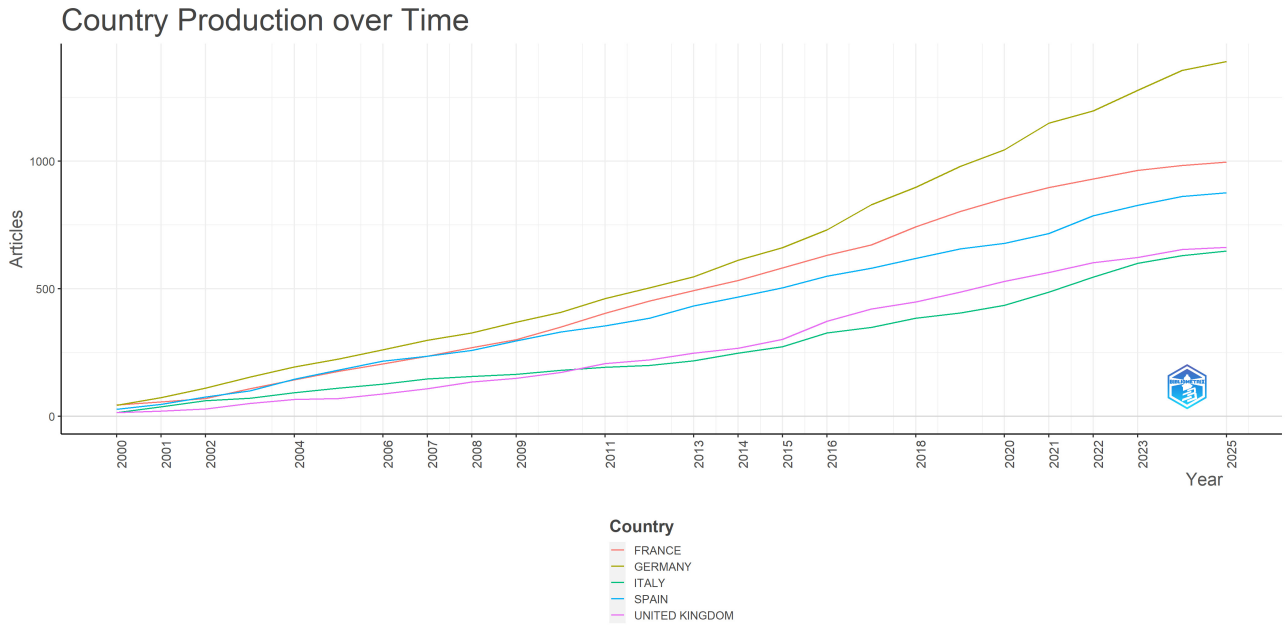

FIGURE 2. Countries' production over time.

publications in 2025, retaining the unassailable lead. Notably, the speed of increase in number of publications grew since 2016 (2016: n=731 → 2025: n=1,390 France: 2000 45th, 2025: 996 2nd. For Spain, the opposite is true; with 28 publications in 2000, Spain has the third place in 2025 with 876 publications. The rate of growth of both countries is very similar in both 2004-2014. Italy's article output is the fourth largest having increased from 14 in 2000 to 648 in 2025. Particularly the sharp rise after 2015 (2015: n=273 → 2025: n=648) is remarkable. The United Kingdom was ranked fifth, The UK started in 2000 with 15 articles to write 662 articles in 2025.

In every country there is a marked acceleration in the number of publications in the 2010-2020 period, with average annual growth rates ranging between 8% and 12% in this period. These figures show that the number of publications decreased in all countries during the pandemic period (i.e., 2020–2022).

These data clearly reveal the leading role of European countries in the Salmonella research, and the rapid increase in research potential of the field, especially in Germany. These trends are visualized in greater detail in Figure 2.

Co-Authorship Analysis between Countries

The Vos Viewer visualization in Figure 3 impressively captures the network of academic cooperation between countries through the thickness of lines, colors and node sizes. The thickness of the lines represents the link strength (Total Link Strength - TLS) between countries, e.g. a thick line between the UK (876 TLS) and Germany (844 TLS) indicates intense academic interaction between these two countries. Nodes of the same color indicate common collaboration groups (e.g. European countries clustered in the same color cluster). The size of the nodes is directly proportional to the number of publications of the countries; large nodes, such as Germany or the USA, emphasize high academic productivity. While the image maintains the dominance of traditional research centers (UK, USA, Germany), it also reveals that countries such as Vietnam (172 TLS) or Ghana (104 TLS) are integrated into the global network with unexpected connectivity. Color groups also make interdisciplinary interactions visible, reflecting geographical or thematic clusters of cooperation. These details show how the image presents not only quantitative but also qualitative dynamics of cooperation. According to Figure

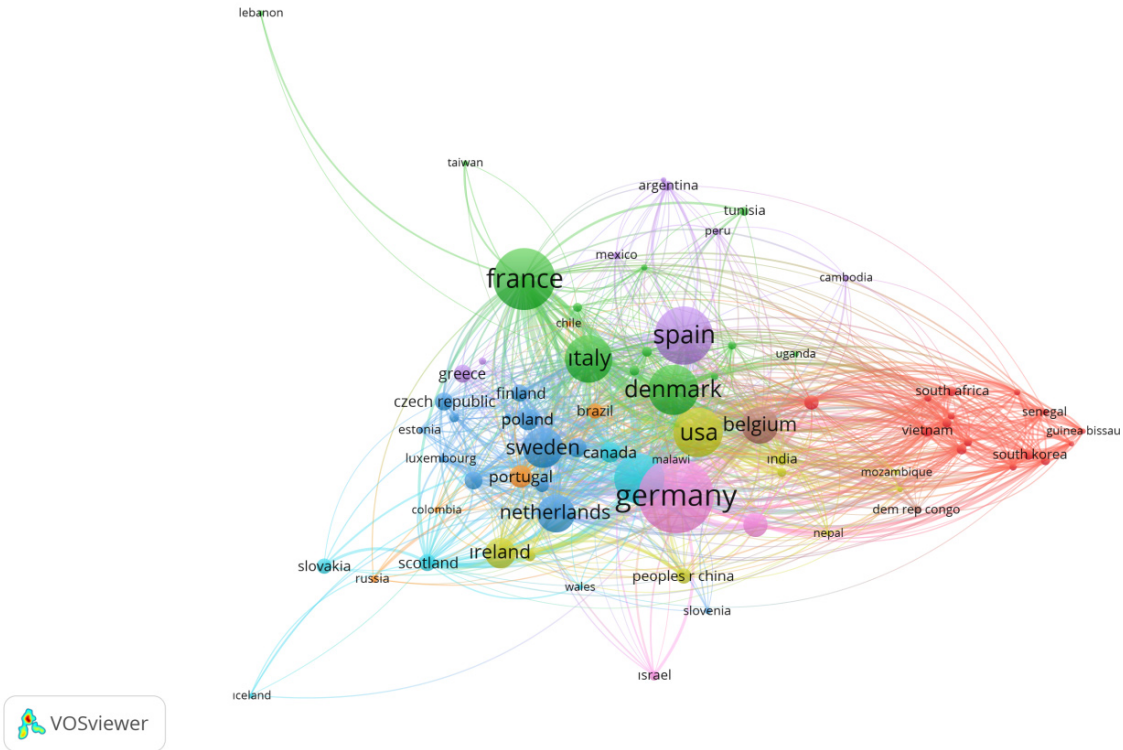

FIGURE 3. Collabrations between countries.

TABLE 5. Top Published Affiliations

Affiliation	Country	Articles
Technical University of Denmark	Denmark	164
Institut Pasteur	France	150
Robert Koch Institute	Germany	128
Statens Serum Institut	Denmark	118
University of Copenhagen	Denmark	104
KU Leuven (Katholieke Universiteit Leuven)	Belgium	79
Karolinska Institutet	Sweden	74
University of Osnabrück	Germany	63
European Centre for Disease Prevention and Control (ECDC)	European Union	62
University of Porto	Portugal	60
University of Oxford	United Kingdom	59
Utrecht University	Netherlands	58
University College Dublin	Ireland	57
Instituto de Salud Carlos III	Spain	55
University of Oviedo	Spain	54
Ghent University	Belgium	53
Hannover Medical School	Germany	51
University of Seville	Spain	50
Free University of Berlin	Germany	49
University of Barcelona	Spain	49
Uppsala University	Sweden	47
Braunschweig University of Technology	Germany	46
National Institute for Public Health and the Environment (RIVM)	Netherlands	46
Institute of Tropical Medicine	Belgium	43
Veterinary Research Institute	Belgium	43

3, the UK stands out as the country with the strongest academic collaboration network (876 total link strength), followed by Germany (n=844) and the USA (n=736). European countries such as France (n=555), Denmark (538) and Italy (380) exhibit strong link networks, while the Netherlands (n=377), Spain (n=358), Belgium (n=329) and Sweden (n=325) also stand out as important centers of academic interaction. Countries such as Ireland (n=280), Switzerland (n=201) and Canada (n=190) have medium-sized but effective networks, while developing countries such as Vietnam (n=172), Bangladesh (n=149) and Ghana (n=104) show surprisingly high connectivity strength. In Africa, Tanzania (n=97), Madagascar (n=94), Senegal (n=94) and Burkina Faso (n=92) have a more active academic cooperation profile than expected, while in Asia, countries such as South Korea (n=139), Thailand (n=73) and Pakistan (n=71) have also gained a foothold in the global network. Large economies such as Brazil (n=76), India (n=75) and Egypt (n=73) exhibit lower than expected connectivity, while countries such as Japan (n=49), Greece (n=46) and Iran (n=32) are represented by relatively weak network structures. Among the smaller countries, Luxembourg (n=90) and Estonia (n=27) have proportionally strong connections, whereas countries such as Turkey (n=21), Russia (n=21) and Chile (n=24) have a limited presence in global academic networks.

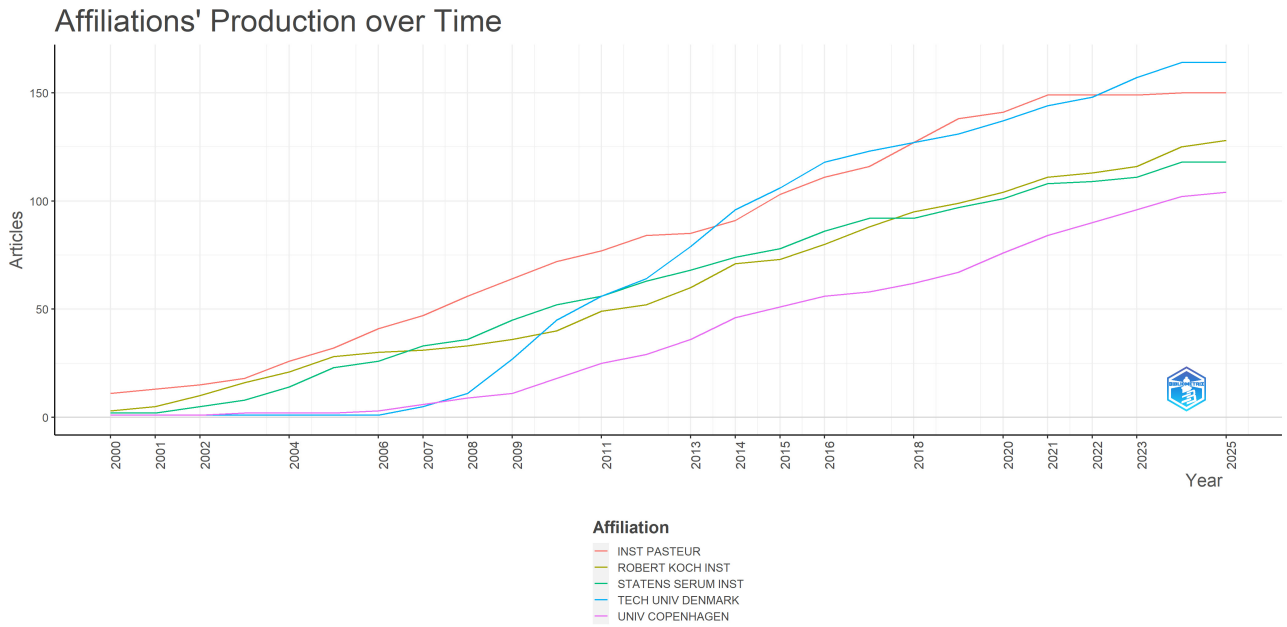

FIGURE 4. Top published affiliations' production over time.

Top Published Affiliations

Denmark appears among the countries with the most publications linked to the Technical University of Denmark (164 articles) and the University of Copenhagen (104 articles), whereas Germany is the country most represented with 5 institutions including the Robert Koch Institute (128 articles). Other major contributors are France (Institute Pasteur, 150 articles), Belgium (KU Leuven, 79 articles) and Sweden (Karolinska Institute, 74 articles). Table 5 also includes institutions as ECDC, European Union institution (62 articles), and the University of Barcelona (Spain) (49 articles). For the most part, Northern and Western European nations appear to be overrepresented in academic publications. Table 5 compares the academic publication impacts of key research institutes within Europe.

Figure 4 compares the research performance of the top five institutions in the publication rankings for 2000-2025. There is a trend towards increasing publication for all organizations, although the rate of growth varied among organizations. Institute Pasteur were the initial leaders with 11 publications in 2000, and rapid expansion was seen at the Technical University of Denmark from 1 in 2006 to 164 in 2024. Also, RKI and Statens Serum Institut both with the same speed to the middle group. The University of Copenhagen came next, seeing a smaller but steady rise from one paper in 2000 to 104 in 2025. The article makes a feature of the different development paths which the institutions have followed since their creation.

Journals

Table 6 summarises the most frequently published journals and their academic impact. For example, *Applied and Environmental Microbiology* (139 articles and h-index of 58 and 9.619 cites) and *Infection and Immunity* (139 articles and h-index of 52, 8. 225 citations) that have achieved a more significant visibility in terms of total publications and impact factor, followed by *Frontiers in Microbiology* (n=145) that present the highest output, but a lower H-index indicating that the articles have not been cited enough; other relevant journals are *Journal of Bacteriology* (H-index: 42), *Antimicrobial Agents and Chemotherapy* (H-index: 45), and *Molecular Microbiology* (H-index: 48), whereas the most recent journals as *Microorganisms and Pathogens* present lower metrics which indicate that they are in their expanding phase. The number of publications in the top five journals over the years is summarised in Figure 5. *Frontiers in Microbiology* began as a new journal in 2013 and demonstrated a very dynamic development, with as many as 145 publications in 2025. *Applied and Environmental*

TABLE 6. Top Published Journals and Their Local Impact

Journal	h_index	g_index	m_index	Total citations	Number of publications	Year of start of publication
Frontiers In Microbiology	30	48	2,308	3239	145	2013
Applied and Environmental Microbiology	58	94	2,231	9619	139	2000
Infection and Immunity	52	85	2	8225	139	2000
Journal of Bacteriology	42	64	1,615	4629	100	2000
Epidemiology and Infection	31	44	1,192	2605	98	2000
Journal of Applied Microbiology	35	54	1,346	3381	94	2000
Journal of Antimicrobial Chemotherapy	44	73	1,692	5507	93	2000
Journal of Clinical Microbiology	42	64	1,615	4489	93	2000
Antımicrobial Agents and Chemotherapy	45	73	1,731	5704	92	2000
Molecular Microbiology	48	81	1,846	7267	81	2000
Microorganisms	13	18	1,857	605	77	2019
Eurosurveillance	26	40	1,529	1837	75	2009
Antıbiotics-Basel	14	21	1,273	589	60	2015
FEMS Microbiology Letters	22	41	0,846	1817	57	2000
PLOS Pathogens	29	50	1,813	3048	50	2010
Emerging Infectious Diseases	28	50	1,077	3025	50	2000
Cellular Microbiology	29	45	1,115	2555	45	2000
BMC Microbiology	24	39	1,263	1592	45	2007
Letters in Applied Microbiology	22	35	0,846	1273	44	2000
PATHOGENS	11	17	1,571	373	42	2019
Microbiology -SGM	22	35	0,88	1231	38	2001
Microbes and Infection	22	37	0,846	1744	37	2000
Journal of Microbiological Methods	16	35	0,615	1271	35	2000
MBIO	22	34	1,467	1164	34	2011
Microbiology Resource Announcements	5	7	0,625	81	33	2018

Microbiology and Infection and Immunity have reached 139 publications each with a continuous increase since 2000, while *Journal of Bacteriology* started from 4 publications in 2000 and plateaued at 100 with a recent increase since 2009. *Epidemiology and Infection* had 10 documents in 2000 and 98 in 2000 with a rate of increase between the years 2010. Overall, *Infection and Immunity and Applied and Environmental Microbiology* are the journals that have shown earlier more publications, while *Frontiers in Microbiology* has shown more publication in recent years. Journals of *Bacteriology and Epidemiology and Infection* maintained similar levels of publications.

Keywords Analysis

The keyword analysis performed with VOSviewer reveals the thematic trends and scientific relationships of research on Salmonella in detail. While the key-

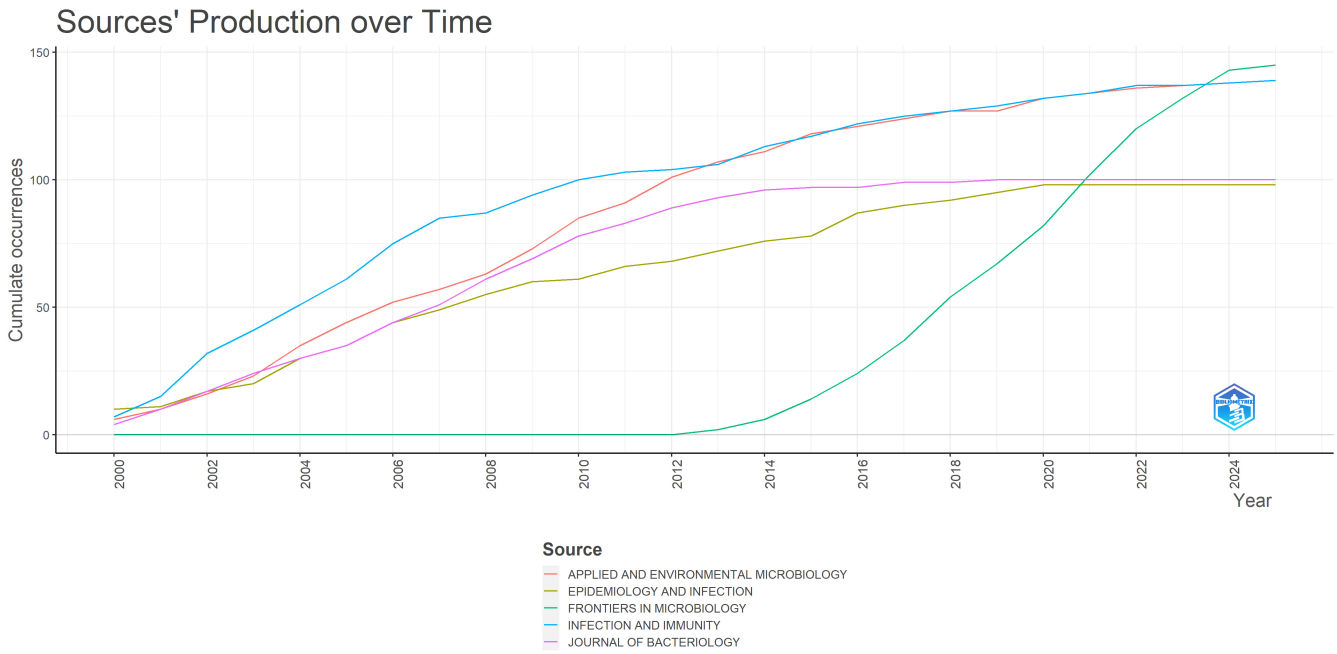

FIGURE 5. Sources' production over time.

word 'Salmonella' (599 occurrences, 1061 TLS) is at the centre of the studies, it is seen that research in this field is mainly concentrated on four main axes.

The primary focus is on antibiotic resistance issues such as 'antimicrobial resistance' (134 occurrences, 363 TLS) and 'antibiotic resistance' (63 occurrences, 138 TLS). In this context, specific resistance mechanisms such as 'multidrug resistance' (43 occurrences, 99 TLS), "fluoroquinolones" (18 occurrences, 46 TLS) and 'colistin resistance' (10 occurrences, 27 TLS) stand out. The second thematic cluster focuses on the molecular characterisation of pathogen species such as '*Salmonella typhimurium*' (123 occurrences, 216 TLS), '*Salmonella enterica*' (96 occurrences, 167 TLS) and 'Salmonella enteritidis' (70 occurrences, 132 TLS). The third and fourth main research areas are epidemiological studies, with molecular typing methods such as PFGE (44 occurrences, 102 TLS), whole genome sequencing (36 occurrences, 93 TLS) and MLST (15 occurrences, 44 TLS) and public health applications such as outbreak (21 occurrences, 36 TLS) and surveillance (33 occurrences, 77 TLS) (Figure 6a).

Figure 6b shows the most frequently used keywords visualised with Worcloud. The Wordcloud analysis generated with Biblioshiny package of Bibliometrix reveals the thematic foci of research on *Escherichia coli* and *Salmonella* species (especially *Typhimurium* and *Enteritidis* serotypes). While '*Escherichia-coli*' (n=620), 'typhimurium' (n=393) and 'identification' (323) are the most frequently encountered terms in the analysis, terms such as "resistance" (n=236), 'antimicrobial resistance' (n=172) and 'antibiotic-resistance' (140), which indicate resistance mechanisms, indicate that a significant portion of the research focuses on antibiotic resistance. Terms related to virulence factors such as 'virulence' (n=221), 'iii secretion system' (n=110) and "invasion" (n=80) indicate that the mechanisms of pathogenicity have been intensively studied, while specific pathogen designations such as 'enterica serovar typhimurium' (203), 'serovar typhimurium' (n=94) reflect the level of taxonomic detail of the studies. Molecular characterisation studies are represented by the term's 'gene' (n=107), 'gene-expression' (n=109), 'sequence' (n=121) and 'molecular characterisation' (n=80), while epidemiological studies are represented by keywords such as 'prevalence' (175), "outbreak" (104) and 'epidemiology' (n=85). The terms 'infection' (n=209), 'infections' (n=160), 'humans' (n=96) and 'disease' (n=75) reflecting the clinical and public health dimension, as well as terms such as "poultry" (n=67) and 'animals' (n=64) indicating zoonotic links, point to the interdisciplinary nature of the studies. In particular, the emphasis on specific resistance models

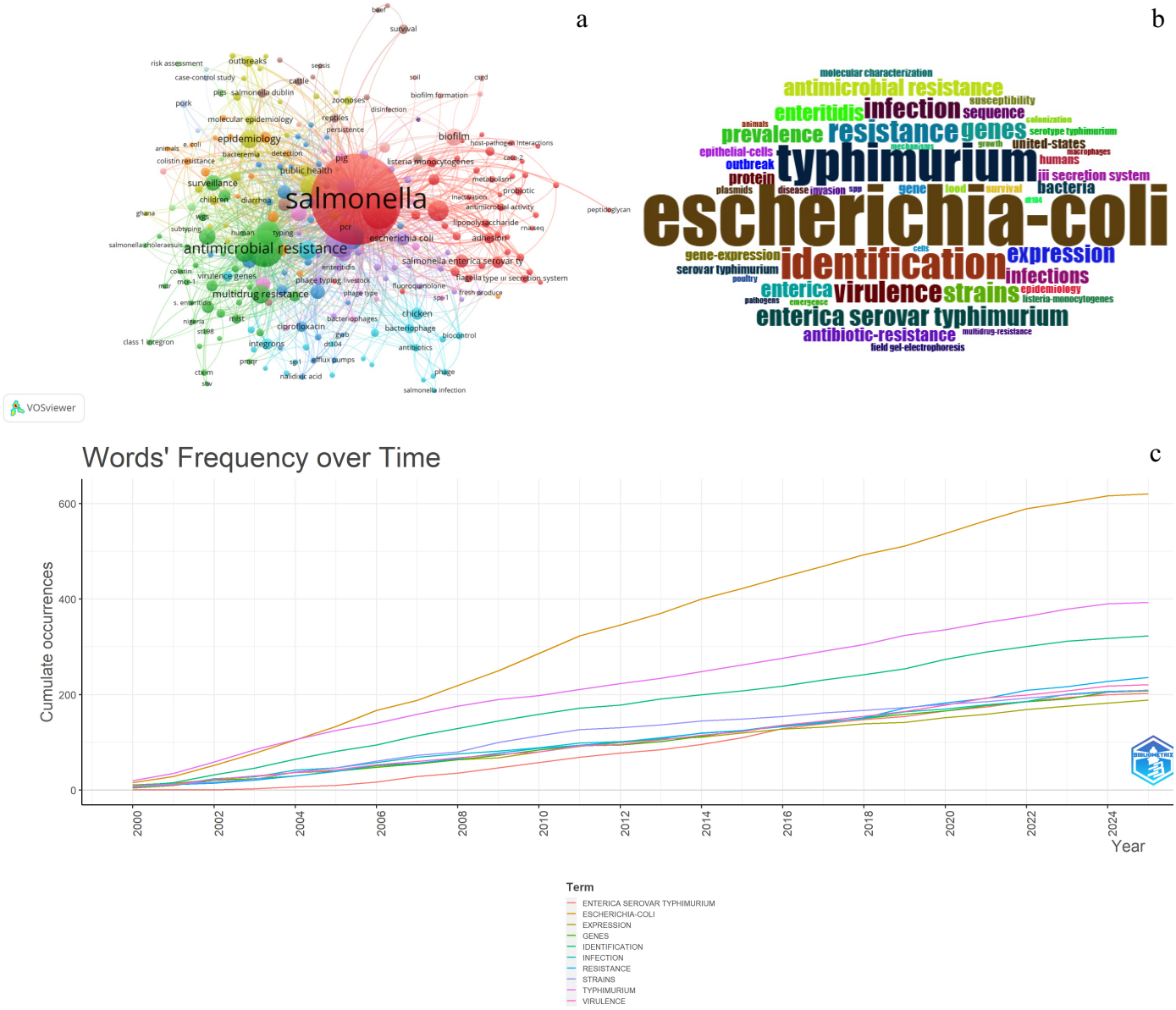

FIGURE 6. **(a) Keyword analysis with Vosviewer, (b) Word cloud and (c) Words' frequency over time.**

such as ‘multidrug resistance’ (n=63) and ‘dt104’ (n=60) indicates that research focuses on current health threats.

Thematic Mapping and Factorial Analysis

When the keyword usage trends in studies conducted between 2000 and 2025 are analyzed, a steady increase is observed for all terms. 'Escherichia-coli' (from 16 in 2000 to 620 in 2025) and “typhimurium” (from 20 to 393) are the two most dominant terms, indicating that studies have largely focused on these pathogens. Increases in the term’s “identification” (9→323), “resistance” (6→236) and “virulence” (7→221) reflect the growing interest in research on pathogen identification, antibiotic resistance and virulence mechanisms. The significant increase in the term ‘enterica serovar typhimurium’ (1→203), especially after 2010, indicates that studies are moving towards more specific pathogen characterization. The steady increase in molecular biology terms such as ‘expression’ (11→189) and ‘genes’ (5→208) indicates that research is focusing on the genetic level. The sharp increases observed for all terms in the 2020-2025 period probably reflect the importance given to post-pandemic infectious disease research. These data quantitatively illustrate the thematic evolution and

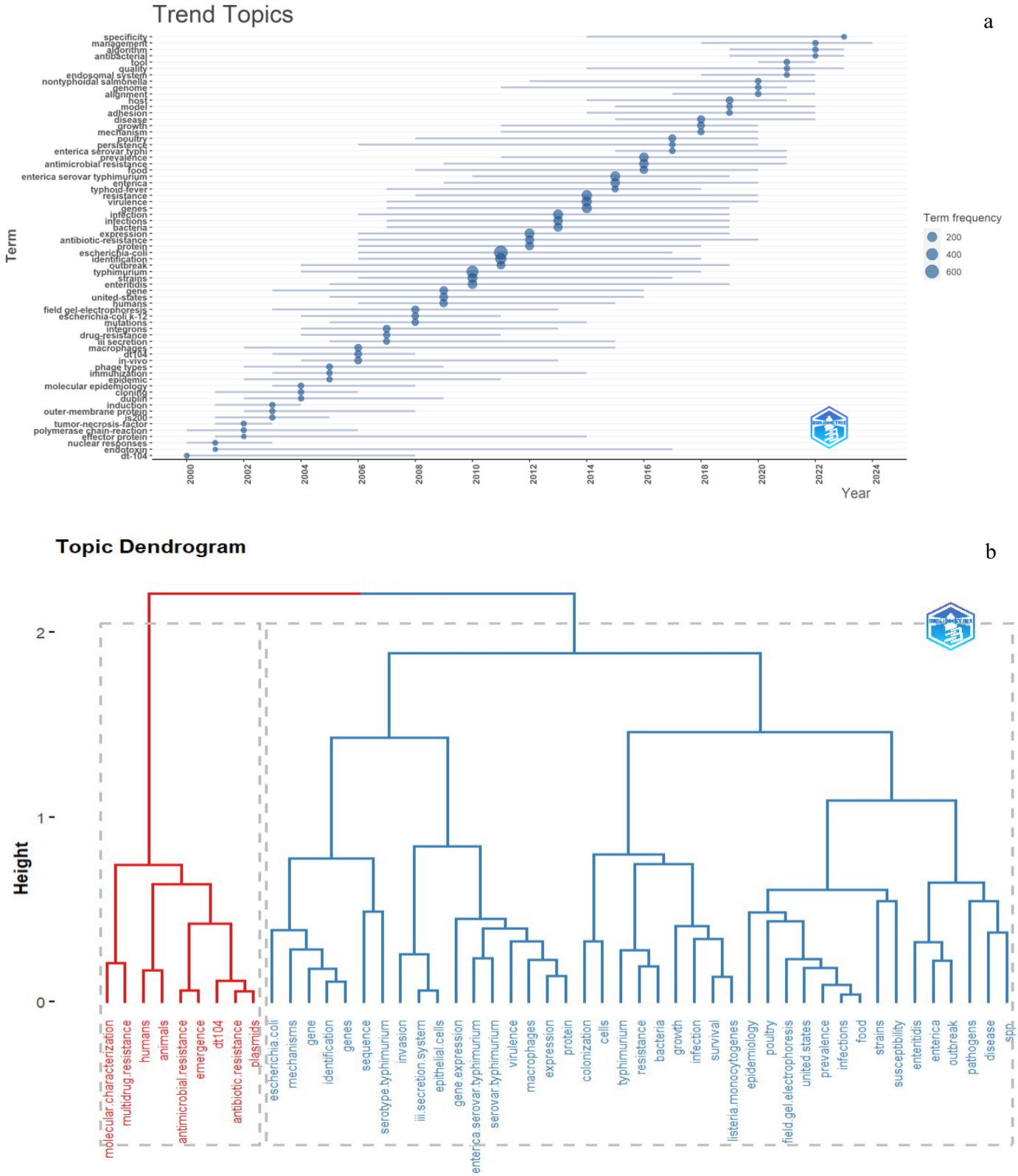

FIGURE 7. (a) Trend topics and (b) Topic dendogram.

change in priorities of microbiology research over a 25-year period. Figure 6c shows a summary of the word frequency analysis by year.

The trend topic analysis and the topic dendrogram (Figure 7a, Figure 7b) described the chronology of the development of microbiology over time, characterizing three main periods: the previous period (early 2000s) in which predominate of molecular basic techniques, such as, "field gel-electrophoresis" (2003-2008-2013) and "gene" (2003-2009-2016), and pathogens identification, such as "%typhimurium%" (2010-2018-2004) and "%enteritidis%" (2010-2019-2005). The rise in the occurrence of "identification" (2006-2011-2018), "expression" (2006-2012-2019), and "protein" (2006-2012-2018) in the intermediate period (2006-2014) is indicative of research moving towards functional genomics and proteomics, whilst the use of "virulence" (2007-2014-2020) and "genes" (2007-2014-2019) suggest an increasing interest in pathogenicity mechanisms. In the more recent era (after 2015), the apparent ascent and dominance of 'disease' (2015 > 2018 > 2022) and to a lesser extent 'antimicrobial resistance' (2009-2021) suggests a growing importance of clinical consequences and resistance mechanisms.

DISCUSSION

In this study, a total of 2900 articles published in the period covering the years 2000-2025 were analyzed and the status and future trends in salmonellosis research were examined in detail. The analysis results reveal that salmonellosis studies have increased significantly, especially in the last 10 years. One of the most striking findings of our study is the sudden increase in the annual number of publications observed in 2021. After a relative decrease in 2020, the first year of the COVID-19 pandemic, the highest number of publications of all time was reached with 144 articles in 2021. This shows that interest in foodborne pathogens has increased in the post-pandemic period. Interestingly, Jangid *et al*.'s study [15] and the current study confirm an increase in the number of publications in the 2020-2021 period despite COVID-19. However, this increase is more pronounced in *E. coli* studies (15.2% increase) [15]. In our study, a 34.5% increase was recorded in 2021, but a rapid decrease was observed in the following years. According to ECDC 2022 data, information on Salmonella serotypes and serogroups was provided in 83.1% of confirmed salmonellosis cases in EU/EEA countries. The four most common *Salmonella* serotypes in 2022 were *S. Enteritidis* (48.9%), *S. Typhimurium* (10.6%), monophasic S. Typhimurium 1, 4, [5], 12: i:- (10.2%) and *S. Infantis* (2.2%), respectively. The number of cases of serotypes other than monophasic *S. Typhimurium* decreased compared to the 2018–2021 average (except for the UK). In the last five years (2018–2022), 108 different serotypes were identified with more than 100 cases each, 26 of which reached their highest number of cases in 2022. Multi-country or national outbreaks have been reported in Europe for 10 of these serotypes. On the other hand, 7 serotypes that reported more than 100 cases between 2018 and 2022 were reported to have reached their lowest number of cases in 2022 [1].

As Salmonellosis is the second most common zoonosis in the EU after campylobacteriosis, and the European Food Safety Authority (EFSA) provides scientific support for reduction targets by combating this disease, which causes over 91,000 cases annually and an economic burden of up to 3 billion euros, through an integrated 'farm-to-fork' approach that addresses the risk of transmission primarily from eggs and raw meat [16]. This could also be a reason for the increasing scientific output.

Comparing the findings of this study with the bibliometric analysis of *Escherichia coli* O157 by Jangid *et al*. [15] reveals striking similarities and differences in foodborne zoonotic disease research. Although both studies focus on pathogens that are critical to food safety, they differ significantly in terms of geographic distribution, research priorities, and methodological approaches. The study by Jangid *et al*. [15] highlighted the global burden of infections caused by *E. coli* O157 (~420,000 deaths per year) and the dominance of the USA in this area. Our study highlights the epidemiological burden of salmonellosis in EU/EEA countries (~155,000 deaths per year) and Europe's research leadership. While our study found that salmonellosis research in EU/EEA countries was led by Germany (1,390 publications) and France (996 publications), Jangid *et al*. [15] reported the absolute dominance of the USA (42.3% contribution) in *E. coli* O157 research. According to the Global Burden of Disease Study

2019, which examined global mortality associated with 33 bacterial pathogens in 2019, Escherichia coli was associated with 950,000 deaths worldwide in 2019, Salmonella species (including typhoid and non-invasive typhoid types) caused a total of 397,000 deaths. These findings demonstrate that these bacteria play a significant role in the global disease burden and are priority pathogens for public health interventions [17].

Abdul's bibliometric analysis study [18] examined the risks of Bacillus cereus to food safety and examined research trends focusing on the toxin production, resistance mechanisms and behavior of this pathogen in different food matrices. The study revealed that China, South Korea and the USA are prominent in publications in this field [18]. The bibliometric study by Sweileh *et al*. on Campylobacter (2000-2015) reveals that the USA (23.6%) and European countries are dominant [19]. Kılıç Altun *et al*. [20]'s bibliometric analysis study on *S. Typhi* examined the global characteristics of *S. Typhi* research over 52 years with WoS data. The USA is the leader with the most publications (1,332 articles) and funding (United States Department of Health and Human Services). Interestingly, both our study and Jangid *et al*.' study [15] show that the UK and China stand out with their strong international collaborations, highlighting the importance of global collaborations in foodborne pathogen research.

While our study highlights the collective contributions of institutional structures such as the Technical University of Denmark (164 publications) and the Institute Pasteur (150 publications), Jangid *et al*.' study [15] shows that individual researchers such as Doyle MP (84 publications, H-Index: 49) lead the field. This reflects the institutional collaboration-based structure of the research culture in Europe.

While Jangid's study [15] highlights the financial contributions of American institutions such as the NIH and USDA, our analysis is dominated by European-based funders such as the European Union (169 publications) and the German Research Foundation (DFG, 132 publications), demonstrating the critical role of regional funding policies in shaping the research agenda.

Among the journals examined in our study, traditional high impact factor journals such as *Applied and Environmental Microbiology* (IF: 5.2) and *Infection and Immunity* (IF: 4.8) were found to be leading journals in salmonellosis research. In contrast, Jangid's analysis [15] revealed that open access journals such as *Frontiers in Microbiology* (IF: 6.1) and Scientific Reports (IF: 4.6) were more preferred (38.4%) in *E. coli* O157 research. Interestingly, Antimicrobial *Agents and Chemotherapy* was in the top 5 in both studies, indicating the importance of antimicrobial resistance research for both pathogens. Also, Kılıç Altun *et al*. [20] reported that the most influential journal was Infection and Immunity.

While new generation technologies such as "CRISPR-CAS", "LAMP" and "phage therapy" were prominent in the word cloud and dendrogram analysis of Jangid *et al*. [15], traditional molecular techniques such as "whole genome sequencing" (23.1%), "PFGE" (18.7%) and "MLST" (12.4%) were dominant in the word frequency analysis of our study. In addition our study showed that, TLS for keywords related to antibiotic resistance had more than doubled after 2015. Our trend topic analysis revealed a significant increase in the terms "antimicrobial resistance" (34.2%) and "multidrug resistance" (28.5%) after 2015, while innovative concepts such as "biosensors" (21.8%) and "nano-diagnostics" (17.3%) were more prominent in Jangid *et al*'s study [15]. Our thematic mapping analysis showed that salmonellosis research was clustered into four main clusters: resistance patterns in clinical isolates (32.6%), foodborne outbreaks (28.4%), molecular epidemiology (25.1%), and animal reservoirs (13.9%). In contrast, Jangid *et al*.'s dendrogram analysis revealed a different distribution of *E. coli* O157 research: rapid diagnostic technologies (39.2%), antibiotic alternatives (31.7%), food processing technologies (19.4%), and genomic epidemiology (9.7%). This comparison highlights the clear differences in research priorities for the two pathogens; salmonellosis research is driven by traditional epidemiology and clinical focus, while *E. coli* O157 research focuses more on diagnostic technologies and treatment alternatives. Abdul's bibliometric analysis study [18] revealed that sustainable control strategies such as probiotics and bacteriocins have received increasing attention in publications on *B. cereus*. In the bibliometric study on *Campylobacter* by Sweileh *et al*. [19], research themes focused on antimicrobial resistance (especially to quinolones) and public health burden. In the study by Kılıç Altun *et al*. (18), typhoid vaccines, systemic infections and diagnostic methods were reported to be the most prominent themes in publications [20].

Strengths and Limitations

The strengths of this study lie primarily in its ability to conduct an in-depth analysis of long-term trends in Salmonella research, thanks to its broad time span of 25 years (2000–2025) and its regional approach focusing on EU/EEA countries. On the methodological front, the use of up-to-date and advanced bibliometric tools such as VOSviewer and R Studio (Biblioshiny) enables the reliable mapping of complex collaboration and thematic relationships through network analysis and clustering. This allows the study to present original and important findings of high strategic value for research policies.

This study also has limitations, such as database selection bias, language- and region-coverage, time delay in the process of a citation analysis and exclusion of grey literature. The review was limited to the WoS Core Collection indexed journals/ source, and this may have excluded potentially relevant studies from non-WoS core indexed journals or non-English languages. As 2025 has not yet been completed, data for this year cannot be fully evaluated. The study did not include important research in other languages, especially from non-English-speaking EU/EEA countries, potentially influencing the generalizability of the findings. The study also excluded conference abstracts, government reports and preprints and those missing because experiences data and preliminary findings may be available in these sources that were not published yet in peer-reviewed journals. The study underscores the involvement of large western European institutions, which does not necessarily reflect the work being performed by smaller and/or less well-endowed centers in eastern and southern Europe. Depending on author-supplied keywords, and WoS's "Keywords Plus" may not adequately reflect all pertinent topics, when *Salmonella* research is approached in an interdisciplinary way such as when discussing Veterinary Medicine, Food Safety, or Environmental Microbiology. Furthermore, the bibliometric approach concentrated on metadata and not full-text, and could potentially overlook subtle discussions of pathogenicity mechanisms, treatments, or case study particulars.

CONCLUSION

This bibliometric study shows emerging trends, collaborations and prominent publication trends in salmonellosis research in EU/EEA countries from 2000 to 2025. In this study, Germany, France and Spain were found to be the most productive in terms of work, together with other countries, especially the UK and the US. Over time, it was understood that the research focus shifted from basic microbiological definitions to antimicrobial resistance, molecular epidemiology and public health applications. Future EU funding frameworks such as Horizon Europe should prioritise collaborative TGD surveillance networks.

Although antibiotic resistance is the most frequently emphasized topic in salmonellosis research, the use of cutting-edge technologies such as whole genome sequencing (WGS) has become very important in pathogen monitoring and outbreak control. However, it was concluded that cutting-edge areas such as synthetic biology, bacteriophage therapies and nanotechnology have not yet received sufficient attention and may be the subject of future research. A 48.38% international co-authorship rate and TLS increases in keywords related to TGD highlight the trend towards collaborative, genome-focused research.
In summary, this analysis provides a comprehensive resource to assess the current status and prospects of Salmonella research, highlighting the importance of interdisciplinary collaboration and creative problem solving in combating foodborne zoonotic diseases. These findings necessitate the strategic prioritisation of EU-wide genomic consortia to combat emerging resistance threats.

Ethics Approval and Consent to Participate

As this study is a bibliometric study using publicly available aggregate data, ethical approval is not required. No human or animal subjects were involved, and anonymity has been preserved.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: CU, SA; Study Design: CU, SA; Supervision: CU, SA; Funding: N/A; Materials: CU, SA; Data Collection and/or Processing: CU, SA;

Statistical Analysis and/or Data Interpretation: CU, SA; Literature Review: CU, SA; Manuscript Preparation: CU, SA; and Critical Review: CU, SA.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. European Centre for Disease Prevention and Control (2022) Salmonella Annual Epidemiological Report for 2022. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/SALM_AER_2022_Report.pdf (Accessed: 1 June 2025).
2. Lu J, Wu H, Wu S, et al. Salmonella: Infection mechanism and control strategies. Microbiol Res. 2025;292:128013. doi: 10.1016/j.micres.2024.128013.
3. Gal-Mor O. Persistent Infection and Long-Term Carriage of Typhoidal and Nontyphoidal Salmonellae. Clin Microbiol Rev. 2018;32(1):e00088-18. doi: 10.1128/CMR.00088-18.
4. Dos Santos AMP, Ferrari RG, Conte-Junior CA. Virulence Factors in Salmonella Typhimurium: The Sagacity of a Bacterium. Curr Microbiol. 2019n;76(6):762-773. doi: 10.1007/s00284-018-1510-4.
5. Li Q. Mechanisms for the Invasion and Dissemination of Salmonella. Can J Infect Dis Med Microbiol. 2022;2022:2655801. doi: 10.1155/2022/2655801.
6. LeLièvre V, Besnard A, Schlusselhuber M, Desmasures N, Dalmasso M. Phages for biocontrol in foods: What opportunities for Salmonella sp. control along the dairy food chain? Food Microbiol. 2019;78:89-98. doi: 10.1016/j.fm.2018.10.009.
7. Ehrhardt K, Becker AL, Grassl GA. Determinants of persistent Salmonella infections. Curr Opin Immunol. 2023;82:102306. doi: 10.1016/j.coi.2023.102306.
8. Brown EW, Bell R, Zhang G, et al. Salmonella Genomics in Public Health and Food Safety. EcoSal Plus. 2021 Dec 15;9(2):eESP00082020. doi: 10.1128/ecosalplus.ESP-0008-2020.
9. UK Government. EU countries and the single market. Available at: https://www.gov.uk/eu-eea (Accessed: 1 June 2025).
10. Perianes-Rodriguez A, Waltman L, van Eck NJ. Constructing Bibliometric Networks: A Comparison between Full and Fractional Counting. J Informetr. 2016;10(4):1178-1195. doi: 10.1016/j.joi.2016.10.006.
11. Aria M, Cuccurullo C. Bibliometrix: An R-Tool for Comprehensive Science Mapping Analysis. J Informetr. 2017;11(4):959-975. doi: 10.1016/j.joi.2017.08.007.
12. Coburn B, Grassl GA, Finlay BB. Salmonella, the host and disease: a brief review. Immunol Cell Biol. 2007;85(2):112-118. doi: 10.1038/sj.icb.7100007. Epub 2006 Dec 5.
13. Deng G, Chen X, Shao L, Wu Q, Wang S. Glycosylation in autoimmune diseases: A bibliometric and visualization study. Heliyon. 2024;10(9):e30026. doi: 10.1016/j.heliyon.2024.e30026.
14. Manoj Kumar L, George RJ, P S A. Bibliometric Analysis for Medical Research. Indian J Psychol Med. 2023;45(3):277-282. doi: 10.1177/02537176221103617.
15. Jangid H, Kumar D, Kumar G, Kumar R, Mamidi N. An Emerging Foodborne Pathogen Spotlight: A Bibliometric Analysis and Scholarly Review of Escherichia coli O157 Research. Antibiotics (Basel). 2024;13(1):60. doi: 10.3390/antibiotics13010060.
16. EFSA (European Food Safety Authority). Salmonella. Available at: https://www.efsa.europa.eu/en/topics/topic/salmonella (Accessed: 7 November 2025).
17. GBD 2019 Antimicrobial Resistance Collaborators. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022;400(10369):2221-2248. doi: 10.1016/S0140-6736(22)02185-7.
18. Abdul ME, Pavoni E. Bacillus cereus in food safety: a bibliometric analysis. Front Microbiol. 2025;16:1574802. doi: 10.3389/fmicb.2025.1574802.
19. Sweileh WM, Al-Jabi SW, Sawalha AF, AbuTaha AS, Zyoud SH. Bibliometric analysis of publications on Campylobacter: (2000-2015). J Health Popul Nutr. 2016;35(1):39. doi: 10.1186/s41043-016-0076-7.
20. Kılıç Altun S, Aydemir ME, Alkan S. A global bibliometric analysis of Salmonella Typhi over the past 52 years (1970-2022). J Infect Dev Ctries. 2025;19(5):691-698. doi: 10.3855/jidc.17946.

The European Research Journal 2026;12(1):51-58 Original Article

DOI: https://doi.org/10.18621/eurj.1792804 *Radiology*

The Frequency of Incidental Findings in Prostate Multiparametric Magnetic Resonance Imaging

Ali Salbas, Atakan Bayir, Cesur Gumus

Department of Radiology, Atatürk Training and Research Hospital, İzmir Katip Çelebi University, İzmir, Türkiye

ABSTRACT

Objectives: This study aimed to evaluate the prevalence, distribution, and clinical significance of incidental findings on multiparametric magnetic resonance imaging (mpMRI) performed for suspected prostate cancer, and to assess differences based on patient age.

Methods: This retrospective study reviewed the mpMRI examinations of 532 patients. Incidental findings were identified and categorized as genitourinary or extragenitourinary, and further classified as clinically significant or non-significant. The prevalence and characteristics of incidental findings were analyzed and compared between two age groups (≤ 65 and > 65 years).

Results: Of the 532 patients, 243 (45.7%) had at least one incidental finding, for a total of 275 findings. The majority (94.9%) were clinically non-significant, with bladder wall thickening (n=58) and fat-containing inguinal hernias (n=40) being the most common. Fourteen findings (5.1%) were deemed clinically significant, including bladder carcinoma (n=3), iliac artery aneurysm (n=2), and rectal cancer (n=1). The prevalence of incidental findings was significantly higher in patients aged >65 years compared to those ≤ 65 years (51.6% vs. 37.2%, P=0.001), although there was no significant difference in the rate of clinically significant findings between the age groups (P=0.128).

Conclusions: Incidental findings are frequently detected in prostate mpMRI, occurring in nearly half of patients. Although most are benign, a small but important proportion (5.1%) are clinically significant and may impact patient management. These results underscore the necessity for radiologists to perform a comprehensive evaluation of the entire imaging field of view.

Keywords: Aneurysm, Incidental Findings, Magnetic Resonance Imaging, Prevalence, Prostatic Neoplasms, Rectal Cancer

Prostate cancer (PCa) is the second most common cancer in men worldwide [1]. The traditional strategy for early detection has relied on serum prostate-specific antigen (PSA) testing and/or digital rectal examination, followed by systematic transrectal ultrasound-guided biopsy [2]. In recent years, one of the most significant advances in the diagnostic pathway for clinically significant prostate cancer (csPCa) has been the implementation of prebiopsy multiparametric magnetic resonance imaging (mpMRI), which enables the identification of suspicious lesions and improves risk stratification [2-5].

Received: September 29, 2025 **Accepted:** November 22, 2025 **Available Online:** November 26, 2025 **Published:** January 4, 2026

How to cite this article: Salbas A, Bayir A, Gumus C. The Frequency of Incidental Findings in Prostate Multiparametric Magnetic Resonance Imaging. Eur Res J. 2026;12(1):51-58. doi: 10.18621/eurj.1792804

Corresponding author: Ali Salbas, MD., Phone: +90 232 244 44 44, E-mail: dralisalbas@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

When performed according to standardized protocols, mpMRI increases accuracy in detecting csPCa. It can also serve as a triage tool by helping to avoid unnecessary biopsies in patients with negative scans and enabling targeted sampling of abnormal areas in those with positive findings [4, 5].

With the growing demand for mpMRI in clinical practice, incidental findings (IFs) unrelated to prostate cancer have become increasingly recognized [6-8]. While many of these findings are benign and clinically insignificant, others may require further diagnostic work-up or intervention, thereby posing important implications for patient management.

Our study aimed to evaluate the prevalence and distribution of incidental findings detected on multiparametric prostate MRI according to their clinical significance, with the intent of contributing additional data to the relatively limited literature on this topic.

METHODS

Ethical approval was obtained from the institutional ethics committee (Date: September 11, 2025; Decision No: 0544). Multiparametric prostate MRI examinations performed between January and June 2025 were retrospectively retrieved from the institutional imaging archive. All patients with clinical suspicion of prostate cancer who underwent mpMRI during this period were included. A total of 560 mpMRI examinations were available. In cases where more than one mpMRI was performed for the same patient (n=5), the most recent examination was considered the index study for analysis. Incomplete MRI examinations (n=23) due to patient intolerance or claustrophobia were excluded from the study. The final study population consisted of 532 patients with 532 mpMRI examinations.

All mpMRI scans were performed in accordance with the Prostate Imaging Reporting and Data System (PI-RADS) version 2.1, at least 6 weeks after any prostate biopsy to minimize postbiopsy hemorrhage and avoid potential diagnostic pitfalls [5]. No enema or antispasmodic agent was administered before the examination.

Imaging was performed using a 3.0-T MRI scanner (Magnetom Lumina, Siemens Healthineers, Erlangen, Germany) with an 18-channel phased-array surface coil. The standard protocol included high-resolution turbo spin-echo T2-weighted imaging in axial (FOV 200 × 200 mm), sagittal (FOV 200 × 200 mm), and coronal planes (FOV 200 × 200 mm); axial diffusion-weighted imaging (b-values: 50, 200, 800, 1400 s/mm²; FOV 200 × 102 mm) with apparent diffusion coefficient (ADC) mapping; axial turbo spin-echo T1-weighted imaging (FOV 300 × 300 mm); and dynamic contrast-enhanced imaging (FOV 220 × 220 mm) after intravenous administration of gadobutrol at a dose of 0.1 mL/kg with an injection rate of 3 mL/sec. In addition, contrast-enhanced fat-suppressed axial T1-weighted imaging (FOV 300 × 300 mm) was acquired up to the level of the aortic bifurcation. All mpMRI reports and images were jointly reviewed by two radiologists with 5 and 3 years of experience in genitourinary MRI. In cases of disagreement, consensus was reached by consultation with a senior radiologist with 10 years of experience in prostate imaging. All extraprostatic findings were recorded. Conditions directly related to prostate cancer staging, such as seminal vesicle invasion, bladder invasion, rectal invasion, bone metastasis, and lymph node metastasis, were not considered IFs.

IFs were categorized as related to the genitourinary system or not related to the genitourinary system. Furthermore, findings that required medical or surgical management or were expected to affect patient prognosis were defined as clinically significant, while those requiring only follow-up without additional imaging or intervention were considered clinically non-significant, based on a classification approach inspired by the work of Emekli and Gundogdu [9]. The study population was stratified into two age groups (≤ 65 years and > 65 years) to evaluate potential differences in the prevalence and clinical significance of IFs [7].

Statistical Analysis

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean±standard deviation (SD) and categorical variables as numbers and percentages. The chi-square (χ^2) test was used to compare the prevalence of incidental findings (IFs) between age groups (≤65 vs. >65 years) and to assess the distribution of genitourinary versus extragenitourinary IFs, as well as the prevalence of clinically significant IFs. The Mann-Whitney U test

was applied to compare the mean number of IFs per patient between age groups, given the non-normal distribution of the data. A P-value of < 0.05 was considered statistically significant.

RESULTS

A total of 532 patients were included in the study. The mean age was 66.9±8.1 years (range, 32-85 years). Of these, 289 patients had no IFs, while 243 (45.7%) patients had at least one IF. Among patients with IFs, 212 (87.2%) had a single IF, 30 (12.3%) had two IFs, and one patient (0.4%) had three IFs.

Of the 275 IFs, 121 (44.0%) were related to the genitourinary system, and 154 (56.0%) were extragenitourinary. In total, 261 IFs (94.9%) were classified as clinically non-significant, whereas 14 IFs (5.1%) were clinically significant. Forty distinct types of IFs were recorded (Figure 1).

The most common IFs were bladder wall thickening (n=58, 21.1%), inguinal hernia (fat-containing) (n=40, 14.5%), Tarlov cysts (n=28, 10.2%), and bladder diverticula (n=16, 5.8%) (Tables 1 and 2).

Clinically significant IFs (n=14, 5.1%) included bladder carcinoma (n=3), inguinal hernia containing intestinal loops (n=3), iliac artery aneurysm (n=2), dilated bowel loops (n=1), femoral hernia (n=1), lesion

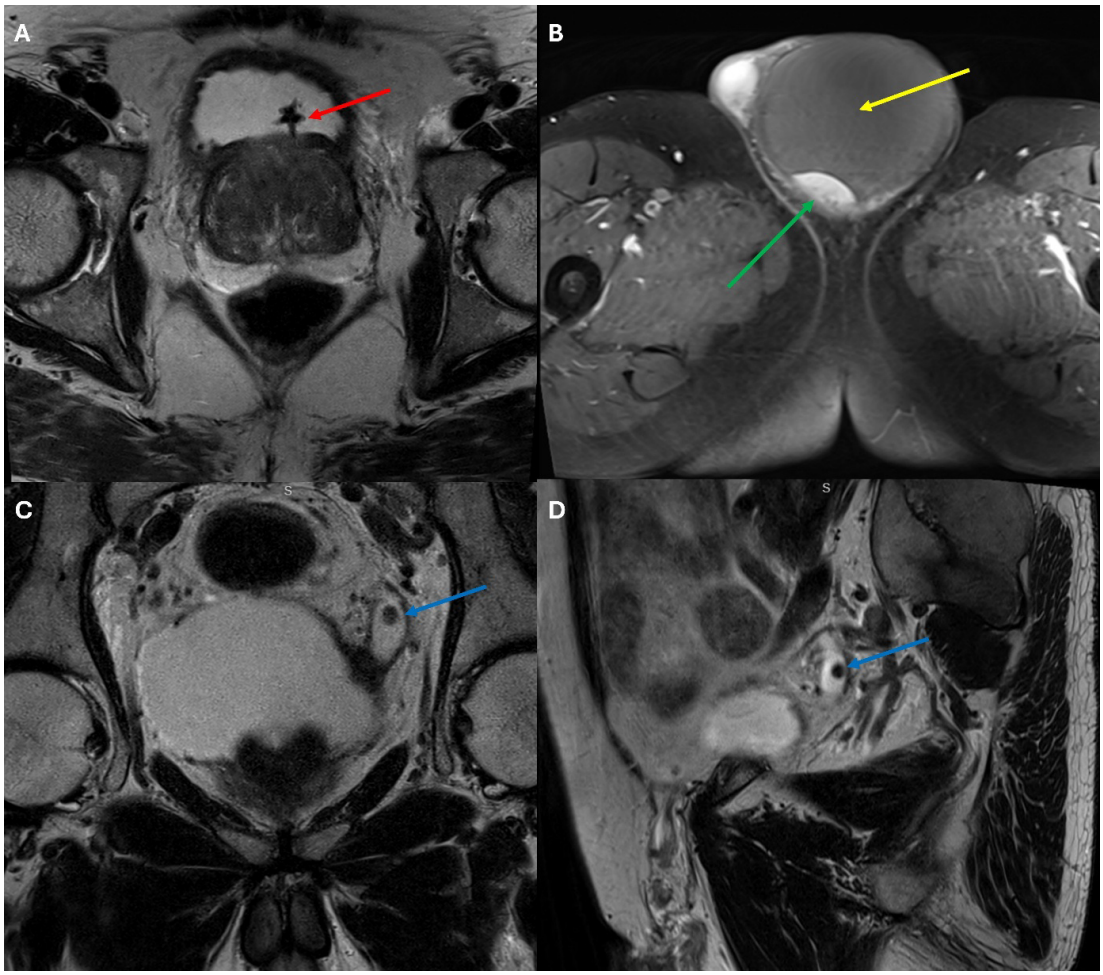

FIGURE 1. Examples of incidental findings detected on prostate mpMRI. A 70-year-old man: axial T2-weighted image demonstrates a low-signal-intensity lesion within the bladder lumen (A), consistent with a calculus (red arrow). A 61-year-old man: contrast-enhanced fat-suppressed axial T1-weighted image shows a left-sided hydrocele (B) (yellow arrow) with posterior displacement of the testicular parenchyma (green arrow). A 68-year-old man: coronal (C) and sagittal (D) T2-weighted images reveal dilatation of the left ureter with an intraluminal signal void at the distal ureter, consistent with a ureteral calculus (blue arrows).

TABLE 1. Genitourinary Incidental Findings

Incidental finding	Number of cases
Bladder wall thickening	58
Bladder diverticula	16
Epididymal cyst	9
Hydrocele	9
Utricle cyst	7
Bladder calculi	6
Seminal vesicle cyst	4
Bladder carcinoma	**3**
Bladder hernia	3
Ectopic kidney	2
Atrophic testis	1
Cyst of the spermatic cord	1
Ureter calculi	1
Varicocele	1
Total	121

Clinically significant findings are shown in bold.

TABLE 2. Extragenitourinary Incidental Findings

Incidental finding	Number of cases
Inguinal hernia (fat-containing)	40
Tarlov cyst	28
Bone alterations (not metastatic)	14
Colon diverticulum	13
Narrowing of the ischiofemoral space	12
Synovial cyst	9
Muscle alterations	7
Pelvic free fluid	4
Rectal wall thickening (not cancer)	4
Inguinal hernia (intestinal loops)	**3**
Avascular necrosis of the femur head	2
Femur herniation pit	2
Iliac artery aneurysm	**2**
Vertebral hemangioma	2
Abdominal wall hernia	1
Dilated bowel loops	**1**
Enchondroma	1
Femoral hernia	**1**
Intramuscular cystic lesion	1
Intramuscular lipoma	1
Lesion suspicious for gossypiboma	**1**
Perianal abscess	**1**
Rectal cancer	**1**
Rectal polyp (pathology not available)	**1**
Rectal prolapse	1
Sacroiliitis	1
Total	154

Clinically significant findings are shown in bold. For the lesion suspicious for gossypiboma, further evaluation was recommended; however, follow-up information was not available.

suspicious for gossypiboma (n=1), perianal abscess (n=1), rectal cancer (n=1), and rectal polyp (n=1) (Figure 2). Pathological confirmation was not available for the rectal polyp. For the lesion suspicious for gossypiboma, further evaluation was recommended; however, follow-up information was not available.

Patients were divided into two groups: ≤ 65 years (n=218) and > 65 years (n=314). Incidental findings were present in 37.2% of patients aged ≤ 65 years and in 51.6% of those aged > 65 years, with a statistically significant difference between the groups ($\chi^2 = 10.23$, P=0.001) (Table 3). The mean number of IFs per patient was 0.40±0.56 in patients aged ≤ 65 years and 0.60±0.63 in those aged > 65 years. This difference was statistically significant (P < 0.001).

Among patients aged ≤ 65 years, 41.6% of IFs were genitourinary and 58.4% were extragenitourinary, while in patients aged > 65 years, 45.2% were genitourinary and 54.8% were extragenitourinary. There was no statistically significant difference between the two age groups (χ^2=0.25, P=0.620).

Clinically significant IFs (n=14) were observed in 9 patients aged ≤ 65 years (4.1%) and 5 patients aged > 65 years (1.6%), with no statistically significant difference between the groups (χ^2=2.32, P=0.128).

DISCUSSION

In this study, IFs were observed in nearly half of the patients undergoing prostate mpMRI, with a total prevalence of 45.7%. Among these, 14 findings (5.1%) were identified as clinically significant. The frequency of incidental findings increased with advancing age.

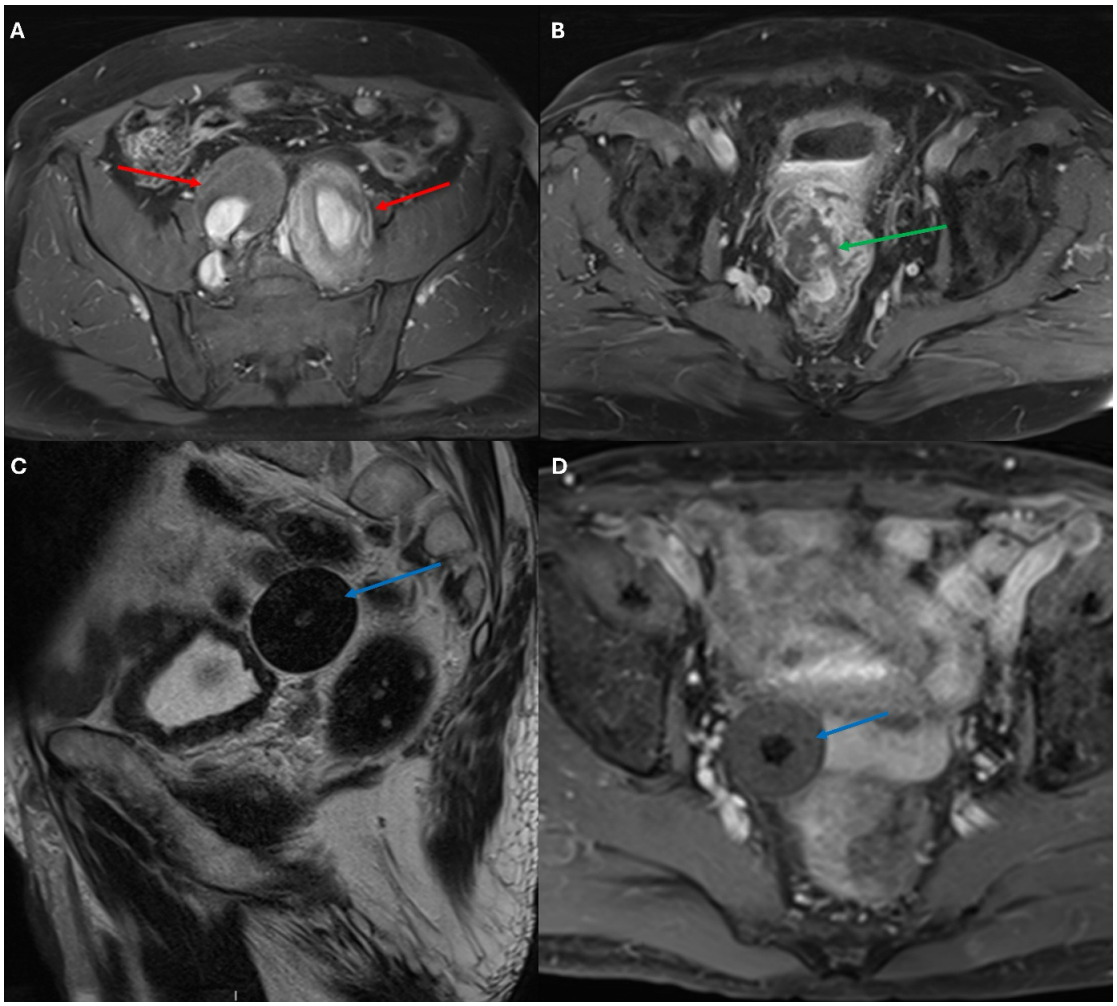

FIGURE 2. Examples of clinically significant incidental findings detected on prostate mpMRI. In a 59-year-old man, contrast-enhanced fat-suppressed axial T1-weighted image (A) demonstrates partially thrombosed aneurysmal dilatation of both iliac arteries (red arrows). In a 72-year-old man, contrast-enhanced fat-suppressed axial T1-weighted image (B) shows asymmetric wall thickening and enhancement of the rectum consistent with a malignant mass (green arrow), which was confirmed as mucinous adenocarcinoma on histopathology. In an 81-year-old man, sagittal T2-weighted image (C) and contrast-enhanced fat-suppressed axial T1-weighted image (D) depict a round lesion with central low signal intensity in the right pelvis (blue arrows), not related to adjacent bowel loops. In the context of prior abdominal surgery, the finding was considered suspicious for gossypiboma; however, no follow-up information was available.

TABLE 3. Comparison of Incidental Findings Between Age Groups

	≤65 years (n=218)	>65 years (n=314)	P-value
Patients with IF	81 (37.2%)	162 (51.6%)	**0.001**
Number of IFs per patient	0.40±0.56	0.60±0.63	**<0.001**
Distribution of IFs (GU / EGU)	37/52	84/102	0.620
Clinically significant IFs	9 (4.1%)	5 (1.6%)	0.128

Data are shown as mean±standard deviation or number or number (percentage) where appropriate. IF, incidental finding; GU, genitourinary; EGU, extragenitourinary. Statistically significant P-values are shown in bold.

IFs are commonly encountered during prostate mpMRI examinations performed for suspected prostate cancer. In the literature, the reported prevalence of IFs ranges between 40% and 52.7% [6, 7, 9, 10]. Sherrer *et al*. identified IFs in 40% of patients (233/580) in a cohort of 580 men undergoing mpMRI [6]. Similarly, in a study including 426 patients, 49.8% were reported to have at least one IF [9]. In a larger series including 647 patients, Cutaia *et al*. found that 52.7% of examinations revealed extraprostatic incidental findings [7]. Interestingly, Wagnerova *et al*. reported a much higher prevalence of 95.2%, which they attributed to epidemiological differences, such as the variable prevalence of diverticulosis across populations, as well as to the systematic evaluation of musculoskeletal structures, potentially broadening the spectrum of recorded findings [11]. These discrepancies among studies may be explained by differences in patient demographics, imaging protocols (including field of view and sequence selection), and population-specific disease prevalence. In our study, the prevalence of IFs was 45.7%, which is consistent with most of the existing literature.

Regarding clinically significant IFs, studies using other imaging modalities have reported higher proportions of clinically significant IFs, ranging between 6% and 12.7% in unenhanced urinary computed tomography (CT) examinations [12, 13] and between 6.9% and 13.3% in trauma patients undergoing emergency CT scans [14, 15]. In contrast, our study demonstrated a prevalence of 5.1%, which is comparable to prior mpMRI-based series reporting lower rates. The relatively lower rates reported in mpMRI studies, including ours, may in part be explained by the more limited field of view inherent to this imaging technique. Previous studies have shown that only about 2–7% of IFs are clinically significant in the context of prostate mpMRI [6, 7, 9-11]. In a cohort of 580 patients undergoing mpMRI, 349 incidental findings were identified, of which only 6.6% were considered clinically significant [6]. In another series including 426 patients, 22 of 321 (6.9%) IFs were clinically significant, with only four of genitourinary origin and the majority (n=18) being extragenitourinary pathologies [9]. In a smaller study of 185 patients, the prevalence of incidental malignancies was 3.4% (3 of 88 patients with IFs) [10]. Typical examples of clinically significant IFs include urinary tract tumors, gastrointestinal neoplasms, and vascular abnormalities. Cutaia *et al*. [7] identified incidental cancers in 12 patients, including bladder carcinoma in 7, testicular tumors in 3, and rectal cancer in 2; notably, 27 (4.2%) patients in their series required surgical treatment for IFs not directly related to prostate cancer. Similarly, other series have highlighted clinically significant IFs such as urothelial carcinomas, colorectal polyps or cancers, iliac artery aneurysms, and inguinal hernias containing bowel loops, all of which may require prompt intervention [9]. More recently, a large study including 1282 patients reported that highly significant IFs resulted in management changes in only 49 (3.8%) cases [16].

In our cohort, 14 clinically significant IFs (5.1%) were detected, including bladder carcinomas, rectal cancer, a rectal polyp, iliac artery aneurysms, inguinal and femoral hernias containing bowel loops, bowel dilatation, a perianal abscess, and a lesion suspicious for gossypiboma. Consistent with the literature, most IFs in our study were benign and required only follow-up. However, early detection of this small subset of clinically significant findings may have important implications for patient outcomes. Although the prevalence of 5.1% may appear modest, the widespread use of prostate mpMRI means that clinically significant incidental findings still represent a relevant consideration in daily practice. Such findings may have important implications not only for patient outcomes but also from a medicolegal perspective. Therefore, it is crucial that radiology reports clearly highlight such findings, indicate their clinical relevance, and ensure appropriate referral for further evaluation or treatment. Importantly, incidental detection of malignancies such as bladder or rectal cancers, or vascular pathologies with rupture risk, may provide an opportunity to improve patient outcomes.

Most incidental lesions identified on prostate mpMRI, however, fall into the non-significant category. Reported series have shown that the most frequent findings include bladder wall thickening, fat-containing inguinal hernias, colonic diverticulosis, bladder diverticula, hydroceles, and Tarlov cysts [6, 7, 9-11]. In our cohort, the most common IFs were bladder wall thickening (21.1%), inguinal hernia (fat-containing) (14.5%), Tarlov cysts (10.2%) and bladder diverticula (5.8%). These findings are broadly consistent with prior literature, although minor differences in relative frequencies were observed. These may reflect epidemiological

differences and variations in patient populations.

Previous research has consistently shown that incidental findings on prostate mpMRI become more frequent with advancing age, both in terms of overall prevalence and in the proportion of clinically significant cases [7, 9, 16], which is biologically expected given the cumulative impact of aging on multiple organ systems. In our study, incidental findings were also more common in older patients, who demonstrated a higher average number of findings per individual, consistent with previous reports. However, unlike previous reports, we did not observe a significant age-related difference in the distribution of clinically significant IFs.

One possible explanation lies in the heterogeneous nature of the "clinically significant" category. Malignancies such as bladder or rectal cancers and vascular pathologies like iliac artery aneurysms typically increase with age, whereas conditions such as hernias containing bowel loops may be less age-dependent and even more common in younger, more physically active patients. This heterogeneity indicates that clinically significant IFs may not behave uniformly with respect to age, as their association likely varies by underlying pathophysiology. Future research that examines these findings in subgroups such as malignant, vascular, infectious, or mechanical could provide a clearer understanding of age-related patterns. Additionally, the absence of a significant association may also reflect the relatively small number of clinically significant IFs (n=14), which limited the statistical power to detect differences.

Strengths and Limitations

This study has several limitations. First, it was conducted as a single-center, retrospective analysis, which may limit the generalizability of the results. Second, only examinations performed over a relatively short period (January–June 2025) were included, potentially restricting the sample size and representativeness. Third, pathological confirmation and follow-up data were not available for all clinically significant IFs, such as rectal polyps and the lesion suspicious for gossypiboma, which may have influenced the accuracy of classification. Finally, there is no universally accepted standard for categorizing incidental findings as clinically significant or non-significant, which may introduce variability in comparisons with previous studies. Despite these limitations, this study contributes valuable data to the relatively limited literature on incidental findings in prostate mpMRI. Future prospective, multicenter studies with longer follow-up and standardized classification systems are needed to validate and expand upon our findings.

Beyond their role in prostate cancer evaluation, prostate mpMRI examinations may also provide an opportunity for broader pelvic health assessment. Given the high prevalence of incidental findings, radiologists should systematically evaluate all structures within the field of view, rather than focusing solely on the prostate. Implementing standardized protocols for reporting and managing such findings, for example through structured templates or checklists, may help ensure that clinically significant abnormalities are consistently highlighted and effectively communicated to referring physicians.

CONCLUSION

Incidental findings are frequently encountered during multiparametric prostate MRI examinations, with nearly half of patients in our cohort demonstrating at least one such finding. Although the vast majority were benign or clinically non-significant, a small proportion (5.1%) represented clinically significant abnormalities with potential implications for patient management. These included malignancies, vascular pathologies, and complex hernias, underscoring the need for careful and systematic evaluation of all structures within the field of view. The increased prevalence of incidental findings with advancing age further emphasizes the importance of comprehensive assessment, particularly in elderly populations undergoing prostate mpMRI.

Ethics Approval and Consent to Participate

This study was approved by the İzmir Katip Çelebi University Health Research Ethics Committee (Decision No: 2025/0544; date: 11.09.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: AŞ, CG, AB; Study Design: AŞ, CG; Supervision: CG; Funding: N/A; Materials: N/A; Data Collection and/or Processing: AB, AŞ; Statistical Analysis and/or Data Interpretation: AŞ, CG; Literature Review: AŞ, AB; Manuscript Preparation: AŞ; and Critical Review: AŞ, CG.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The authors acknowledge the use of ChatGPT for grammar and spelling checks. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the final version of the manuscript.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Raychaudhuri R, Lin DW, Montgomery RB. Prostate Cancer: A Review. JAMA. 2025;333(16):1433-1446. doi: 10.1001/jama.2025.0228.
2. Triquell M, Campistol M, Celma A, et al. Magnetic Resonance Imaging-Based Predictive Models for Clinically Significant Prostate Cancer: A Systematic Review. Cancers (Basel). 2022;14(19):4747. doi: 10.3390/cancers14194747.
3. Coskun M, Mehralivand S, Shih JH, et al. Impact of bowel preparation with Fleet's™ enema on prostate MRI quality. Abdom Radiol (NY). 2020;45(12):4252-4259. doi: 10.1007/s00261-020-02487-6.
4. Kasivisvanathan V, Rannikko AS, Borghi M, et al; PRECISION Study Group Collaborators. MRI-Targeted or Standard Biopsy for Prostate-Cancer Diagnosis. N Engl J Med. 2018;378(19):1767-1777. doi: 10.1056/NEJMoa1801993.
5. Turkbey B, Rosenkrantz AB, Haider MA, et al. Prostate Imaging Reporting and Data System Version 2.1: 2019 Update of Prostate Imaging Reporting and Data System Version 2. Eur Urol. 2019;76(3):340-351. doi: 10.1016/j.eururo.2019.02.033.
6. Sherrer RL, Lai WS, Thomas JV, Nix JW, Rais-Bahrami S. Incidental findings on multiparametric MRI performed for evaluation of prostate cancer. Abdom Radiol (NY). 2018;43(3):696-701. doi: 10.1007/s00261-017-1237-x.
7. Cutaia G, Tosto G, Cannella R, et al. Prevalence and clinical significance of incidental findings on multiparametric prostate MRI. Radiol Med. 2020;125(2):204-213. doi: 10.1007/s11547-019-01106-9.
8. Rayn KN, Hale GR, Bloom JB, et al. Incidental bladder cancers found on multiparametric MRI of the prostate gland: a single center experience. Diagn Interv Radiol. 2018;24(5):316-320. doi: 10.5152/dir.2018.18102.
9. Emekli E, Gündoğdu E. Evaluation of the frequency of incidental findings and their clinical significance in multiparametric prostate magnetic resonance imaging examination. Pol J Radiol. 2022;87:e409-e414. doi: 10.5114/pjr.2022.118312.
10. Ediz SS, Gunduz N. The Relationship between PI-RADS Categories and Incidental Findings in Multiparametric Prostate MRI. J Coll Physicians Surg Pak. 2021;31(9):1030-1034. doi: 10.29271/jcpsp.2021.09.1030.
11. Wagnerova M, Macova I, Hanus P, et al. Quantification and significance of extraprostatic findings on prostate MRI: a retrospective analysis and three-tier classification. Insights Imaging. 2023;14(1):215. doi: 10.1186/s13244-023-01549-9.
12. Hoppe H, Studer R, Kessler TM, Vock P, Studer UE, Thoeny HC. Alternate or additional findings to stone disease on unenhanced computerized tomography for acute flank pain can impact management. J Urol. 2006;175(5):1725-1730; discussion 1730. doi: 10.1016/S0022-5347(05)00987-0.
13. Samim M, Goss S, Luty S, Weinreb J, Moore C. Incidental findings on CT for suspected renal colic in emergency department patients: prevalence and types in 5,383 consecutive examinations. J Am Coll Radiol. 2015;12(1):63-69. doi: 10.1016/j.jacr.2014.07.026.
14. van Vugt R, Dekker HM, Deunk J, et al. Incidental Findings on Routine Thoracoabdominal Computed Tomography in Blunt Trauma Patients. J Trauma Acute Care Surg. 2012;72(2):416-421. doi: 10.1097/TA.0b013e3182166b4b.
15. Bedel C, Korkut M, Erman K. Evaluation of incidental findings of whole body computed tomography in multiple trauma patients in emergency department. Ann Med Res. 2019;26(6):1075-1079. doi: 10.5455/annalsmedres.2019.03.122.
16. Porões F, Karampa P, Sartoretti T, et al. Additional findings in prostate MRI. Cancer Imaging. 2025;25(1):29. doi: 10.1186/s40644-025-00846-4.

The European Research Journal 2026;12(1):59-67 Original Article

DOI: https://doi.org/10.18621/eurj.1748832 *Radiology and Organ Imaging*

Trochlear Dysplasia and Its Structural Correlates on Knee Magnetic Resonance Imaging: A Multiparametric Analysis

Gizem Timoçin Yığman[1], Hande Özen Atalay[1]

[1]*Department of Radiology, Koç University Hospital, İstanbul, Türkiye*

ABSTRACT

Objectives: To investigate associations between trochlear dysplasia severity and various morphometric and degenerative findings on knee Magnetic Resonance Imaging (MRI)

Methods: A total of 211 knee MRIs from 181 patients diagnosed with trochlear dysplasia between November 2012 and March 2025 were retrospectively reviewed. Morphometric parameters - sulcus angle, trochlear depth, tibial tuberosity to trochlear groove (TT-TG) distance, patellar angle, and patellar position - were measured. Chondromalacia patella (grades 0-4), meniscal degeneration and tear, and Anterior Cruciate Ligament (ACL) mucoid degeneration were also recorded. Statistical analyses included ANOVA, Kruskal–Wallis, and chi-square tests.

Results: Trochlear dysplasia type A was the most common (61.1%). Significant associations were found between trochlear dysplasia severity and sulcus angle, TT-TG distance, and trochlear depth ($P<0.0001$ for all). While chondromalacia presence did not differ significantly across trochlear dysplasia types ($P=0.203$), its severity correlated with trochlear dysplasia grade ($P=0.036$). Meniscal degeneration was also significantly associated with trochlear dysplasia ($P=0.0004$), particularly when both menisci were involved ($P=0.003$). No significant associations were found for patellar angle, patellar position, meniscal tear, or ACL mucoid degeneration.

Conclusions: Trochlear dysplasia is significantly linked to specific morphologic and degenerative MRI findings. A comprehensive MRI-based assessment may aid in the diagnosis and management of patients with anterior knee pain and patellofemoral instability.

Keywords: Trochlear Dysplasia, Chondromalacia, Meniscal Degeneration, Patella Alta, ACL Mucoid Degeneration, Patellofemoral Instability

Trochlear dysplasia (TD) is a key anatomical risk factor for patellofemoral instability and anterior knee pain, particularly among young and active individuals [1]. It is characterized by abnormal morphology of the femoral trochlear groove which can result in insufficient guidance for patellar tracking, and recurrent dislocation [2]. It has been reported that the prevalence of TD to be 3.2% in the general population, whereas this rate can be as high as 96% in patients with patellar instability [3].

Magnetic resonance imaging (MRI) is the preferred imaging modality for the diagnosis and evaluation of morphological features of TD. Basic morphometric measurements such as sulcus angle,

Received: July 22, 2025 **Accepted:** October 18, 2025 **Available Online:** November 13, 2025 **Published:** January 4, 2026

How to cite this article: Timoçin Yığman G, Özen Atalay H. Trochlear Dysplasia and Its Structural Correlates on Knee Magnetic Resonance Imaging: A Multiparametric Analysis. Eur Res J. 2026;12(1):59-67. doi: 10.18621/eurj.1748832

Corresponding author: Gizem Timoçin Yığman, MD., Phone: +90 850 250 8 250, E-mail: gizemtimocin1@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

trochlear depth and tibial tubercle to trochlear groove (TT-TG) distance provide quantitative assessment of anatomy and patellofemoral alignment [4]. In addition, structural abnormalities such as chondromalacia patella, meniscal degeneration and anterior cruciate ligament (ACL) changes are frequently present among the accompanying pathologies in patients with TD [5-7]. However, the relationship between the severity of TD and these intra-articular structural changes has not yet been fully elucidated.

Although individual parameters related to TD have been addressed in previousstudies, the number of studies that comprehensively examine TD severity and a large number of radiological findings in the same patient group is quite limited. Therefore, the aim of this study; to better understand the structural implications of this anatomical anomaly by investigating the relationship between TD severity and morphometrical and structural changes assessed on knee MRI.

METHODS

This retrospective study was conducted in accordance with the Declaration of Helsinki and was approved by our institution's Biomedical Research Ethics Committee (ethics committee approval number: 2025.223.IRB2.103). Before imaging procedures, written informed consent is obtained from all patients at our institution, and this consent includes permission for the use of images for research purposes.

The study included patients who underwent knee MRI examinations at our institution between November 2012, and March 2025. The Inclusion criteria were individuals with trochlear dysplasia over the age of 18. Exclusion criteria included a history of previous knee surgery, nondiagnostic image quality due to movement or artifact, and incomplete MRI protocol or insufficient clinical information.

All images were obtained using devices with 1.5T or 3T magnetic field strength and coils specially designed for the knee. The routine imaging protocol included sagittal proton density (PD) sequences (fat suppressed and non-fat suppressed), axial fat suppressed PD, and coronal T1-weighted or T2-weighted fat suppressed sequences. Slice thickness varied between 2.5-3.5 mm, and interslice spacing between 0.3-0.5 mm.

All MR images were independently evaluated by a single radiologist with 10 years of experience in musculoskeletal radiology. TD was evaluated using the Dejour classification (Types A-D) and was coded as a numerical grade between 1-4 in the analyses.

The sulcus angle was measured as the angle between tangential lines drawn on the anterior cortical surfaces of the medial and lateral femoral condyles on the axial image at the deepest point of the trochlear groove. Trochlear depth was calculated by subtracting the average height of the medial and lateral femoral condyles at the same level from the depth of the trochlear groove. Values lower than 3 mm were accepted in favor of TD. TT-TG distance was measured as the horizontal distance between the deepest point of the trochlear groove and the center of the tibial tubercle on axial images. The patellar angle was calculated as the angle between lines drawn on the medial and lateral facets of the patella on axial sections. Patellar position was classified as normal, alta (high position), or baja (low position) by measuring the vertical position of the patella in relation to the femoral trochlear groove and the tibial tubercle on sagittal MRI images. This measurement was based on anatomical reference points in accordance with the Insall-Salvati method. Chondromalacia patella was graded from 0 to 4 based on to T2-weighted high signal intensity and cartilage surface irregularities. Meniscal degeneration was identified as T2 high signal into menisci and was classified as medial, lateral, bilateral or absent; meniscus tear was considered present if the intrameniscal T2 high signal reached the articular surface. ACL mucoid degeneration was defined as increased signal and thickening of the ligament on T2-weighted images.

Statistical Analysis

All statistical analyses were performed using the Python programming language. One-way ANOVA was used for continuous variables with normal distribution, Kruskal-Wallis test was used for ordinal variables, and chi-square test was used for categorical variables. The value of $P<0.05$ was accepted as the limit of statistical significance.

RESULTS

A total of 257 knee MRI examinations from 227 different patients were initially included in the study. Based on the exclusion criteria, 14 patients were excluded from the study due to previous surgical history, 20 patients due to poor image quality, and 12 patients due to incomplete protocol. The final analysis was performed with a total of 211 knee MRIs from 181 different patients. The patient cohort consisted of 111 female (132 knees) and 70 male (79 knees) individuals, and the mean age was calculated as 35.13 years (ranging 18 to 76) (Table 1).

According to the Dejour classification, the most common dysplasia type was Type A, detected in 129 (61.14%) knees. This was followed by Type B (56 knees, 26.54%), Type C (22 knees, 10.43%) and Type D (4 knees, 1.9%). This distribution indicates that milder forms of dysplasia (Types A and B) were significantly more common than advanced stages (Types C and D) in the patient group in our study.

When TD degrees were evaluated separately in

TABLE 1. Distribution of MRI-Based Knee Parameters and Their Association with Trochlear Dysplasia

Parameters		Data (n=211)	P-value
Gender distribution (Female/male)		111/70	0.386
Sulcus angle (°)		151 (145-172)	**<0.0001**
Patellar angle (°)		124 (101-148)	0.498
TT-TG distance (mm)		12.9 (4.0-36.0)	**<0.0001**
Trochlear depth (mm)		2.5 (1.1-6.6)	**<0.0001**
Chondromalacia patella	Grade 0	44 (20.85%)	**0.036**
	Grade 1	62 (29.38%)	
	Grade 2	44 (20.85%)	
	Grade 3	24 (11.37%)	
	Grade 4	37 (17.54%)	
Chondromalacia patella	Present	167 (79.15%)	0.203
	Absent	44 (20.85%)	
Meniscal degeneration	Present	96 (45.5%)	**0.0004**
	Absent	115 (54.5%)	
Meniscal degeneration	Medial meniscus	82 (38.86%)	**0.003**
	Lateral meniscus	2 (0.95%)	
	Both	12 (5.69%)	
Meniscal tear	Medial meniscus	23 (10.9%)	0.821
	Lateral meniscus	8 (3.79%)	
	Both	2 (0.95%)	
	None	178 (84.36%)	
ACL mucoid degeneration	Present	31 (14.69%)	0.194
	Absent	180 (85.31%)	
Patellar position	Normal	68 (32.2%)	0.3035
	Alta	137 (64.9%)	
	Baja	6 (2.8%)	

Data are shown as median (range) or n (%) where appropriate. ACL, anterior cruciate ligament; MRI, magnetic resonance imaging; TT-TG, tibial tuberosity to trochlear groove. Statistically significant P-values are shown in bold.

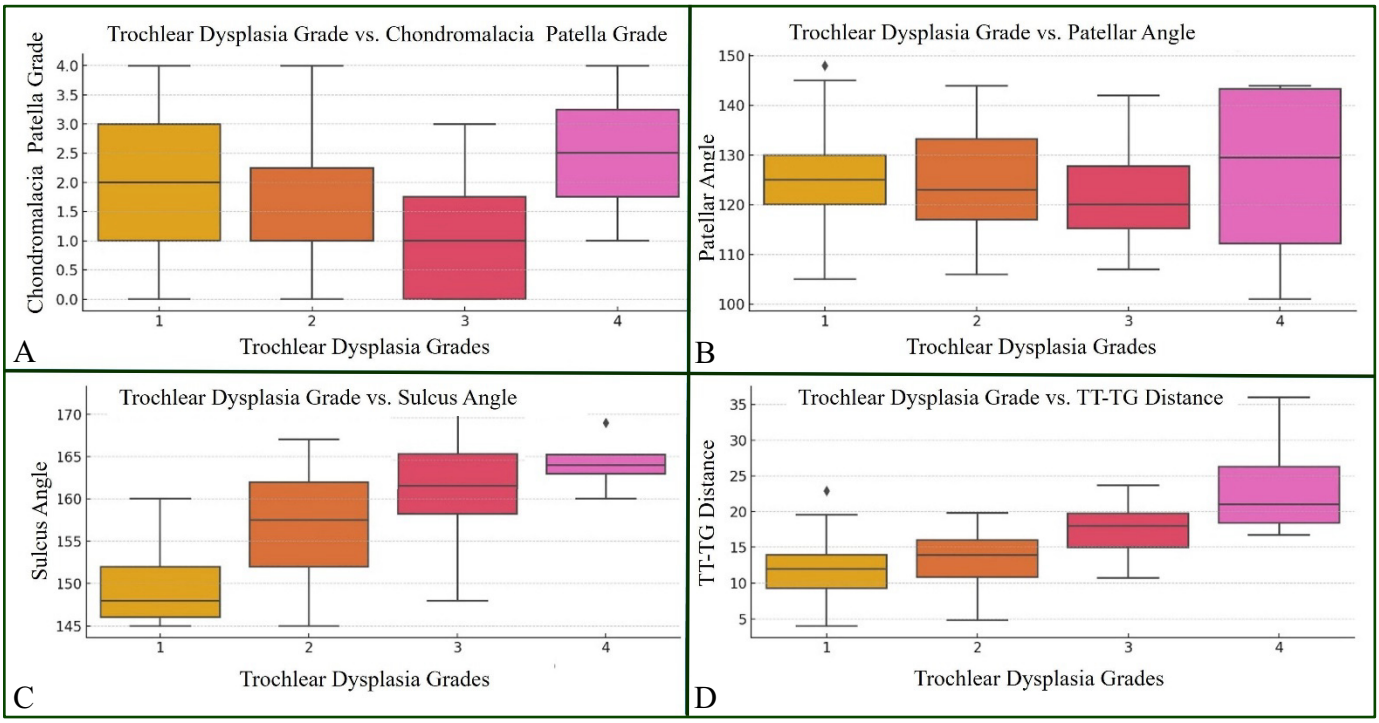

FIGURE 1. Boxplot comparison of trochlear dysplasia grades with MRI-based quantitative parameters. (A) Trochlear dysplasia vs. chondromalacia patella grade; (B) Trochlear dysplasia vs. pateller angle; (C) Trochlear dysplasia vs. sulcus angle and (D) Trochlear dysplasia vs. TT-TG distance.

female and male patients; Type A was detected in 86 female and 43 male knees. Type B was detected in 31 female and 25 male knees; Type C was observed in 12 female and 10 male knees; Type D was observed in 3 female and 1 male knees. No statistically significant difference was observed in the distribution of TD types according to gender (P=0.386).

The sulcus angle was measured as a median of 151° (range: 145°-172°). A statistically significant difference was found between the sulcus angles according to the TD degrees (P<0.0001); an increase in the sulcus angle was observed with more advanced dysplasia degrees (Figure 1). This supports the fact that the trochlear groove becomes progressively flatter. The median value of the patellar angle was found as 124° (range: 101°-148°). However, no statistically significant relationship was shown between the TD degrees and the patellar angle (P=0.498) (Figure 1). This finding suggests that the patellar angle may be insufficient to reflect trochlear morphology in isolation.

The TT-TG distance was calculated as a median of 12.9 mm (range: 4.0-36.0 mm). As the degree of TD increased, a significant increase in the TT-TG distance was observed (P<0.0001), suggesting lateralization of the tibial tubercle, which may contribute to patellar maltracking (Figure 1). A strong statistical relationship was found between trochlear depth and TD degrees. Median trochlear depth was measured as 2.5 mm (range: 1.1-6.6 mm), and the difference between the groups was found to be highly significant when evaluated with one-way ANOVA (P<0.0001).

Patellar position was classified as normal, alta and baja in sagittal MRI images. 68 (32.2%) of 211 knees were evaluated as normal, 137 (64.9%) as patella alta and 6 (2.8%) as patella baja. No statistically significant relationship was found between patellar position and TD degrees (P=0.3035). Chondromalacia patella was observed in 167 (79.15%) knees. The distribution of cases is as follows: Grade 0 (normal): 44 (%20.85) knees , Grade 1: 62 (%29.38) knees , Grade 2: 44 (%20.85) knees, Grade 3: 24 (%11.37) knees, Grade 4: 37 (%17.54) knees. Although no significant difference was found between the presence of chondromalacia and the degree of TD (P=0.203), a

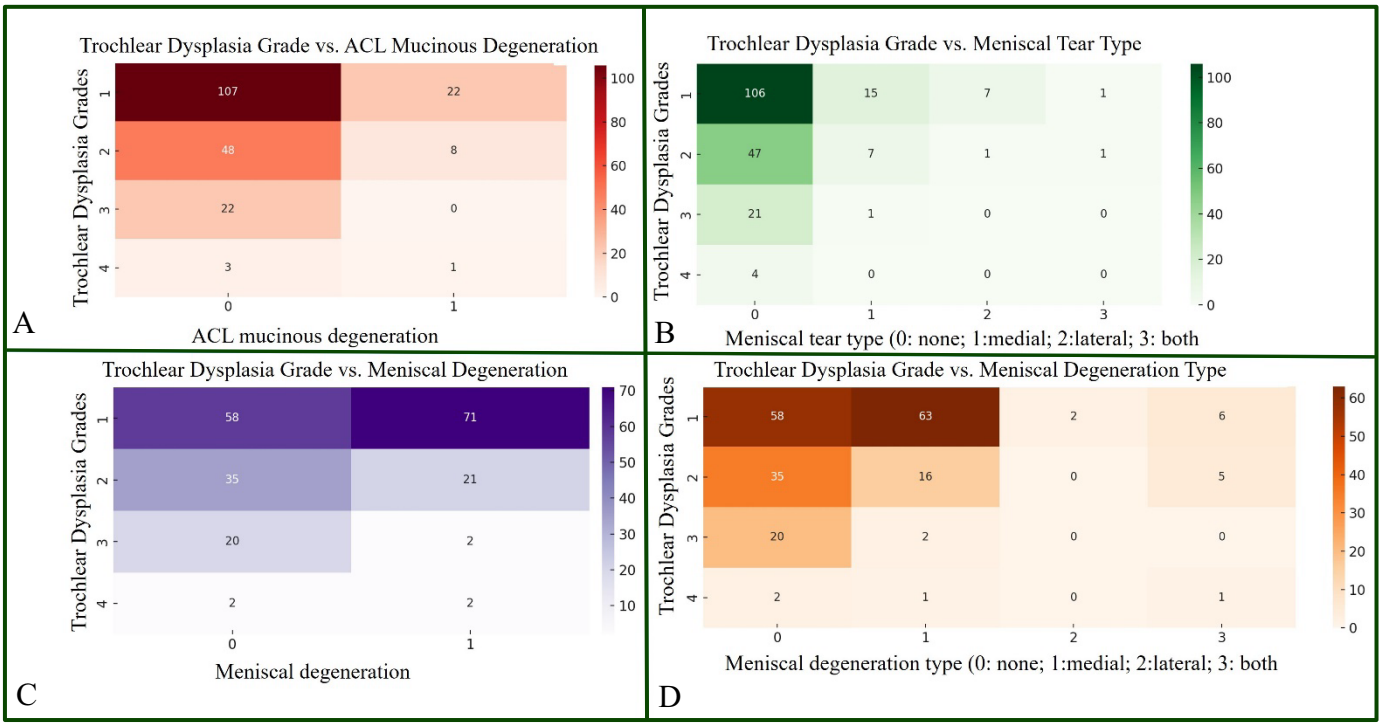

FIGURE 2. **Heatmaps of ligamentous and meniscal abnormalities by trochlear dysplasia grade. (A) Trochlear dysplasia grade vs. ACL mucinous degeneration; (B) Trochlear dysplasia grade vs. meniscal tear type; (C) Trochlear dysplasia grade vs. meniscal degeneration and (D) Trochlear dysplasia grade vs. meniscal degeneration type.**

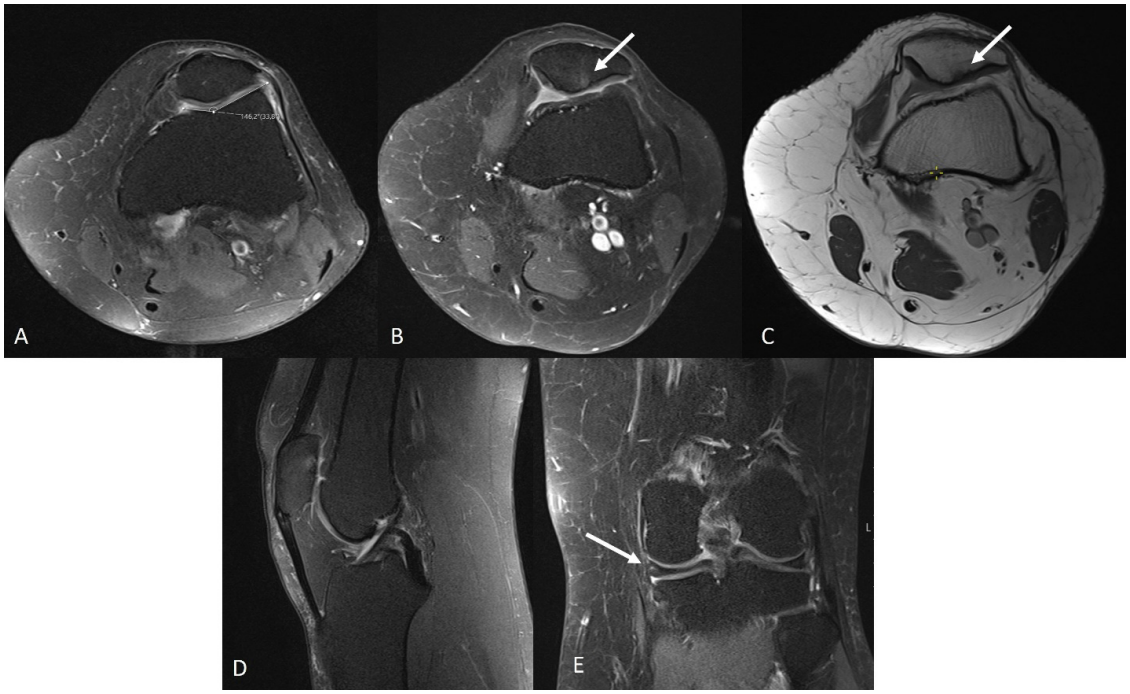

FIGURE 3. **MRI findings of a 45-year-old patient with Type A trochlear dysplasia. (A) Axial proton density-weighted fat-suppressed image shows the measurement of sulcus angle. (B-C) Axial proton density-weighted and T1 weighted images indicating grade 4 chondromalacia patella (white arrows) and (B) the full-thickness cartilage loss with underlying bone reactive changes. (D-E) Sagittal and coronal proton density-weighted images showing increased signal intensity within the medial meniscus (white arrow), indicative of medial meniscus degeneration.**

significant relationship was found between the degrees of chondromalacia and the severity of TD (P=0.036) (Figure 1). This shows that as the severity of TD increases, cartilage damage may be more advanced.

Meniscus degeneration was observed at 96 (45.5%) knees, and no degeneration findings were detected in 115 (54.5%) knees. Of the cases with degeneration, 82 (85.42%) were medial, 2 (2.08%) were lateral, and 12 (12.5%) were both medial and lateral menisci. A significant relationship was observed between TD degrees and the presence of meniscus degeneration (P=0.0004) (Figure 2). A significant relationship was also detected in the evaluation made according to the location of meniscal degeneration (P=0.003), and it was determined that degeneration seen together in both menisci was more common in advanced dysplasia degrees (Figure 2).

Meniscus tears were detected in 33 (15.64%) knees and were not found in 178 (84.36%) knees. In 23 (10.9%) of the cases with tears, only medial, 8 (3.79%) only lateral, and 2 (0.95%) both menisci were observed. No statistically significant relationship was found between TD grades and the presence or type of meniscal tear (P=0.821), suggesting that tears may develop independently of trochlear morphology (Figure 2).

Anterior cruciate ligament mucoid degeneration was detected in 31 (14.69%) knees. No statistically significant relationship was observed between TD grades and ACL mucoid degeneration (P=0.194), indicating that such ligament changes may not be directly related to trochlear structural disorders (Figure 2).

DISCUSSION

This study investigated the relationship between TD and various structural disorders observed in the knee joint. In particular, significant relationships were observed between chondromalacia patella grading, meniscal degeneration (both presence and location), sulcus angle, patellar angle, TT-TG distance, trochlear depth and TD degrees; these findings, despite some differences, were generally consistent with the literature.

One of the most striking findings of our study is the significant relationship between TD degree and chondromalacia patella severity. While there was no significant difference between TD degrees in terms of chondromalacia presence (present/absent), the grading of chondromalacia (0-4) showed a significant correlation with TD severity (Figures 3 and 4). This finding supports the observation that patellofemoral joint morphology plays an important role in cartilage degeneration. This is consistent with the findings of Tuna *et al.* [8], who attributed the high prevalence of chondromalacia in TD patients to increased sulcus angle and reduced trochlear depth.

Meniscal degeneration, especially in cases with both medial and lateral involvement, showed a significant relationship with the TD grade (Figures 3 and 4). This finding is consistent with previous

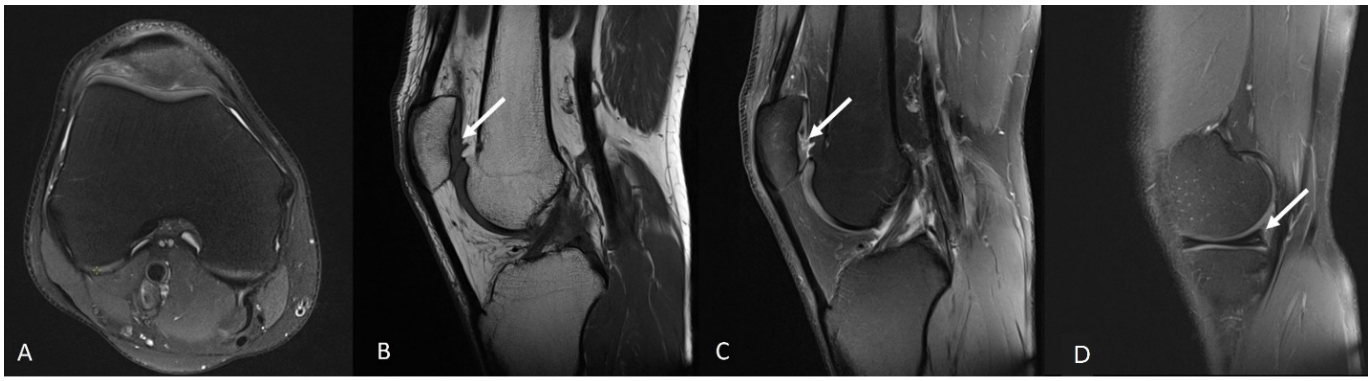

FIGURE 4. MRI findings of a 30-year-old patient with Type B trochlear dysplasia. (A) Axial proton density-weighted fat-suppressed image shows trochlear dysplasia. (B-C) Sagittal proton density-weighted and T1 weighted images indicating grade 3 chondromalacia patella (white arrows). In figure C, full-thickness cartilage loss is shown. (D) Sagittal proton density-weight image shows increased signal intensity within the medial meniscus (white arrow), indicative of medial meniscus degeneration.

research which showed that the frequency of meniscal degeneration increased in knees with dysplastic trochlea morphology [6]. However, when analyzed in terms of meniscal tears, no significant relationship was found with the TD grade. Although meniscal tears were detected in 15.64% of the cases, this distribution was not statistically significant among the TD groups. This suggests that TD may affect degenerative processes over time, but acute or focal tears may be associated with different mechanical traumas.

ACL mucoid degeneration was observed in 14.7% of the cohort, but it was not statistically associated with the TD grade. In a study by Yigman *et al*. [5], the ACL mucoid degeneration rate in patients with TD was reported as higher at 31.6%. The lower rate and lack of a significant relationship in our study may be due to differences in patient selection, MRI criteria, and population.

A significant increase in sulcus angle was observed with advancing TD severity in our study. While the normal sulcus angle is considered to be 138° on average, values above 145° are compatible with dysplasia [9, 10]. This progressive increase supports the notion that trochlear flattening becomes more pronounced with higher degrees of dysplasia, contributing to patellofemoral instability. TT-TG distance and patellar angle are both key indicators of patellofemoral alignment. In our study, a significant increase in TT-TG distance was observed with increasing TD severity, which may reflect altered alignment of the extensor mechanism that may predispose to patellar maltracking or instability. In contrast, although the patellar angle assesses patellar orientation relative to the femoral trochlea and has been previously linked to TD, no significant relationship was observed in our cohort (P=0.498). This suggests that the patellar angle alone may not be sufficient to reflect the severity of TD.

Trochlear depth is an important measurement in the morphological evaluation of dysplastic trochlea. In this study, an inverse and highly significant relationship was found between TD severity and trochlear depth. The median depth was measured as 2.5 mm (range 1.1-6.6 mm), and this value is considered to be in favor of dysplasia when < 3 mm [8]. Our findings support the trochlear depth as a simple but reliable parameter in the diagnosis and classification of TD.

It is accepted that patella alta may be associated with TD [12]. However, in our study, no statistical significance was observed between patellar position and TD degrees (P=0.3035). Although patella alta was observed in 64.9% of the cases, this distribution was balanced according to TD types. Similarly, there are studies in some literatures reporting that patella alta, although frequently observed, is not sufficient to predict TD severity [13, 14]. Therefore, patellar height assessment should be considered together with other parameters such as sulcus angle and TT-TG.

This study contributes to the growing body of evidence that TD is associated with multiple intra-articular structural abnormalities. In particular, revealing the relationship between TD and chondral and meniscal degeneration emphasizes the importance of detailed morphological evaluation in patients with anterior knee pain or patellofemoral instability. Recognition of TD may contribute to determining the risk level of patients and may be helpful in shaping both conservative and surgical treatment approaches.

Strengths and Limitations

This study possesses numerous significant strengths. One of its primary advantages is the relatively substantial sample size, enabling a comprehensive multiparametric assessment of trochlear dysplasia through knee MRI. Concurrent evaluation of standardized morphometric measurements and related degenerative characteristics facilitates a comprehensive and quantitative assessment of dysplasia severity. Furthermore, establishing clinically significant correlations with cartilage and meniscal degeneration increases the study's practical relevance and its contribution to the understanding of patellofemoral pathology.

There are some limitations to this study. First, its retrospective and cross-sectional design may introduce selection bias and prevents the establishment of cause-effect relationships. The inclusion of patients based on clinical imaging records may also limit the generalizability of the findings. Second, all imaging evaluations and measurements were performed by a single radiologist, which may have introduced observer bias; inter-reader agreement was not assessed. Additionally, while a relatively large sample was included, few subgroup sizes were smaller, potentially reducing the statistical power. Finally, this

was a single-center study, and further prospective studies in larger and more diverse populations, including clinical outcome correlations, are warranted to validate our findings. Therefore, prospective studies in larger patient groups and correlation analyses to be performed with clinical outcomes will strengthen the validity of the current findings.

CONCLUSION

This study demonstrated that TD is significantly associated with anatomical changes and as well as meniscal degeneration and chondromalacia patella grading as a degenerative change observed in the knee joint. Morphological and structural abnormalities such as increased sulcus angle, increased TT-TG distance, decreased trochlear depth, advanced chondromalacia patellae, and meniscal degeneration reveal the contribution of TD to patellofemoral biomechanical imbalance. Although parameters such as patellar angle and position were not significantly associated with TD, it is thought that these variables may also contribute to comprehensive radiological evaluation. In general, our findings support the adoption of a multiparameter MRI-based approach in the identification of TD and related pathologies. Early and accurate identification of these structural changes may contribute to the planning of time-sensitive treatment strategies in patients presenting with anterior knee pain and instability and may improve clinical outcomes.

Key points

•TD is significantly associated with anatomical changes and as well as meniscal degeneration and chondromalacia patella grading as a degenerative change observed in the knee joint.

•Morphological and structural abnormalities such as increased sulcus angle, increased TT-TG distance, decreased trochlear depth, advanced chondromalacia patellae, and meniscal degeneration reveal the contribution of TD to patellofemoral biomechanical imbalance.

•Early and accurate identification of these structural changes may contribute to the planning of time-sensitive treatment strategies.

Ethics Approval and Consent to Participate

This study was approved by the Koç University Biomedical Research Ethics Committee (Decision No: 2025.223.IRB2.103; date: 13.05.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. The study had retrospective design, no additional procedures were performed. Informed consent forms are obtained from each patient before the radiological examination in our institution as a clinical routine.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: GTY, HÖA; Study Design: GTY; Supervision: GTY; Funding: N/A; Materials: GTY, HÖA; Data Collection and/or Processing: GTY; Statistical Analysis and/or Data Interpretation: GTY; Literature Review: GTY, HÖA; Manuscript Preparation: GTY, HÖA; and Critical Review: GTY.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Only AJ, Arendt EA, Hinckel BB. Anatomic Risk Factors for Lateral Patellar Instability. Arthroscopy. 2024;40(11):2642-2644. doi: 10.1016/j.arthro.2024.08.009.
2. Ackermann J, Caliskan B, Hartmann M, Vlachopoulos L, Fucentese SF. The Effect of a Supratrochlear Spur on Patellofemoral Cartilage in Patients With Trochlear Dysplasia. Am J Sports Med. 2025;53(5):1127-1132. doi: 10.1177/03635465251323806.
3. Morello V, Zingg M, Tscholl P. The prevalence of radiographic trochlear dysplasia in patients with patellar fractures. BMC Musculoskelet Disord. 2024;25(1):1091. doi: 10.1186/s12891-024-08204-4.
4. Figueroa F, Guiloff R, Bolton S, Figueroa D, Tapasvi S, Stocker E. Specific considerations in female patients with patellar instability: current concepts. J ISAKOS. 2024;9(3):457-463. doi: 10.1016/j.jisako.2024.03.014.
5. Yigman GT, Toprak H. Coexistence of Trochlear Dysplasia and Anterior Cruciate Ligament Mucoid Degeneration and Relationship Between Dysplasia Degree and Mucoid Degeneration. Acad Radiol. 2022;29(5):685-688. doi: 10.1016/j.acra.2020.07.007.
6. Atay M, Yılmaz TF, Sarı L, Toprak H. Association of trochlear dysplasia with knee meniscal-cartilage damage and anterior cruciate ligament mucoid degeneration. Clin Radiol. 2023;78(1):e1-e5. doi: 10.1016/j.crad.2022.08.123.
7. Usal C, Çilengir AH, Sarıoğlu O, Dirim B, Tosun Ö. The Relationship Between Patellar Chondromalacia and Patellofemoral Joint Anatomical Variations. Curr Res MRI 2023;2(1):11-15. doi: 10.5152/CurrResMRI.2023.22044.
8. Tuna BK, Semiz-Oysu A, Pekar B, Bukte Y, Hayirlioglu A. The association of patellofemoral joint morphology with chondromalacia patella: a quantitative MRI analysis. Clin Imaging. 2014;38(4):495-498. doi: 10.1016/j.clinimag.2014.01.012.
9. Mofidi A, Veravalli K, Jinnah RH, Poehling GG. Association and impact of patellofemoral dysplasia on patellofemoral arthropathy and arthroplasty. Knee. 2014r;21(2):509-513. doi: 10.1016/j.knee.2013.09.009.
10. Tanaka MJ, LaPorte ZL, Perry NPJ, Velasquez Hammerle MV, Nukala V, Liu F. Association of Trochlear Length on Sagittal MRI to Trochlear Dysplasia in Knees With Patellar Instability. Orthop J Sports Med. 2023 5;11(6):23259671231169730. doi: 10.1177/23259671231169730.
11. Tscholl PM, Wanivenhaus F, Fucentese SF. Conventional Radiographs and Magnetic Resonance Imaging for the Analysis of Trochlear Dysplasia: The Influence of Selected Levels on Magnetic Resonance Imaging. Am J Sports Med. 2017;45(5):1059-1065. doi: 10.1177/0363546516685054.
12. Lippacher S, Reichel H, Nelitz M. Radiological criteria for trochlear dysplasia in children and adolescents. J Pediatr Orthop B. 2011;20(5):341-344. doi: 10.1097/BPB.0b013e3283474c8b.
13. Biedert RM. Patella Alta: When to Correct and Impact on Other Anatomic Risk Factors for Patellofemoral Instability. Clin Sports Med. 2022;41(1):65-76. doi: 10.1016/j.csm.2021.07.002.
14. Colatruglio M, Flanigan DC, Harangody S, Duerr RA, Kaeding CC, Magnussen RA. Identifying Patients With Patella Alta and/or Severe Trochlear Dysplasia Through the Presence of Patellar Apprehension in Higher Degrees of Flexion. Orthop J Sports Med. 2020;8(6):2325967120925486. doi: 10.1177/2325967120925486.

The European Research Journal 2026;12(1):68-75 Original Article

DOI: https://doi.org/10.18621/eurj.1799369 *Rheumatology and Arthritis*

HALP Score and Disease Activity in Psoriatic Arthritis: Comparison with Healthy Controls

Sezgin Zontul[1], Servet Yolbaş[2], Elif İnanç[2], Emrah Şimşek[3], Ahmet Kadir Arslan[4]

[1]Department of Physical Medicine and Rehabilitation, Division of Rheumatology, Faculty of Medicine, Inonu University, Malatya, Türkiye; [2]Department of Internal Medicine, Division of Rheumatology, Faculty of Medicine, Inonu University, Malatya, Türkiye; [3]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Inonu University, Malatya, Türkiye; [4]Department of Biostatistics and Medical Informatics, Faculty of Medicine, Inonu University Malatya, Türkiye

ABSTRACT

Objectives: This study evaluated the relationship between the haemoglobin-albumin-lymphocyte-platelet (HALP) score and disease activity in patients with psoriatic arthritis (PsA), and compared HALP scores between PsA patients and healthy controls.

Methods: This single-centre, cross-sectional study included 73 PsA patients and 59 healthy controls. Demographic, clinical and laboratory data were collected. Disease activity was assessed using the Ankylosing Spondylitis Disease Activity Score (ASDAS) based on C-reactive protein (CRP), the Disease Activity index for Psoriatic Arthritis (DAPSA), the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the visual analogue scale (VAS), and the Psoriasis Area and Severity Index (PASI). HALP was calculated as haemoglobin × albumin × lymphocyte / platelet. Group comparisons, correlation analyses and ROC analyses were performed.

Results: Compared with controls, PsA patients had higher CRP, erythrocyte sedimentation rate and platelet values, and lower albumin (all $P<0.05$). HALP scores did not differ significantly between groups ($P=0.232$). HALP correlated positively with age at diagnosis ($r = 0.250$; $P=0.031$) and negatively with ASDAS-CRP ($r = -0.259$; $P=0.026$). ROC analysis showed limited diagnostic performance (AUC = 0.561, $P=0.228$).

Conclusions: Although HALP showed a significant inverse correlation with ASDAS-CRP, the association was weak and its diagnostic performance was poor. HALP alone has limited value in reflecting PsA disease activity or distinguishing patients from controls. This may relate to PsA's heterogeneous inflammation and treatment status. Larger prospective studies in different PsA subgroups are needed to clarify the potential role of HALP as an objective biomarker.

Keywords: Psoriatic Arthritis, HALP Score, Inflammation, Biomarker, Disease Activity

Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory arthropathy characterized by inflammation of the joints and entheses, including the axial skeleton [1]. The incidence of PsA in patients with psoriasis ranges from 0.27 to 2.7 per 100 person-years, depending on study design and outcome definitions. PsA typically affects individuals between 30 and 60 years of age and occurs equally in men and women [2]. Peripheral manifestations include polyarthritis, oligoarthritis, distal inter-

Received: October 8, 2025 **Accepted:** November 10, 2025 **Available Online:** November 28, 2025 **Published:** January 4, 2026

How to cite this article: Zontul S, Yolbaş S, İnanç E, Şimşek E, Arslan AK. HALP Score and Disease Activity in Psoriatic Arthritis: Comparison with Healthy Controls. Eur Res J. 2026;12(1):68-75. doi: 10.18621/eurj.1799369

Corresponding author: Sezgin Zontul., MD., Assist. Prof., Phone: +90 422 3410660, ext. 3606, E-mail: sezginzontul@hotmail.com, sezgin.zontul@inonu.edu.tr

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

phalangeal arthritis and, less commonly, the arthritis mutilans subtype. Periarticular features such as dactylitis and enthesitis are frequent. Axial PsA, also known as the spondylitis subtype, can be limited to the spine and sacroiliac joints or coexist with peripheral disease [3].

Regular and accurate assessment of disease activity is essential in PsA because uncontrolled inflammation leads to structural damage, functional loss and reduced quality of life [4]. Several clinical indices have been developed for this purpose. The Disease Activity Index for Psoriatic Arthritis (DAPSA) primarily focuses on peripheral joint involvement, while the Psoriatic Arthritis Disease Activity Score (PASDAS) includes a broader range of domains but is complex and time-consuming, limiting its routine use [5–7]. For axial involvement, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Ankylosing Spondylitis Disease Activity Score (ASDAS) are commonly used, but they are not specific to PsA and do not fully capture peripheral or skin manifestations [5]. Minimal Disease Activity (MDA) criteria take a multidomain approach but rely on binary outcomes, which may not reflect the full spectrum of disease activity [8]. These limitations highlight the need for more practical and objective biomarkers to assess PsA disease activity.

Recently, several inflammatory ratios derived from complete blood counts and routine biochemistry have been investigated in rheumatic diseases, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and monocyte-to-HDL ratio (MHR) [9-11]. The haemoglobin–albumin–lymphocyte–platelet (HALP) score, originally proposed as a prognostic marker in malignancies [12], has more recently been explored in autoimmune and inflammatory diseases [13, 14]. HALP reflects nutritional, inflammatory, immune and haematologic status through a simple calculation, making it a potentially useful biomarker in chronic inflammatory conditions. Psoriatic arthritis is characterized by both systemic inflammation and metabolic alterations that may influence the components of the HALP score. Low haemoglobin and albumin levels are common in chronic inflammation and have been linked to disease activity and poor nutritional status [15]. Reduced lymphocyte counts may reflect immune dysregulation, while elevated platelet counts are associated with systemic inflammation and endothelial activation [16, 17]. Therefore, evaluating the HALP score in PsA may provide an integrated measure of inflammatory and nutritional status, potentially serving as a simple, objective indicator of disease activity.

Given these considerations, there remains a need to investigate the role of HALP in PsA. This study aimed to evaluate the relationship between the HALP score and PsA disease activity, and to compare HALP levels between PsA patients and healthy controls, in order to explore its potential clinical utility as an objective biomarker.

METHODS

This study was designed as a single-centre, cross-sectional, observational study. Patients aged 18-65 years who were being followed up at our rheumatology outpatient clinic and had been diagnosed with PsA according to the CASPAR criteria were included in the study. The control group consisted of healthy individuals who did not have any rheumatic disease and who met the exclusion criteria. Those with liver disease, haematological or other malignancies, kidney diseases such as glomerulonephritis, acute or chronic infections, gastrointestinal diseases causing protein loss, other rheumatological diseases or a history of pregnancy were excluded. Approval for the study was obtained from the İnönü University Clinical Research Ethics Committee (numbered 2025/7251 and dated 11 March 2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to inclusion in the study.

The age, gender, body mass index (BMI), smoking and alcohol consumption habits, duration of illness, clinical characteristics, and medications used of all participants were recorded. Routine laboratory tests included haemoglobin, white blood cell, lymphocyte, neutrophil and platelet counts, as well as albumin, erythrocyte sedimentation rate (ESR) and CRP levels. Additionally, the NLR and PLR were calculated.

PsA disease activity in participants was assessed using the Visual Analogue Scale (VAS), DAPSA, Psoriasis Area and Severity Index (PASI), ASDAS-CRP and BASDAI. The HALP score was calculated using the formula ‘HALP = haemoglobin (g/L) × albumin (g/L) × lymphocytes (/L)/platelets (/L)’ [18].

Statistical Analysis

According to the theoretical power analysis findings, when the test power is 80%, the effect size is 0.8 and the alternative hypothesis (H1) is two-tailed, the required sample size to detect a statistically significant difference between groups using an independent two-sample t-test is at least 52 per group (104 in total) at a 5% significance level. This analysis was performed using the WSSPAS (Web-Based Sample Size & Power Analysis Software) tool, which was developed by the Department of Biostatistics and Medical Informatics at the Faculty of Medicine, İnönü University [19].

The variables used in the study were summarized using arithmetic mean, standard deviation, median, 25th-75th percentiles, and number and percentage statistics. Quantitative data were analyzed for normal distribution using the Shapiro-Wilk test. The independent samples t-test and Mann-Whitney U test were used for between- group comparisons of quantitative data. Pearson's chi-square and Fisher's exact tests were used for between-group comparisons of qualitative data. Receiver operating characteristic (ROC) analysis was used to calculate the cut-off point and area under the curve (AUC) for quantitative data. P≤0.05 was accepted as the statistical significance level. R Project (version 4.1.2) software was used for analyses.

RESULTS

The study included 59 individuals in the control group and 73 patients in the PsA group. There was no significant difference in mean age or BMI between the two groups (P=0.148 and P=0.059, respectively). Serum albumin levels were lower in the PsA group than in the control group (4.3 [4.1-4.6] vs. 4.4 [4.3-4.6] g/dL,

TABLE 1. Comparison of Clinical and Laboratory Parameters Between Groups

Variables		Control Group (n=59)	PsA Group (n=73)	P-value
Age (years),		44±12	47±11	0.148*
BMI (kg/m²)		27.06 (24.2-30.4)	28.6 (25.5-32.8)	0.059**
Gender	Female	40 (67.8%)	55 (75.3%)	0.337†
	Male	19 (32.2%)	18 (24.7%)	
Smoking	No	39 (66.1%)	52 (71.2%)	0.526†
	Yes	20 (33.9%)	21 (28.8%)	
Alcohol	No	58 (98.3%)	70 (95.9%)	0.628††
	Yes	1 (1.7%)	3 (4.1%)	
Albumin (g/dL)		4.4 (4.3–4.6)	4.3 (4.1-4.6)	**0.029****
CRP (mg/dL)		0 (0-0.0)	0.3 (0-1.05)	**<0.001****
Haemoglobin (g/dL)		13.90±1.62	13.36±1.78	0.075*
Lymphocyte (cells/µL)		2220 (1860-2470)	2390 (1870-2940)	0.067**
Neutrophil (cells/µL)		4130 (3160-5260)	4090 (3310-5640)	0.449**
NLR		1.95 (1.58-2.39)	1.76 (1.35-2.45)	0.335**
PLR		123.12 (100.3-140.5)	119 (95.2-151.1)	0.814**
ESR (mm/h)		6 (2-9)	12 (5-24)	**<0.001****
Platelets (x10³/µL)		249 (218-307)	303 (244-349)	**0.006****
HALP Score		50.73 (42.5-63.56)	48 (34.4-62.64)	0.232**

Data are shown as mean±standard deviation or number (percent) or median (25th-75th percentiles) where appropriate.

BMI, body mass index; CRP, C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; ESR, erythrocyte sedimentation rate; HALP, hemoglobin-albumin lymphocyte-platelet.

*Independent-samples t-test, **Mann-Whitney U test, †Pearson chi-square, ††Fisher's exact test.

Statistically significant P-values are shown in bold.

P=0.029). CRP and ESR levels were higher in the PsA group (CRP: 0.3 [0-1.05] vs. 0 [0-0.0] mg/dL, P<0.001; ESR: 12 [5-24] vs. 6 [2-9] mm/h, P<0.001). Platelet counts were also higher in the PsA group (303 [244-349] vs. 249 [218-307] $\times 10^3/\mu L$, P=0.006). Other parameters, including lymphocyte and neutrophil counts, the neutrophil-to-lymphocyte ratio, and the platelet-to-lymphocyte ratio, did not differ significantly between the two groups (P>0.05 for all). Gender distribution, smoking status, and alcohol consumption were similar between groups (P=0.337, P=0.526, and P=0.628, respectively). No significant difference was found in HALP scores between PsA and control groups (48 [34.4-62.64] vs. 50.73 [42.5-63.56], P=0.232). These findings are summarized in Table 1.

Among the 73 PsA patients, 53.4% (n= 39) had peripheral involvement, 42.5% (n=31) had both axial and peripheral disease, and 4.1% had purely axial involvement. Regarding treatment, 20.5% were untreated, 43.8% (n=32) were on conventional synthetic DMARDs (csDMARDs), and smaller proportions received anti-TNF agents (11.0%) or IL-17 inhibitors (9.6%). Corticosteroids were used by 28.8% (n=21) of patients. The median disease duration was 4.0 years (P25–P75: 0.3–7.0). The median VAS, DAPSA, PASI, and BASDAI scores were 6.0 (5.0-8.0), 16.0 (10.0-21.0), 0.3 (0.0-2.4), and 6.1 (4.5-7.1), respectively. The mean ASDAS-CRP score was 2.65± .06 (Table 2).

TABLE 2. Clinical Characteristics, Treatment Distribution and disease Activity Scores of Patients

Characteristics		Value
Involvement type	Axial	3 (4.1)
	Peripheral	39 (53.4)
	Axial + Peripheral	31 (42.5)
	Total	73 (100)
DMARD treatment	No treatment	15 (20.5)
	csDMARD	32 (43.8)
	>1 csDMARD	4 (5.5)
	anti-TNF	8 (11.0)
	IL-17 inhibitor	7 (9.6)
	csDMARD+anti-TNF	6 (8.2)
	csDMARD+IL-17 inhibitor	1 (1.4)
	Total	73 (100)
Steroid use	No	52 (71.2)
	Yes	21 (28.8)
	Total	73 (100)
Age at diagnosis (years)		4.0 (0.3-7.0)
VAS score,		6.0 (5.0-8.0)
DAPSA score		16.0 (10.0-21.0)
PASI score		0.3 (0.0-2.4)
ASDAS-CRP score		2.65±1.06
BASDAI score		6.1 (4.5-7.1)

Data are shown as mean±standard deviation or number (percent) or median (25th-75th percentiles) where appropriate. DMARD, disease-modifying antirheumatic drug; csDMARD, conventional synthetic DMARD; TNF, tumor necrosis factor; IL-17, interleukin-17; VAS, visual analogue scale; DAPSA, disease activity in psoriatic arthritis; PASI, psoriasis area and severity index; ASDAS-CRP, ankylosing spondylitis disease activity score using C-reactive protein; BASDAI, bath ankylosing spondylitis disease activity index.

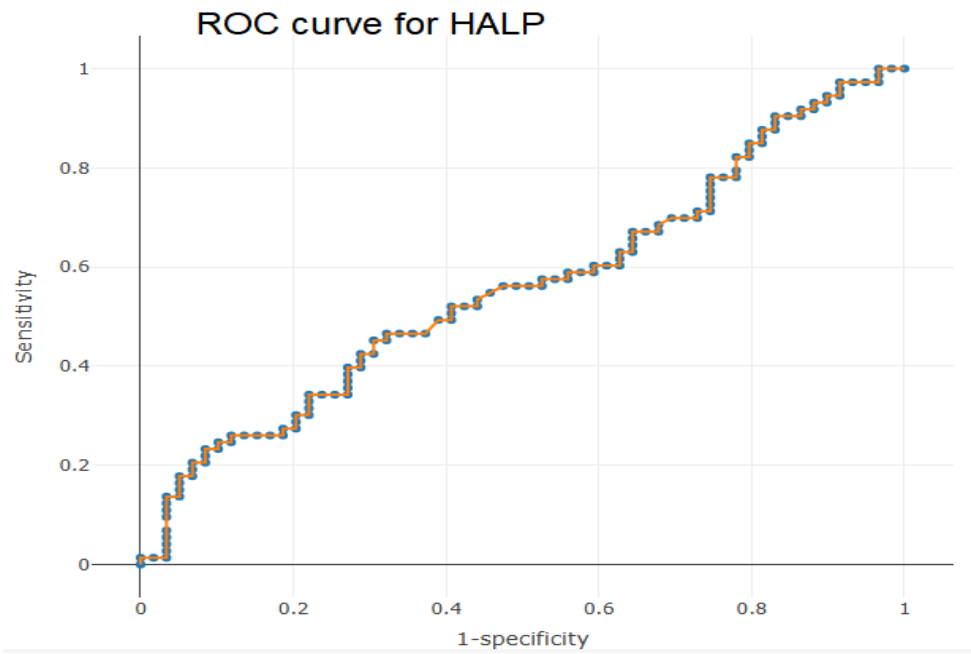

FIGURE 1. Receiver operating characteristic (ROC) curve of the HALP score for distinguishing PsA patients from healthy controls. The area under the curve (AUC) was 0.561 (95% CI: 0.462-0.659), with a sensitivity of 23.3% and a specificity of 91.5%, indicating limited diagnostic discrimination.

In the correlation analysis conducted on the PsA group, a positive and significant correlation was found between the HALP score and age at diagnosis (r=0.250, 95% CI: 0.022-0.456, P=0.031). By contrast, a negative and significant correlation was found between the HALP score and the ASDAS-CRP score (r=−0.259, 95% CI: -0.462 to -0.034, P=0.026).

ROC analysis showed an AUC of 0.561 (95% CI: 0.462-0.659), with an optimal cut-off value of 31.25, corresponding to a sensitivity of 23.3% and a specificity of 91.5% (P=0.228). These values indicate that the discriminative performance of HALP for distinguishing PsA from controls was not statistically significant. The ROC curve is presented in Figure 1.

DISCUSSION

This study examined the prognostic value of the HALP score in relation to the clinical and laboratory characteristics of patients with PsA. Our findings showed that there were significant differences in CRP, ESR, platelet count and serum albumin levels in the PsA group compared to the control group, but the HALP score did not show a statistically significant difference. Furthermore, ROC curve analysis showed that the HALP score had limited discriminatory power. These results suggest that the HALP score may not be a reliable PsA biomarker on its own.

In cases of systemic inflammation, the number of platelets often increases, while haemoglobin, albumin and lymphocyte levels tend to decrease. Inflammatory cytokines increase the number of neutrophils in circulation while decreasing lymphocyte count and albumin levels [20, 21]. Interleukin (IL)-1β and tumour necrosis factor (TNF)-α suppress the release of erythropoietin from the kidneys. Additionally, IL-6 increases hepcidin production in the liver. This reduces iron absorption from the intestine and iron release from macrophages, leading to decreased haemoglobin production and chronic disease anaemia in patients [22, 23]. Therefore, a decrease in the HALP score is expected in cases of systemic inflammation.

Previous studies have demonstrated that the HALP score may be useful for predicting outcomes in different disease groups. In recent years, attention has been drawn to the HALP score due to its ability to assess inflammation, nutrition, immune response and coagulation processes simultaneously. The ease with which it can be calculated also makes it attractive for

use, particularly in diseases involving intense inflammation [24, 25]. Notably, a low HALP score has been reported to be strongly associated with survival in solid tumours, including gastric and colorectal cancer [12, 18]. Furthermore, the HALP score has been identified as a meaningful biomarker for predicting renal relapses in lupus nephritis and for determining disease activity in patients with ankylosing spondylitis [13, 14]. These findings suggest that the HALP score could play a role in both malignant and inflammatory processes. In our study, HALP showed a statistically significant negative correlation with ASDAS-CRP, which may indicate a partial relationship with inflammatory activity in PsA. However, this association was weak, and no clear correlations were observed with other disease activity indices. These results imply that the clinical utility of HALP in PsA may be more limited than in other diseases.

One possible explanation for the limited performance of the HALP score in PsA is that systemic inflammatory responses tend to be less pronounced in PsA compared to other inflammatory arthritides such as rheumatoid arthritis (RA). Recent studies have shown that levels of CRP, serum amyloid A (SAA), and adhesion molecules (sICAM-1, sVCAM-1) are significantly higher in RA than in PsA [26]. In PsA, the inflammatory response is often localized to tissues such as the entheses and synovium, and type 3 immune pathways, particularly IL-23/IL-17 signaling, are thought to play a central role in this process. As a result, classical systemic inflammatory markers are not always elevated [1, 2]. This pathophysiological difference may partly explain the limited performance of systemic inflammation-based indices, such as the HALP score, in PsA.

Several studies have investigated the NLR and PLR as markers of systemic inflammation in PsA. For example, Kim *et al*. [27] reported in a retrospective Korean study that both NLR and PLR were significantly higher in PsA patients compared to healthy controls and patients with psoriasis alone, and that NLR was particularly effective in predicting the presence of PsA. Similarly, a Japanese study demonstrated that pre-treatment NLR and PLR levels were significantly higher in PsA than in psoriasis, and that both ratios decreased in parallel with CRP following biologic therapy [28]. These findings suggest that NLR and PLR may serve as useful indicators of systemic inflammation in PsA. In our study, most patients were receiving treatment, and ESR and CRP levels were not markedly elevated. This may partly explain the limited performance of both NLR/PLR and the HALP score. Although HALP reflects not only inflammation but also nutritional and hematologic status, the localized inflammatory response characteristic of PsA, together with treatment-related suppression, may have reduced its ability to distinguish disease activity. These findings suggest that HALP alone may not be sufficient as a biomarker in PsA, but its combined use with other hematological ratios (e.g., NLR, PLR, MHR) or novel biological markers could enhance its clinical relevance.

Furthermore, recent studies in other rheumatologic diseases have demonstrated a growing interest in inflammation-related hematologic and biochemical ratios. For example, one study evaluated monocyte-to-HDL and CRP-to-albumin ratios in Takayasu arteritis, demonstrating that these markers may reflect disease activity and vascular inflammation [29]. From this perspective, HALP can be viewed as part of a broader group of composite ratios being investigated in various rheumatologic conditions to quantify inflammatory burden and monitor disease activity.

Strengths and Limitations

To our knowledge, this is the first study to directly examine the relationship between the HALP score and disease activity in patients with PsA, offering a new perspective to the current literature. A major strength of our study is its comparative design with a healthy control group and the multidimensional evaluation of disease activity using different clinical indices. However, several limitations should be considered. The relatively small sample size, cross-sectional design, and heterogeneity of treatment regimens may have influenced our findings. In addition, evaluating HALP in different PsA subtypes (axial, peripheral, dactylitis-dominant) or across various treatment groups (e.g., bDMARDs, IL-17 inhibitors) may help to better define its potential clinical applications. These findings also underline the limited value of the HALP score in PsA and emphasize the need for cautious interpretation of systemic inflammation markers in clinical practice. Therefore, further confirmation in larger, prospective cohorts is warranted. Future multicentre studies will help to more clearly define the biomarker potential of the HALP score in psoriatic arthritis.

CONCLUSION

In conclusion, the HALP score demonstrated a limited association with disease activity in PsA, likely reflecting the unique inflammatory profile of this disease. Although it may not serve as a stand-alone biomarker, its accessibility and simplicity suggest potential value as an exploratory or hypothesis-generating parameter rather than a clinically established marker. Larger, prospective, and multicentre studies are needed to confirm these preliminary findings and to better define the role of HALP in disease monitoring and assessment in PsA.

Ethics Approval and Consent to Participate

This study was approved by the the İnönü University Scientific Research and Publication Ethics Committee (Decision No: 2025/7251; date: 11.03.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all participants prior to inclusion in the study.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: SZ, SY, MBB; Study Design: SZ, SY; Supervision: SZ, SY; Funding: N/A, BS; Materials: SZ, EŞ, Eİ; Data Collection and/or Processing: SZ, Eİ, EŞ; Statistical Analysis and/or Data Interpretation: AKA, SZ; Literature Review: SZ; Manuscript Preparation: SZ, AKA; and Critical Review: SY, Eİ, EŞ, AKA.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Veale DJ, Fearon U. The pathogenesis of psoriatic arthritis. Lancet. 2018;391(10136):2273-2284. doi: 10.1016/S0140-6736(18)30830-4.
2. FitzGerald O, Ogdie A, Chandran V, et al. Psoriatic arthritis. Nat Rev Dis Primers. 2021 Aug 12;7(1):59. doi: 10.1038/s41572-021-00293-y.
3. Ocampo D V, Gladman D. Psoriatic arthritis. F1000Res. 2019;8:F1000 Faculty Rev-1665. doi: 10.12688/f1000research.19144.1.
4. Coates LC, Moverley AR, McParland L, et al. Effect of tight control of inflammation in early psoriatic arthritis (TICOPA): a UK multicentre, open-label, randomised controlled trial. Lancet. 2015;386(10012):2489-2498. doi: 10.1016/S0140-6736(15)00347-5.
5. Kerschbaumer A, Smolen JS, Aletaha D. Disease activity assessment in patients with psoriatic arthritis. Best Pract Res Clin Rheumatol. 2018;32(3):401-414. doi: 10.1016/j.berh.2018.08.004.
6. Schoels M, Aletaha D, Funovits J, Kavanaugh A, Baker D, Smolen JS. Application of the DAREA/DAPSA score for assessment of disease activity in psoriatic arthritis. Ann Rheum Dis. 2010;69(8):1441-1447. doi: 10.1136/ard.2009.122259.
7. Helliwell PS, FitzGerald O, Fransen J, et al. The development of candidate composite disease activity and responder indices for psoriatic arthritis (GRACE project). Ann Rheum Dis. 2013;72(6):986-991. doi: 10.1136/annrheumdis-2012-201341.
8. Coates LC, Fransen J, Helliwell PS. Defining minimal disease activity in psoriatic arthritis: a proposed objective target for treatment. Ann Rheum Dis. 2010;69(1):48-53. doi: 10.1136/ard.2008.102053.
9. Mercan R, Bitik B, Tufan A, et al. The Association Between Neutrophil/Lymphocyte Ratio and Disease Activity in Rheuma-

toid Arthritis and Ankylosing Spondylitis. J Clin Lab Anal. 2016;30(5):597-601. doi: 10.1002/jcla.21908.
10. Gasparyan AY, Ayvazyan L, Mukanova U, Yessirkepov M, Kitas GD. The Platelet-to-Lymphocyte Ratio as an Inflammatory Marker in Rheumatic Diseases. Ann Lab Med. 2019;39(4):345-357. doi: 10.3343/alm.2019.39.4.345.
11. Varkal G, Türk I, Yetişir A, Ağbuga ÖD, Mete B, Özbek S. The Relationship Between the Monocyte-to-High-Density Lipoprotein-Cholesterol Ratio and Disease Activity in Patients with Psoriatic Arthritis. Eur J Rheumatol. 2025;12(2):1-5. doi: 10.5152/eurjrheum.2025.24052.
12. Farag CM, Antar R, Akosman S, Ng M, Whalen MJ. What is hemoglobin, albumin, lymphocyte, platelet (HALP) score? A comprehensive literature review of HALP's prognostic ability in different cancer types. Oncotarget. 2023;14:153-172. doi: 10.18632/oncotarget.28367.
13. Marín-Corte JA, Castillo-Sigales AA, Fragoso-Loyo H, Cimé-Aké E. HALP Score for Predicting Renal Relapse in Lupus Nephritis: A Nested Case-Control Study. J Clin Rheumatol. 2025;31(7):e158-e165. doi: 10.1097/RHU.0000000000002244.
14. Cüre O. Association between disease activity and HALP score in ankylosing spondylitis patients. Eur Res J. 2025;11(2):251-259. doi: 10.18621/eurj.1609765.
15. Fan M, Zhang L, Yin Q, Bao F, Zhou Y, Zhao E. Association between hemoglobin albumin lymphocyte and platelet (HALP) score and psoriasis: Evidence from NHANES. J Natl Med Assoc. 2025 Sep 4:S0027-9684(25)00299-8. doi: 10.1016/j.jnma.2025.08.099.
16. Schulze-Koops H. Lymphopenia and autoimmune diseases. Arthritis Res Ther. 2004;6(4):178-180. doi: 10.1186/ar1208.
17. Scherlinger M, Richez C, Tsokos GC, Boilard E, Blanco P. The role of platelets in immune-mediated inflammatory diseases. Nat Rev Immunol. 2023;23(8):495-510. doi: 10.1038/s41577-023-00834-4.
18. Chen XL, Xue L, Wang W, et al. Prognostic significance of the combination of preoperative hemoglobin, albumin, lymphocyte and platelet in patients with gastric carcinoma: a retrospective cohort study. Oncotarget. 2015;6(38):41370-41382. doi: 10.18632/oncotarget.5629.
19. Arslan AK, Yaşar Ş, Çolak C, Yoloğlu S. WSSPAS: an interactive web application for sample size and power analysis with R using Shiny. Turkiye Klin J Biostat. 2018;10(3):224-246. doi: 10.5336/biostatic.2018-62787.
20. Xin Y, Wang Y, Shu Y, Liang H, Yang Y. Hemoglobin, albumin, lymphocyte, and platelet (HALP) score predict prognosis in patients with atrial fibrillation and acute coronary syndrome or undergoing percutaneous coronary intervention. BMC Cardiovasc Disord. 2025;25(1):507. doi: 10.1186/s12872-025-04968-2.
21. Huang L, Li X, Zhou W, et al. The Clinical Value of the Neutrophil-to-Lymphocyte Ratio, the C-Reactive Protein-to-Albumin Ratio, the Systemic Inflammatory Index, and the Systemic Inflammatory Response Index in Patients with the Anti-Synthetase Syndrome. J Inflamm Res. 2024;17:3617-3628. doi: 10.2147/JIR.S460610.
22. Coşkun BN, Öksüz MF, Ermurat S, et al. Neutrophil lymphocyte ratio can be a valuable marker in defining disease activity in patients who have started anti-tumor necrosis factor (TNF) drugs for ankylosing spondylitis. Eur J Rheumatol. 2014;1(3):101-105. doi: 10.5152/eurjrheumatol.2014.034.
23. Germolec DR, Shipkowski KA, Frawley RP, Evans E. Markers of Inflammation. Methods Mol Biol. 2018;1803:57-79. doi: 10.1007/978-1-4939-8549-4_5.
24. Tian M, Li Y, Wang X, et al. The Hemoglobin, Albumin, Lymphocyte, and Platelet (HALP) Score Is Associated With Poor Outcome of Acute Ischemic Stroke. Front Neurol. 2021;11:610318. doi: 10.3389/fneur.2020.610318.
25. Xu SS, Li S, Xu HX, et al. Haemoglobin, albumin, lymphocyte and platelet predicts postoperative survival in pancreatic cancer. World J Gastroenterol. 2020;26(8):828-838. doi: 10.3748/wjg.v26.i8.828.
26. Veale JD, Gorman Á, Veale DJ, Fearon U, Orr C, Marzaioli V. Investigation of serum biomarkers in rheumatoid and psoriatic arthritis patients for disease-specific signatures. Arthritis Res Ther. 2025;27(1):147. doi: 10.1186/s13075-025-03608-6.
27. Kim DS, Shin D, Lee MS, et al. Assessments of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in Korean patients with psoriasis vulgaris and psoriatic arthritis. J Dermatol. 2016;43(3):305-310. doi: 10.1111/1346-8138.13061.
28. Asahina A, Kubo N, Umezawa Y, Honda H, Yanaba K, Nakagawa H. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio and mean platelet volume in Japanese patients with psoriasis and psoriatic arthritis: Response to therapy with biologics. J Dermatol. 2017;44(10):1112-1121. doi: 10.1111/1346-8138.13875.
29. Kılınç EA, Varkal G, Kırmızıer G, Türk İ, Özer HTE. Are monocyte-to-HDL and C-reactive protein-to-albumin ratios useful for the diagnosis and follow-up of Takayasu arteritis? Rev Assoc Med Bras (1992). 2024;70(5):e20231683. doi: 10.1590/1806-9282.20231683.

The European Research Journal 2026;12(1):76-85 Original Article

DOI: https://doi.org/10.18621/eurj.1792589 *Ophthalmology*

Evaluation of the Epidemiological and Clinical Features of Childhood Open Globe Injuries

Ülkü Demir[1]

[1]*Department of Ophthalmology, Faculty of Medicine, İnönü University, Malatya, Türkiye*

ABSTRACT

Objectives: To determine the epidemiological and clinical characteristics of open globe injuries causing permanent visual loss in children.

Methods: In our study, the files of 45 pediatric patients aged 1-18 years who were followed up in the ophthalmology clinic due to open globe injuries were retrospectively scanned. Patients' age, gender, traumatized eye, localization of the incision, initial and final visual acuity, place of the trauma, tools causing the trauma, pathologies accompanying the trauma, surgeries after the trauma, time between the trauma and repair, and follow-up period were recorded.

Results: Fifteen (33.3%) patients were female and 30 (66.6%) were male. The mean age was 10.4±4.4 years. The mean time between trauma and hospitalisation was 3.84±2.88 hours, and the mean follow-up period was 8.24±6.47 months. The right eye was traumatised in 25 (55.6%) patients and the left eye in 20 (44.4%) patients. Twenty (44.4%) patients had corneal, 18 (40%) corneascleral, and 7 (15.6%) scleral injuries. Trauma occurred at home in 31 (68.9%) patients. Penetrating injuries were detected in 39 (86.7%) patients. No rupture was observed in any patient. The most common instrument causing trauma was a knife in 14 (31.1%) patients.

Conclusions: Open globe injuries were mostly observed in boys. Corneal and penetrating injuries were observed, mainly in the right eye. It was noted that most of the injuries occurred at home and with sharp objects. Our findings highlight the importance of preventive education and parental awareness to reduce the risk of ocular trauma in children.

Keywords: Open Globe Injury, Pediatric, Penetrating Eye Injury, Ocular Trauma

Open globe injury is a full-thickness damage to the cornea or sclera. It is the most common cause of acquired unilateral blindness in children [1, 2]. Eye injuries are divided into two as: open globe injuries and closed globe injuries. Open globe injuries are classified as penetrating injuries, perforating injuries, ruptured globe, and intraocular foreign body injuries [1-3]. Visual outcomes in open globe injuries in children are worse than closed globe injuries. Studies reported that 27-48% of all open globe injuries are seen in children. The incidence of open globe injuries in pediatric patients is estimated to be 7.93 per 1,000,000 people [1, 2, 4, 5]. Although studies have been conducted on eye injuries in developed countries, information on the incidence of eye injuries in developing countries is insufficient.

Received: September 28, 2025 **Accepted:** November 8, 2025 **Available Online:** November 28, 2025 **Published:** January 4, 2026

How to cite this article: Demir Ü. Evaluation of the Epidemiological and Clinical Features of Childhood Open Globe Injuries. Eur Res J. 2026;12(1):76-85. doi: 10.18621/eurj.1792589

Corresponding author: Ülkü Demir, MD., Assist. Prof., Phone: +90 422 341 06 60, E-mail: ulkukohle@hotmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

Data on the outcomes of open globe injuries are also limited [5-7].

After ocular traumas, visual outcome is worse in children compared with adults because visual development is incomplete in children, the risk of amblyopia increases and due to poor communication and cooperation with children, diagnosis may be difficult [7, 8].

The diagnosis of ocular injuries is usually made only by history and clinical examination. If possible, the initial visual acuity of the patient is evaluated. Anterior segment examination is performed with a slit lamp. Infants and patients with poor communication and cooperation may need to be examined under general anaesthesia. If the diagnosis of open globe injury is definite, scleral depression, eye pressure measurement, and pupil dilatation, which will cause the intraocular contents to come out, should be avoided [9, 10]. Diagnostic imaging is performed especially in the presence of an intraocular foreign body. The main imaging modalities are B-scan ultrasonography and computed tomography. X-ray and magnetic resonance imaging are sometimes used. Computed tomography has been reported to be 90-100% sensitive for intraocular foreign bodies. While metal and glass appear hyperdense on computerised tomography, wood fragments give a hypodense image [10-12].

This study aims to determine the etiology, epidemiology and clinical characteristics of open globe injuries that cause permanent visual loss in children. The occurrence of open globe injuries can be reduced by investigating this issue in detail and informing the family and the community.

METHODS

In our study, the files of 45 pediatric patients aged 1-18 years who were followed up in the ophthalmology clinic for open globe injury between January 2018 and January 2023 were retrospectively reviewed. This study was conducted in accordance with the Declaration of Helsinki. Ethics committee approval (number: 5358, date: 26 December 2023) was obtained from the Instutional Review Board of İnönü University. Consent was obtained from the parents of the patients in a signed form.

A detailed history of trauma was obtained from the parents. Visual acuity was measured with Snellen's chart, and visual acuity was recorded with the logMAR scale in all children. Ocular motility, slit-lamp biomicroscopy, anterior segment examination, and fundus examination were performed. During the initial examination, any pressure on the eye was avoided as it might expel intraocular contents, and no intraocular pressure measurement was performed. Further investigations such as orbital and cranial computed tomography and magnetic resonance imaging were performed in selected patients when necessary.

The Ocular Trauma Score (OTS), proposed by Kuhn *et al*. [13] and colleagues, is a simple system incorporating several variables to predict the final visual outcome of an injured eye following ocular trauma. The OTS variables were (initial visual acuity, globe rupture, endophthalmitis, penetrating injury, retinal detachment, and afferent pupillary defect). Each of these variables is assigned a specific numerical value (raw number). The sum of the raw scores constitutes the OTS score. The OTS score ranges from 1 (more serious injury and poorer prognosis) to 5 (less serious injury and better prognosis). Acar *et al*. [14] reported the prognostic value of a new OTS in pediatric penetrating injuries. The Pediatric Ocular Trauma Score (POTS) variables (initial visual acuity, age, wound localisation, iris prolapse, hyphaema, organic/unclean injury, delay of surgery (>48h), traumatic cataract, vitreous haemorrhage, retinal detachment, endophthalmitis). As afferent pupillary defects could not be assessed in children, they were not included in the scoring. Patient variables such as age and wound location were evaluated as important parameters and included in the scoring. Patients were divided into five groups based on their trauma assessment score, with higher scores indicating a better prognosis [14].

The variables specified in this study have been determined by taking into account OTS and POTS variables. Afferent pupillary defect examination was not included in this study because it is very difficult in children. Age, gender, traumatised eye, localisation of the incision, initial and final visual acuity, place of trauma, vehicles causing trauma, pathologies accompanying trauma, post-traumatic operations, time between trauma and repair, and follow-up period were recorded.

The localization of the trauma was divided into zones as corneal, corneascleral, and scleral injury according to the standardised international classification of ocular trauma [2, 3, 15]. The site of injury was classified as home, outdoor, and school. Patients with open globe injury and under the age of 18 were included in the present study. Patients without open globe injury, over the age of 18, and with missing data were excluded from the study. Initial and final visual acuities were categorized as ≤0.05, 0.05-0.5, ≥ 0.5 [13-15]. Initial and final visual acuity levels of the patients whose visual acuity could not be analysed were not evaluated statistically.

Primary repair was performed under general anaesthesia in all patients participating in this study. Necrotic and infected tissues at the wound site were excised. If there was vitreous prolapse at the wound site, a vitrectomy was performed. Lensectomy was performed in eyes with capsular rupture. Enucleation was not performed in any patient. After surgery, broad-spectrum intravenous antibiotics were administered for seven days, topical antibiotics every hour and cyclopentolate twice a day. After one week, systemic antibiotics and cyclopentolate were discontinued. The topical antibiotic was decreased and continued for a month. Postoperative ocular ultrasound was performed in all patients.

Statistical Analysis

Statistical analysis of the data was performed with SPSS 26.0 programme (SPSS Inc., Chicago, IL). Power analysis was performed with G-Power v.3.1.9.7 software (Franz Faul, Universität Kiel, Germany). For this study, an alpha error of 5% was accepted, and P<0.05 was considered statistically significant. A power analysis was performed before the study. Due to similar studies in the literature, the statistical power (1-β) was accepted as 80% and the total sample size was determined as 45 pediatric patients. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and as number of cases and (%) for categorical variables. Frequency analyses, chi-square test, and Pearson correlation analyses were used in this study. Binary logistic regression analyses were used to evaluate the relationships between variables. Results were considered statistically significant for P<0.05.

RESULTS

In our study, 45 eyes of 45 pediatric patients who were followed up for open globe injury in our clinic were evaluated retrospectively. Fifteen (33.3%) patients were girls, and 30 (66.6%) were boys. The number of male patients was twice the number of female patients. The mean age was 10.4±4.4 years (1-18 years). Children were divided into three groups according to their ages. Six (13.3%) were 0-6 years old, 24 (53.3%) were 7-12 years old, and 15 (33.3%) were 13-18 years old (Table 1). There was no statistically significant difference between the ages and genders of the patients (P=0.407). The mean time between trauma and hospitalisation was 3.84±2.88 hours, and the mean follow-up period was 8.24±6.47 months. A statistically significant negative correlation was found between the time to hospitalisation and the duration of the follow-up period (P=0.014).

The right eye was traumatised in 25 (55.6%) patients and the left eye in 20 (44.4%) patients. No patient had involvement of both eyes. Corneal injury was detected in 20 (44.4%), corneascleral injury in 18 (40%), and scleral injury in 7 (15.6%) patients. Thirty-one (68.9%) patients were exposed to trauma at home,

TABLE 1. Distribution of Patients According to Age and Gender

	Female		Male		
Age (years)	**n**	**(%)**	**n**	**(%)**	**P-value***
0-6	3	10.0	3	20.0	0.407
7-12	18	60.0	6	40.0	
13-18	9	30.0	6	40.0	
Total	30	100.0	15	100.0	

*Chi-square analysis

TABLE 2. Characteristics of Patients According to Gender and age Groups

Characteristics	0-6 years		7-12 years		13-18 years		
	M	F	M	F	M	F	P-value*
Traumatized eye							
Right eye	2	2	11	3	4	3	0.977
Left eye	1	1	7	3	5	3	
Localization of trauma							
Corneal	3	2	8	2	3	2	0.088
Corneascleral			9	4	3	2	
Scleral		1	1		3	2	
Place of trauma							
Home	3	3	12	4	4	5	0.712
Outdoor			5	1	5		
School			1	1	1		
Type of trauma							
Penetrating	2	3	16	6	6	6	0.261
Perforating	1		2	-	1	-	
Foreign body	-				2		
Rupture	-						
Total	6		24		15		

Data are shown as number (frequency). M, Male; F, Female.
*Chi-square analysis

11 (24.4%) patients were exposed to trauma outdoors and 3 (6.7%) patients were exposed to trauma at school. Most of the injuries occurred at home Penetrating injuries were detected in 39 (86.7%) patients, perforating injuries in 4 (8.9%) patients, and intraocular foreign bodies in 2 (4.4%) patients. No rupture was observed in any patient. No statistically significant correlation was found between the eye in which the trauma occurred, the localisation of the trauma, the place where the trauma occurred, the type of trauma and the age and gender of the patients (Table 2). Trauma was most commonly seen in boys aged 7-12 years and in the cornea.

The most common tools causing trauma in this study were a knife in 14 (31.1%) patients, glass in 7 (15.5%) patients, and stone in 3 (6.6%) patients. The other tools causing trauma were 2 (4.4%) scissors, 2 (4.4%) pieces of wood, 2 (4.4%) toys, 2 (4.4%) pens, 1 (2.2%) spectacle lenses, 2 (4.4%) gun bullets, 2 (4.4%) fork, 2 (4.4%) tree branches, 2 (4.4%) dart darts, 1 (2.2%) wooden kite stick, 1 (2.2%) wire, 1 (2.2%) nail, and 1 (2.2%) thorn.

Initial and final best visual acuity could not be determined in three female patients aged 0-6 years because they were too young and could not cooperate during the examination. Initial visual acuity was ≤ 0.05 in 27 (64.3%) patients, between 0.05-0.5 in 11 (26.2%) patients and ≥ 0.5 in 4 (9.5%) patients. The best final visual acuity was ≤ 0.05 in 11 (26.2%) patients, between 0.05-0.5 in 13 (30.9%) patients, and ≥ 0.5 in 18 (42.9%) patients. There was a statistically significant difference between initial visual acuity and final corrected best visual acuity according to the patients' age and gender (P<0.001) (Table 3).

The findings revealed that the most common ocular pathologies were iris prolapse in 19 (42.22%) patients, traumatic cataract in 12 (26.67%) patients, and hyphaema in 5 (11.11%) patients. There was no statistically significant correlation between ocular pathologies accompanying globe injuries and the age

TABLE 3. Visual Acuity According to Age Groups and Gender

		Age (years)			
		0-6	7-12	13-18	P-value*
Gender	**Initial visual acuity**				
Female	≤0.05	-	5	4	**<0.001**
	0.05-0.5	-	-	1	
	≥0.5	-	1	1	
Male	≤0.05	2	13	3	
	0.05-0.5	-	4	6	
	≥0.5	1	1	-	
Gender	**Final best corrected visual acuity**				
Female	≤0.05	-	3	1	**<0.001**
	0.05-0.5	-	2	2	
	≥0.5	-	1	3	
Male	≤0.05	-	6	1	
	0.05-0.5	1	4	4	
	≥0.5	2	8	4	

Data are shown as number (frequency). (-) patient whose visual acuity cannot be analyzed.
*Chi-square analysis. Statistically significant P-values are shown in bold.

and gender of the patients (P=0.475) (Table 4).

Outcome visual acuity was categorised as ≤0.05 and >0.05. Both univariate and binary logistic regression analyses were performed with outcome visual acuity ≤0.05 and >0.05 as the dependent variable. There was a statistically significant difference in age between the two visual acuity groups (Table 5).

TABLE 4. Ocular Pathologies Associated with Open Eye İnjuries According to Gender and Age Groups

Factors	0-6 years		7-12 years		13-18 years		P-value*
	M	F	M	F	M	F	
Aphakia		1	1		1		0.475
Endophthalmitis					1		
Hyphema			3	1	1		
Iris prolapse	1		9	3	3	3	
Choroidal detachment		1					
Optik nerve damage			1				
Retinal detachment	1			1			
Retinal haemorrhage			1			1	
Traumatic cataract		1	4	2	4	1	
Vitreous haemorrhage	1		1			1	

Data are shown as number (frequency).
*Chi- square analysis.

TABLE 5. Variables Affecting Visual Prognosis

Factors	Final best visual acuity ≤0.05%	Final best visual acuity >0.05%	P-value	OR (regression analysis)
Gender				
Male	7 (23.3)	23 (76.6)	0.085	0.251
Female	4 (33.3)	8 (66.6)		
Age				
0-6		3 (100)	**0.049**	3.958
7-12	9 (37.5)	15 (62.5)		
13-18	2 (13.3)	13 (86.7)		
Traumatized eye				
Right eye	5 (22.7)	17 (77.3)	0.409	1.989
Left eye	6 (30)	14 (70)		
Localization of trauma				
Corneal	2 (11.1)	16 (88.9)	0.458	0.715
Corneascleral	8 (44.4)	10 (55.6)		
Scleral	1 (16.7)	5 (83.3)		
Place of trauma				
Home	8 (28.6)	20 (71.4)	0.284	0.500
Outdoor	3 (27.3)	8 (72.7)		
School		3 (100)		
Type of trauma				
Perforating	1 (25)	3 (75)	0.379	0.296
Penetrating	8 (22.2)	28 (77.8)		
Foreign body	1 (50)	1 (50)		
Rupture				
Iris prolapse	2 (10.5)	17(89.5)	0.498	0.915
Hyphema	2 (40)	3 (60)		
Vitreous haemorrhage	1 (33.3)	2 (66.7)		
Retinal haemorrhage	1 (50)	1 (50)		
Choroidal detachment	1 (100)			
Retinal detachment	2 (100)			
Traumatic cataract	4 (33.3)	8 (66.7)		
Aphakia		3 (100)		
Optic nerve damage	1 (100)			
Endophthalmitis	1 (100)			
Initial vision ≤0.05	27 (64.3)	15 (35.7)		
Presence of intraocular foreign body	1 (50)	1 (50)		

Data are shown as number (%).
Statistically significant P-value is shown in bold.

Primary repair was performed in all patients. 8-0 vicryl suture was used for scleral repair, and 10-0 nylon suture was used for corneal repair. After the first operation, 15 (44.11%) patients had lensectomy, 12 (35.29%) patients had intraocular lens (IOL) implantation, 5 (14.70%) patients had pars plana vitrectomy, and 2 (5.88%) patients had pupilloplasty.

The best final visual examination revealed corneal scarring in 5 (11.11%) patients, aphakia in 3 (6.66%) patients, optic atrophy in 1 (2.22%) patient, retinal scarring in 1 (2.22%) patient, and retinal detachment in 2 (4.44%) patients.

DISCUSSION

In our study, trauma was most commonly seen in boys aged 7-12 years and in the cornea. Penetrating injury was the most common and most injuries occurred at home with sharp objects. There was no significant difference in the frequency of right and left eye involvement, bilateral eye involvement was 0%. Initial visual acuity was ≤ 0.05 in 64.3% of patients, while final best visual acuity was ≥ 0.5 in 42.9% of patients.

It has been reported that most of the open globe injuries (53.1%) occur during play because children spend more of their time playing and are exposed to potentially dangerous substances in their environment and are vulnerable during play. Socioeconomic and sociocultural conditions are important factors in eye injuries occurring during play. The incidence rate is higher among families who are poor, less educated, and live in rural areas. It is observed twice more in boys than in girls. The higher prevalence in boys is attributed to boys being more aggressive than girls [8,16]. In our study, similarly, open globe injuries were observed twice more in boys than in girls.

Eye injuries have been reported to occur with sharp objects such as scissors, glass, nails, knives and keys. Young children, whose motor skills are not yet fully developed, are prone to bumping into hard objects and falling while walking or running. In some cases, it is not known how the child was injured because the child could not express himself/herself [1, 8, 11, 15, 17]. In our study, it was recorded that open globe injuries occurred mostly with knife (31.1%), scissors (15.5%) and stone (6.6%).

The most common type of open globe injury in children worldwide is penetrating injury (48.4%-83%). The most common injury localisation is corneal incision, followed by corneascleral incision [1, 8, 11, 15, 17-20]. Similar to other studies, the most common type of injury in our study was penetrating injury (86.7%). Corneal incision (44.4%) and corneascleral incision (40%) were the most common injury localisation.

In their study, Guo *et al*. [15] reported that the rate of open globe injuries was higher in children aged 0-6 years for both sexes than in children in other age groups and that injuries were more common in boys than in girls and in the right eye than in the left eye. In the same study, it was reported that injuries occurred due to glass, scissors and fireworks in pre-school children and most injuries occurred with pencils in primary school age. Since children are protected from the working environment, unlike adults, perforation with an intraocular foreign body is less common. In their study, the rate of perforation with intraocular foreign body in children was 6.4% [15].

Tok *et al*. [21] reported that 54 of 82 patients were boys, and 37.8% were between the ages of 3-6 years. They reported that the most common tool causing trauma was pointed metallic objects, and injuries occurred most frequently in the street [21]. Gupta *et al*. [22] reported that open globe injuries were observed in (85%) of males and (15%) of females, and the mean age of traumatised patients was 9.8 years. In our study, the mean age of the traumatised patients was 10.4±4.4 years.

The findings accompanying open globe injuries in children include hyphema, vitreous haemorrhage, uveal prolapse, cataract, lens subluxation, iridodialysis, retinal detachment and endophthalmitis [11, 23]. In studies, it has been reported that poor visual prognosis is related to poor initial visual acuity, presence of an afferent pupillary defect, posterior location and long wound, scleral injury, lens involvement, retinal detachment, vitreous and uveal prolapse, hyphema and endophthalmitis [9, 11, 15, 21, 24]. The most common findings in our study were iris prolapse (42.22%), traumatic cataract (26.67%) and hyphaema (11.11%). There was no statistically significant relationship between the findings recorded in the study and final visual acuity. Since retinal detachment, endophthalmitis, optic nerve damage and choroidal detachment are rarely seen, it is thought that there is no significant relationship with visual acuity.

Traumatic cataract is the most common

complication after open globe injuries. Other complications observed are endophthalmitis, retinal detachment, vitreous haemorrhage, sympathetic ophthalmia, chronic intraocular foreign body toxicity, amblyopia, corneal scar and astigmatism [8, 11, 15]. In our study, cataract (26.67%) and corneal scar (11.11%) were observed most frequently.

Azimi *et al*. [2] reported that 33.6% of the cases were operated twice, and 12.7% were operated three times in their study. They reported that lensectomy and deep vitrectomy were performed most frequently after the first operation [2]. In open globe injuries, vitrectomy provides a reconstruction of the posterior segment, clears vitreous opacity and prevents phakisis. A vitrectomy should be performed within the first 14 days after ocular trauma [23]. In our study, lensectomy (44.11%), intraocular lens implantation (35.29%), pars plana vitrectomy (14.70%) and pupilloplasty (5.88%) were performed after the first operation.

The risk of endophthalmitis after open globe injuries in children is 4.9-54.2%. The risk of endophthalmitis increases in the presence of an intraocular foreign body, injury in a rural area, contact with organic material in the wound and if more than 24 hours have passed since the injury. The agents of endophthalmitis after trauma are frequently streptococcus, staphylococcus epidermis, bacillus sp., fungal infection may occur in injuries caused by tree branches [2, 11]. Sul *et al*. [9] found endophthalmitis in 22 patients (12.1%) in their study. In 16 of 22 patients, primary surgical intervention was performed after 24 hours. This shows the importance of primary surgical intervention in less than 24 hours in terms of endophthalmitis development. Final visual acuity was also worse. Retinal detachment was observed in 11 patients who developed endophthalmitis [9]. Narang *et al*. [25] reported that endophthalmitis developed in 39 (54.16%) of 72 patients after open globe injuries in children. They reported that delayed repair for more than 24 hours, injury with arrow and domestic injuries were associated with a high risk of endophthalmitis, while the size and location of the wound, injury of the lens, iris, vitreous prolapse and presence of intraocular foreign body were not associated with endophthalmitis [25]. In another study by Ghadeer *et al*. [26], it was reported that endophthalmitis developed in 23 eyes. Zheng *et al*. [27] reported that most of the open globe injuries were observed in pre-school children and boys, frequently occurred with sharp objects, such as knives, scissors and broken glasses, and the risk of endophthalmitis increased in primary repairs performed over 24 hours. In our study, endophthalmitis occurred in one patient who was injured in the street by a gunshot.

Gursoy *et al*. found that pars plana vitrectomy, iris, ciliary body and choroidal damage, posttraumatic aphakia were associated with poor prognosis. It was reported that traumatic cataract was not associated with poor prognosis in children with appropriate intervention. Incisions of the prolapsed vitreous and penetrating injuries were associated with better visual prognosis compared to other injuries [28]. Wen *et al*. [29] found that severe intraocular haemorrhage, retinal detachment and choroidal damage occurred in pediatric patients with no light sensation in the first vision.

In our study, there was no statistically significant correlation between pathologies accompanying open globe injury, initial visual acuity ≤0.05, presence of the intraocular foreign body, type of trauma, place of trauma, traumatised eye and localisation of trauma in the eye and final visual acuity (P>0.05). There was a statistically significant difference between initial visual acuity and final corrected best visual acuity according to the patients' age and gender (P<0.001). There was no difference between the pathologies accompanying trauma (P=0.475), traumatised eye (P=0.977), place of trauma (P=0.712), localisation of trauma (P=0.088), type of trauma (P=0.261), age and gender.

Jandeck *et al*. [4] reported that the average follow-up period for perforating globe injuries was 15.5 months. Tabatabaei *et al*. [20] reported that in 66.6% of patients, the time between the occurrence of eye trauma and hospital admission was less than 12 hours. In our study, the mean time between trauma and hospitalisation was 3.84±2.88 hours, and the mean follow-up period was 8.24± 6.47 months. A statistically significant negative correlation was found between the time to hospitalisation and the duration of follow-up period. As there is a high risk of blindness in perforating eye injuries, follow-up periods may be lengthy even if patients arrive at the hospital early.

Strengths and Limitations

Since this study was retrospective, it has limitations. The single-centre nature of the study. The

small number of patients, the fact that patient information is limited to data recorded during the patient's hospital stay, the absence of standardised long-term visual follow-up, and the data obtained during follow-up after the patient's discharge limit this study. This study supports the findings of previous studies. Understanding the epidemiological and clinical characteristics of open globe injuries in children can raise awareness among families and the community, and these injuries, which can lead to amblyopia and blindness, can be prevented by taking simple precautions.

CONCLUSION

As a result, open globe injuries cause more surgical procedures and complications, patients are hospitalised longer and the visual prognosis is worse compared to closed globe injuries. It is important to ensure that children stay away from sharp-edged, pointed, explosive objects. Our findings highlight the importance of preventive education and parental awareness to reduce the risk of ocular trauma in children. To determine the prognostic factors and treatment of the disease, multicentre, prospective studies involving a larger number of cases are required.

Ethics Approval and Consent to Participate

This study was approved by the İnönü University Health Sciences Non-Interventional Clinical Research Ethics Committee (Decision No: 2023/5358-19; date: 26.12.2023). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the parents of the patients included in the study.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: ÜD; Study Design: ÜD; Supervision: ÜD; Funding: ÜD; Materials: ÜD; Data Collection and/or Processing: ÜD; Statistical Analysis and/or Data Interpretation: ÜD; Literature Review: ÜD; Manuscript Preparation: ÜD; and Critical Review: ÜD.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Ava S, Erdem S, Karahan M, Dursun ME, Hazar L, Keklikçi U. Epidemiology and demography of open globe injury in children. Dicle Med J. 2021;48(3):416-424. doi: 10.5798/dicletip.987880.

2. Azimi A, Abdollahi F, Sadeghi E, Reza Farsiani A, Moshksar S, Nadi M. Epidemiological and clinical features of pediatric open globe injuries: a report from Southern Iran. J Ophthalmic Vis Res. 2023;18(1):88-96. doi: 10.18502/jovr.v18i1.12729.

3. Schörkhuber MM, Wackernagel W, Riedl R, Schneider MR, Wedrich A. Ocular trauma scores in paediatric open globe injuries. Br J Ophthalmol. 2014;98(5):664-668. doi: 10.1136/bjophthalmol-2013-304469.

4. Jandeck C, Kellner U, Bornfeld N, Foerster MH. Open globe

injuries in children. Graefes Arch Clin Exp Ophthalmol. 2000;238(5):420-426. doi: 10.1007/s004170050373.
5. Wong JCY, Yonekawa Y. Pediatric open-globe injury: Current perspectives, surgical principles, and literature review. Taiwan J Ophthalmol. 2024;15(2):225-235. doi: 10.4103/tjo.TJO-D-24-00061.
6. Kaçer EÖ, Kaçer İ. Ocular trauma in the pediatric age group: a systematic review. Egypt Pediatr Assoc Gaz. 2022;70:28. doi: 10.1186/s43054-022-00125-y.
7. Jacobson A, Liles N, Besirli CG, Bohnsack BL. Predictors of visual outcomes in pediatric open globe injuries. J AAPOS. 2020;24(6):360.e1-360.e8. doi: 10.1016/j.jaapos.2020.08.010.
8. Abdelazeem K, Al-Hussaini AK, El-Sebaity DM, Kedwany SM. Epidemiology, etiologies, and complications of playtime open globe injuries in children. J Pediatr Ophthalmol Strabismus. 2021;58(6):385-389. doi: 10.3928/01913913-20210426-01.
9. Sul S, Gurelik G, Korkmaz S, Ozdek S, Hasanreisoglu B. Pediatric open-globe injuries: clinical characteristics and factors associated with poor visual and anatomical success. Graefes Arch Clin Exp Ophthalmol. 2016;254(7):1405-10. doi: 10.1007/s00417-015-3087-y.
10. Zhou Y, DiSclafani M, Jeang L, Shah AA. Open Globe Injuries: Review of evaluation, management, and surgical pearls. Clin Ophthalmol. 2022;16:2545-2559. doi: 10.2147/OPTH.S372011.
11. Li X, Zarbin MA, Bhagat N. Pediatric open globe injury: A review of the literature. J Emerg Trauma Shock. 2015;8(4):216-223. doi: 10.4103/0974-2700.166663.
12. Sung EK, Nadgir RN, Fujita A, et al. Injuries of the globe: what can the radiologist offer? Radiographics. 2014;34(3):764-776. doi: 10.1148/rg.343135120.
13. Kuhn F, Maisiak R, Mann L, Mester V, Morris R, Witherspoon CD. The ocular trauma score (OTS). Ophthalmol Clin North Am. 2002;15(2):163-165, vi. doi: 10.1016/s0896-1549(02)00007-x.
14. Acar U, Tok OY, Acar DE, Burcu A, Ornek F. A new ocular trauma score in pediatric penetrating eye injuries. Eye (Lond). 2011;25(3):370-374. doi: 10.1038/eye.2010.211.
15. Guo Y, Guo Y, Gu R, et al. Characteristics and visual prognosis of pediatric open globe injury in Shanghai, China. Ophthalmic Res. 2022;65(3):328-334. doi: 10.1159/000523814.
16. Grieshaber MC, Stegmann R. Penetrating eye injuries in South African children: aetiology and visual outcome. Eye (Lond). 2006;20(7):789-795. doi: 10.1038/sj.eye.6702003.
17. Batur M, Seven E, Akaltun MN, Tekin S, Yasar T. Epidemiology of open globe injury in children. J Craniofac Surg. 2017;28(8):1976-1981. doi: 10.1097/SCS.0000000000004033.
18. Aksu-Ceylan N, Özbilen KT, Karakiraz A. Characteristics of pediatric open globe injuries in preschool-aged and school-aged children. Can J Ophthalmol. 2023;58(6):559-564. doi: 10.1016/j.jcjo.2022.10.013.
19. Gunes A, Kalayc M, Genc O, Ozerturk Y. Characteristics of open globe injuries in preschool children. Pediatr Emerg Care. 2015;31(10):701-703. doi: 10.1097/PEC.0000000000000433.
20. Tabatabaei SA, Khameneh EA, Soleimani M, Baramaki A. Open globe injuries in children under 7 years referred to a tertiary center in Iran from 2006 to 2016. Eye (Lond). 2021;35(4):1235-1239. doi: 10.1038/s41433-020-1023-9.
21. Tok O, Tok L, Ozkaya D, Eraslan E, Ornek F, Bardak Y. Epidemiological characteristics and visual outcome after open globe injuries in children. J AAPOS. 2011;15(6):556-561. doi: 10.1016/j.jaapos.2011.06.012.
22. Gupta A, Rahman I, Leatherbarrow B. Open globe injuries in children: factors predictive of a poor final visual acuity. Eye (Lond). 2009;23(3):621-625. doi: 10.1038/eye.2008.32.
23. Lee CH, Lee L, Kao LY, Lin KK, Yang ML. Prognostic indicators of open globe injuries in children. Am J Emerg Med. 2009;27(5):530-535. doi: 10.1016/j.ajem.2008.04.004.
24. Globocnik Petrovic M, Lumi X, Drnovsek Olup B. Prognostic factors in open eye injury managed with vitrectomy: retrospective study. Croat Med J. 2004;45(3):299-303.
25. Narang S, Gupta V, Simalandhi P, Gupta A, Raj S, Dogra MR. Paediatric open globe injuries. Visual outcome and risk factors for endophthalmitis. Indian J Ophthalmol. 2004;52(1):29-34.
26. Al Ghadeer H, Khandekar R. Characteristics, etiological factors, and visual outcomes of pediatric open globe injuries in central Saudi Arabia: a 22-year retrospective study. Clin Ophthalmol. 2023;17:2909-2917. doi: 10.2147/OPTH.S430394.
27. Zheng L, Tan J, Liu R, et al. The impact of primary treatment on post-traumatic endophthalmitis in children with open globe injuries: A Study in China. Int J Environ Res Public Health. 2019;16(16):2956. doi: 10.3390/ijerph16162956.
28. Gursoy H, Bilgec MD, Sahin A, Colak E. A possible regression equation for predicting visual outcomes after surgical repair of open globe injuries. J Ophthalmol. 2017;2017:1320457. doi: 10.1155/2017/1320457.
29. Wen X, Yuan M, Li C, et al. Risk factors and prognosis of pediatric open globe injuries without initial light perception. Ophthalmologica. 2021;244(2):165-172. doi: 10.1159/000513337.

The European Research Journal 2026;12(1):86-96 Original Article

DOI: https://doi.org/10.18621/eurj.1764519 *Clinical Oncology*

Can Immature Granulocytes Guide Chemotherapy Decisions in Later Lines of Therapy for HER2-Negative Breast Cancer?

Mustafa Ersoy[1]

[1]*Department of Internal Medicine, Faculty of Medicine, Kütahya Health Sciences University, Kütahya, Türkiye*

ABSTRACT

Objectives: In human epidermal growth factor receptor 2 (HER2)-negative breast cancer, the need for chemotherapy often continues from the 3rd line onwards. However, these patients may experience side effects without the desired benefit due to prolonged treatments and low response rates. Our study examined immature granulocytes, routinely checked in hemogram results, for their ability to predict treatment response and toxicity.

Methods: Data from patients who received third-line or later chemotherapy for HER2-negative breast cancer between 2015 and 2024 were analyzed to determine if immature granulocytes could predict treatment response, progression-free survival, or toxicity.

Results: A total of 41 chemotherapy administrations were analyzed, and significant differences were observed in initial immature granulocyte counts (IG#) (P=0.046) and percentages (IG%) (P=0.006) across disease response categories. A statistically significant inverse correlation was identified between progression-free survival and both IG# (r= -0.332, P=0.034) and IG% (r= -0.323, P=0.039), indicating that elevated immature granulocyte levels were associated with shorter progression-free survival. However, regression analysis did not demonstrate a statistically significant predictive effect of IG# or IG% on progression duration (R^2= 0.103, F= 2.47, P=0.098). Grade 3-4 toxicity occurred in 18 of 41 patients, but no statistically significant relationship was observed with IG#, and IG%. P=0.12 for IG# and P=0.24 for IG%).

Conclusions: Pre-treatment immature granulocytes may indicate prognosis and chemotherapy resistance in breast cancer patients. They could potentially help avoid unnecessary chemotherapy, guiding patients towards targeted therapies or best supportive care.

Keywords: Breast Cancer, Immature Granulocytes, Progression-Free Survival, Predictive Biomarker, Overtreatment, Human Epidermal Growth Factor Receptor 2 (HER2)

Breast cancer is a globally prevalent malignancy, presenting a significant clinical challenge in establishing effective and personalized treatment strategies [1]. This complexity is particularly pronounced in advanced-stage, the human epidermal growth factor receptor 2 (HER2)-negative subtypes, where the inherent heterogeneity of the disease and inter-individual variations in tumor biology contribute to unpredictable treatment responses and the development of resistance [2]. Chemotherapy remains

Received: August 14, 2025 **Accepted:** September 16, 2025 **Available Online:** September 27, 2025 **Published:** January 4, 2026

How to cite this article: Ersoy M. Can Immature Granulocytes Guide Chemotherapy Decisions in Later Lines of Therapy for HER2-Negative Breast Cancer? Eur Res J. 2026;12(1):86-96. doi: 10.18621/eurj.1764519

Corresponding author: Mustafa Ersoy, MD., Assist. Prof., Phone: +90 274 260 00 43, E-mail: mustafa.ersoy@ksbu.edu.tr

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

a frequently utilized therapeutic option in clinical practice for patients with HER2-negative breast cancer, even for those with an otherwise favorable prognosis [3]. The persistent reliance on chemotherapy, even in later lines of therapy, is notable given its often diminishing efficacy and the potential for cumulative toxicities [4]. This underscores the critical need for improved predictive biomarkers to optimize patient selection and prevent overtreatment [5].

In this context, there is a continued need for reliable biomarkers that can predict treatment response, particularly as patients progress to later lines of therapy where the balance between efficacy and toxicity becomes increasingly critical. In recent years, immature granulocytes (IGs), defined as early myeloid precursors including promyelocytes, myelocytes, and metamyelocytes released into the peripheral blood during enhanced bone marrow activity, have garnered significant attention as readily accessible indicators of systemic inflammatory response and immune system activation across various malignancies [6]. Their role as potential prognostic or predictive biomarkers has been extensively investigated, offering insights into disease progression, treatment efficacy, and even the likelihood of adverse events [7]. Specifically, in the complex landscape of HER2-negative breast cancer, where treatment individualization is paramount, understanding the predictive capacity of IGs could refine patient stratification and guide critical decisions regarding the continuation or modification of chemotherapy regimens, ultimately aiming to optimize outcomes while mitigating unnecessary toxicity and overtreatment [8].

This study endeavors to evaluate the specific role of IGs, determined before the initiation of third-line and subsequent chemotherapy regimens, in predicting treatment response among patients diagnosed with HER2-negative metastatic breast cancer. This investigation is particularly relevant given the critical need for effective biomarkers in later lines of therapy, where treatment decisions become increasingly complex. The anticipated findings are expected to shed significant light on the clinical utility of IGs as a potential predictive tool. This tool could serve to guide more personalized treatment decisions, ultimately helping to prevent unnecessary overtreatment and thereby improving patient outcomes by optimizing therapeutic strategies.

METHODS

Study Design and Participants

This retrospective study involved the analysis of patient medical records from Kütahya Health Sciences Evliya Çelebi Training and Research Hospital and Kütahya City Hospital, covering the period from 2015 to 2024. Eligible patients were identified, and a total of 41 distinct treatment protocols were documented for individuals who had received third-line or subsequent lines of therapy. Patients who had undergone multiple lines of treatment were not excluded from this analysis. Therapies administered during the metastatic phase were specifically documented, whereas neoadjuvant or adjuvant treatments were not categorized as separate lines of therapy. Similarly, non-chemotherapeutic interventions, such as hormonal or targeted therapies, were not counted as distinct lines of treatment. Patients diagnosed with HER2-positive disease or those who discontinued treatment for reasons unrelated to disease progression were excluded from the study.

Measurement of Immature Granulocytes

Immature granulocyte count (IG#) and immature granulocyte percentage (IG%) were routinely quantified as part of the comprehensive blood count panel. These parameters were analyzed utilizing a Mindray BC-6000 analyzer, employing a focusing flow-direct current detection methodology. This technique facilitates the three-dimensional characterization of target cells through their interaction with specific reagents, thereby generating dual-angle laser scatter signals and fluorescence, a process designated as the 'SF Cube'. The resultant three-dimensional scatter diagrams, derived from data encompassing cellular size, intracellular complexity, and nucleic acid (DNA/RNA) content, enable clinical experts to accurately identify and differentiate various cell populations.

Chemotherapy Regimens

Patients who had received a minimum of two distinct lines of chemotherapy, administered either as monotherapy or in combination regimens, during the metastatic phase and subsequently demonstrated disease progression were included. Additionally, patient inclusion criteria encompassed those who had discontinued anthracycline-based treatment owing to

cumulative dosage, even in the absence of disease progression. The retrospective analysis focused on patients who had adhered to their prescribed treatment protocols by receiving the full planned doses and had undergone subsequent response evaluations. Eligibility further required patients to have completed their treatment and to possess documented progression-free survival (PFS) data derived from the outcomes of their response evaluations. The specific chemotherapy regimens subjected to analysis in this study included Gemcitabine, Taxane, Platinum combinations, Anthracycline-cyclophosphamide, Liposomal doxorubicin, Vinorelbine, Ixabepilone, Eribulin, and Capecitabine.

Response Evaluation

The treatment responses of all patients were meticulously evaluated at the study centers. Patients' treatment responses were systematically classified as complete response, partial response, stable disease, and progressive disease based on the initial imaging evaluations performed 8 to 16 weeks after the start of treatment, according to the Response Evaluation Criteria in Solid Tumors guidelines. The overall response rate was calculated by combining the rates of complete and partial responses. These detailed response assessments were critical for subsequent analyses, including the determination of pro PFS data.

Toxicity Analysis

The toxicities documented in patients' medical records underwent thorough evaluation. Exclusion criteria included patients who discontinued treatment because of toxicity. Toxicities were classified using the Common Terminology Criteria for Adverse Events criteria, and both the incidence of grade 3 or higher toxicities and the specific types of adverse events were assessed.

Ethical Considerations

The study received approval from the Non-Interventional Research Ethics Committee of Bezmiâlem Vakıf University (Decision No.: 2025/31-03; date: 05.02.2025), with all methodologies adhering to the principles outlined in the Helsinki Declaration. Since this was a retrospective study, informed consent was not obtained from the participants.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were utilized to summarize patient demographics, treatment characteristics, and laboratory values. Categorical variables were presented as frequencies and percentages, whereas continuous variables were reported as either mean ±standard deviation (SD) for normally distributed data or as median (interquartile range, IQR) for non-normally distributed variables. To evaluate the association between baseline IG# and IG% with disease response categories (progressive disease, stable disease, and partial response), the Kruskal–Wallis test, a non-parametric method for comparing more than two independent groups, was employed due to the non-normal distribution of the data. The relationship between PFS and both IG# and IG% was assessed using Pearson's correlation coefficient, allowing the evaluation of linear relationships between continuous variables. Additionally, a multiple linear regression analysis was conducted to determine whether IG# and IG% independently predicted PFS, adjusting for potential collinearity and assessing the overall contribution of each predictor to the model. The coefficient of determination (R^2) was calculated to estimate the proportion of variance in PFS explained by the model. Additionally, the Mann–Whitney U test was utilized to compare immature granulocyte metrics (IG# and IG%) between patients with and without Grade 3–4 adverse events reflecting non-parametric analysis due to the non-normal distribution of these variables. All statistical tests were two-tailed, and a P-value of less than 0.05 was considered to indicate statistical significance. The results were interpreted in the context of clinical relevance and existing literature.

RESULTS

Patient Characteristics and Treatment Regimens

Table 1 summarizes the treatment regimens and patient demographics. The study included a total of 41 chemotherapy administrations for 25 patients. The patients had a mean age of 54.2 years, and only 1 patient was male, with the rest being female. The treatment agents used included gemcitabine (34.1%),

TABLE 1. Treatment Regimens and Patient Demographics

Variables	n	Percentage
Treatment		
Gemcitabine	14	34.1%
Taxane	1	2.4%
Platinum combination	4	9.8%
Anthracycline-cyclophosphamide	3	7.3%
Liposomal doxorubicin	2	4.9%
Vinorelbine	6	14.6%
Ixabepilone	2	4.9%
Eribulin	1	2.4%
Capecitabine	8	19.5%
Hormone status		
Positive	10	40%
Negative	15	60%
Line of therapy		
3rd	25	61.0%
4th	12	29.3%
5th	4	9.8%

taxane (2.4%), platinum combinations (9.8%), anthracycline-cyclophosphamide (7.3%), liposomal doxorubicin (4.9%), vinorelbine (14.6%), ixabepilone (4.9%), eribulin (2.4%), and capecitabine (19.5%). Patients were also categorized by hormone receptor status, with 40% being hormone receptor-positive and 60% being hormone receptor-negative. The line of therapy was reported, with 61% receiving treatment in the third line, 29.3% in the fourth line, and 9.8% in the fifth line.

Treatment Response and PFS

Table 2 summarizes treatment responses and PFS. Overall, as the line of therapy advanced, outcomesworsened, with higher rates of progressive disease and progressively shorter median PFS (4.6 months in third line vs. 2.8 and 2.1 months in fourth and fifth lines, respectively). Hormone receptor–negative patients tended to achieve slightly better outcomes than hormone receptor–positive patients. Among treatment regimens, platinum-based combinations were associated with more favorable responses and longer median PFS, while most other agents demonstrated modest efficacy.

IGs and Disease Response

Table 3 and Figure 1 presents the association between IG parameters and disease response groups. The mean initial IG# at treatment start was 0.17±0.22 in the progressive disease group, 0.048±0.075 in the stable disease group, and 0.016±0.016 in the partial response group. The overall comparison showed a statistically significant difference among the groups (P=0.046), with post hoc analysis indicating a significant difference between progressive disease and partial response (P=0.040), while the other pairwise comparisons were not statistically significant. Similarly, the mean initial IG% at treatment start was 1.65±1.78 in the progressive disease group, 0.56±1.06 in the stable disease group, and 0.40±0.28 in the partial response group. The overall Kruskal–Wallis test demonstrated a statistically significant difference (P=0.006), and pairwiseanalysis revealed a significant difference between progressive disease and stable disease (P=0.030), whereas the other subgroup comparisons did not reach statistical significance.

Correlation Between IGs and PFS

Pearson correlation analysis revealed a statistically significant inverse association between PFS and both IG# (r= –0.332, P=0.034) and IG% (r= –0.323, P = 0.039), indicating that higher baseline IG levels were associated with shorter PFS (Table 4). Consistent with these findings, Kaplan-Meier curves stratified by IG levels demonstrated significantly reduced PFS in patients with elevated IGs (Figure 2).

Regression Analysis of Progression Duration

Table 5 presents the regression coefficients for the dependent variable, progression duration. The overall regression analysis results for the model show an R^2 of 0.103, an F-value of 2.47, and a p-value of 0.098. In this model, while the constant coefficient (b= 129.4, SE= 9.81, 95% CI: 109.60-149.33, t= 13.19, P<0.001) was statistically significant, neither the IG# (b= -54.69, SE= 81.46, 95% CI: -219.60-110.23, t= -0.67,

TABLE 2. Treatment Responses and Progression-Free Survival Across Lines of Therapy, Hormone Status, and Regimens

Variables	Progressive disease	Stable disease	Partial response	Complete response	Median progression-free survival (months)[#]
Line of treatment					
3rd	13	6	6	0	4.6
4th	11	1	0	0	2.8
5th	3	1	0	0	2.1
Hormone status					
Positive	14	3	2	0	2.8
Negative	13	5	4	0	3
Regimen					
Gemcitabine	11	3	0	0	3
Taxane	1	0	0	0	3
Platinum combination	0	2	2	0	6
AC	1	1	1	0	5
Liposomal doxorubicin	2	0	0	0	2.1
Vinorelbine	6	0	0	0	2.8
Ixabepilone	1	0	1	0	2.1
Eribulin	1	0	0	0	2
Capecitabine	4	2	2	0	3

Data are shown as median or number of patients. AC, anthracycline-cyclophosphamide.
[#]A Kaplan-Meier analysis was performed.

P=0.506) nor the IG% (b= -4.44, SE= 9.88, 95% CI: -24.44-15.57, t= -0.45, P=0.656) showed a statistically significant effect on progression duration.

Grade 3-4 Toxicity Outcomes

Among the 41 chemotherapy administrations analyzed, Grade 3-4 toxicity was observed in 18 (43.9%) cases , while 23 (56.1%) cases exhibited no such severe adverse events. The most frequently reported high-grade toxicities included hematologic complications such as severe neutropenia, anemia, and thrombocytopenia. In addition, patients experienced other significant toxicities, including gastrointestinal disturbances (e.g., mucositis, persistent nausea/vomiting), fatigue, peripheral neuropathy, and dermatologic reactions. To investigate whether there was an association between baseline IGs and severe toxicity, a comparative analysis was performed. The mean IG# and IG% were higher in patients who developed Grade 3-4 adverse events compared to those who did not (IG# mean rank: 24.08 vs. 18.59; IG% mean rank: 23.44 vs. 19.09). However, these differences did not reach statistical significance (P=0.12 for IG# and P=0.24 for IG%), indicating that initial IG metrics may not reliably predict the occurrence of high-grade toxicities in this cohort (Table 6).

DISCUSSION

The present study unequivocally demonstrates a discernible inverse correlation between the number of chemotherapy lines administered and both treatment response rates and median PFS. This finding highlights a significant clinical dilemma: as patients advance to fourth and fifth lines of systemic therapy, the probability of deriving substantial therapeutic benefit from intensive chemotherapy regimens markedly diminishes. Illustratively, the median PFS,

TABLE 3. Association of Immature Granulocytes with Disease Response Groups

Variables	Progressive disease (n=27)	Stable disease (n=8)	Partial response (n=6)	Overall P-value	Pairwise comparisons P-value
Initial IG# (×10³/µL)	0.17±0.22	0.048±0.075	0.016±0.016	**0.046**	PD vs SD = 0.143; PD vs PR = **0.040**; SD vs PR = 0.491
Initial IG (%)	1.65±1.78	0.56±1.06	0.40±0.28	**0.006**	PD vs SD = **0.03**; PD vs PR = 0.072; SD vs PR = 0.282

Data are shown as mean±standard deviation. IG#, Immature granulocyte count; IG%, Immature granulocyte percentage.
Overall P values were calculated using the Kruskal-Wallis test.
Pairwise subgroup comparisons were performed using the Mann-Whitney U test with Bonferroni correction. Statistically significant P-values are shown in bold.

which stood at 4.6 months for the third-line setting, notably decreased to 2.8 and 2.1 months in the fourth and fifth lines, respectively. This observed trajectory of progressively poorer outcomes with successive lines of therapy is well-documented in the existing literature, underscoring the inherent difficulties associated with managing advanced-stage malignancies and the diminishing efficacy of cumulative chemotherapy interventions [9]. Consequently, these results emphasize the imperative for a more selective approach to patient stratification, particularly when considering advanced-line chemotherapy.

This investigation considered the possible role of IGs as potential indicators of chemotherapy outcomes and PFS, primarily in advanced HER2-negative breast cancer. Our findings revealed statistically significant differences in IG# and IG% levels among the progressive disease, stable disease, and partial response groups. In the existing literature, elevated IG levels are generally recognized as indicators of systemic inflammation, bone marrow stress, or tumor-associated myelopoiesis [10]. In cancer patients, this elevation may reflect an immunological imbalance

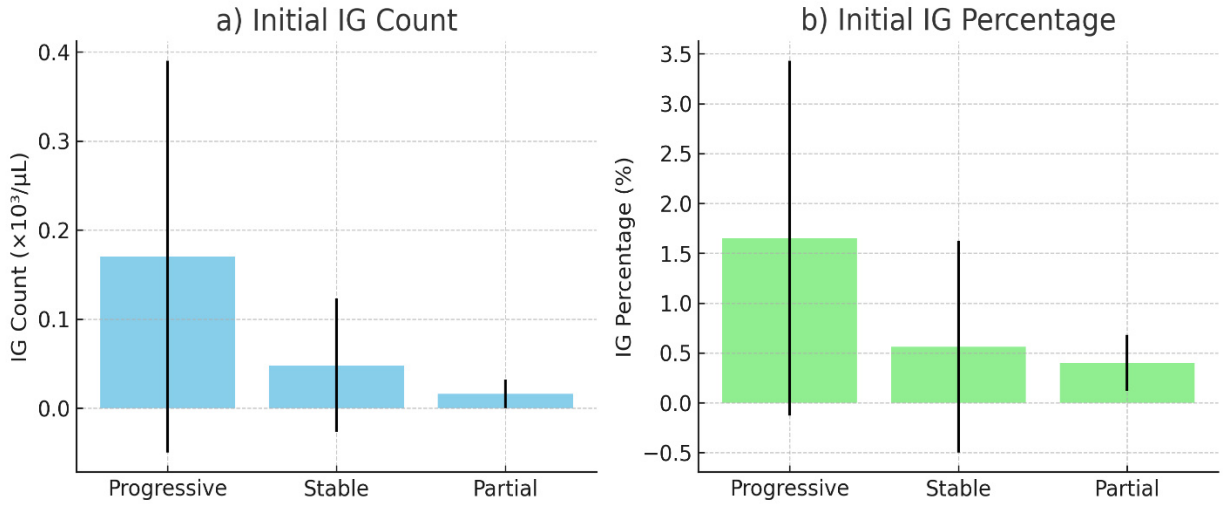

FIGURE 1. Baseline immature granulocyte (IG) levels by treatment response groups. a) Mean IG count (×10³/µL, ±SD) for progressive disease, stable disease, and partial response groups. b) Mean IG percentage (%, ±SD) for the same groups. Error bars represent standard deviations.

TABLE 4. Correlation between progression-free survival and Immature Granulocyte Parameters

Variable	R	P-value
Immature granulocyte count	–0.332	**0.034**
Immature granulocyte percentage	–0.323	**0.039**

Pearson correlation analysis was used to evaluate the relationship between progression-free survival and immature granulocyte parameters. Negative r values indicate an inverse correlation. Statistically significant P-values are shown in bold.

driven by chronic inflammatory processes and the tumor itself [11]. This connection is rooted in the continuous interplay between cancer cells and the host immune system, where the tumor, through various signaling molecules like granulocyte-colony stimulating factor and IL-6, can induce extramedullary hematopoiesis and accelerate myelopoiesis in the bone marrow, leading to an increased release of immature myeloid cells into circulation [12]. These circulating IGs, while often a consequence of systemic inflammation, can also actively contribute to it by releasing pro-inflammatory cytokines and chemokines, thus perpetuating a vicious cycle that supports tumor growth and progression [13]. Cancer-associated inflammation not only facilitates tumor survival and proliferation but also promotes invasion and metastasis by shaping a microenvironment that supports tumor progression [14]. While inflammation is a fundamental defense mechanism of the immune system, in the context of malignancy, it can paradoxically accelerate tumor growth by fostering an immunosuppressive tumor microenvironment, stimulating angiogenesis, and enhancing metastatic potential [15]. IGs, as early myeloid precursors, may contribute to this immunosuppressive state by serving as a source of myeloid-derived suppressor cells(MDSCs), a heterogeneous population of cells known for their potent immunosuppressive functions [16]. These MDSCs, through their unique metabolic and enzymatic activities, actively subvert anti-tumor immunity [17]. Specifically, MDSCs interfere with anti-tumor immunity through multiple mechanisms, including the production of arginase 1 and inducible nitric oxide synthase (iNOS), which deplete essential amino acids necessary for T-cell proliferation and function, leading to T-cell anergy [18]. Furthermore, MDSCs generate reactive oxygen species that induce oxidative stress, causing DNA damage and nitration

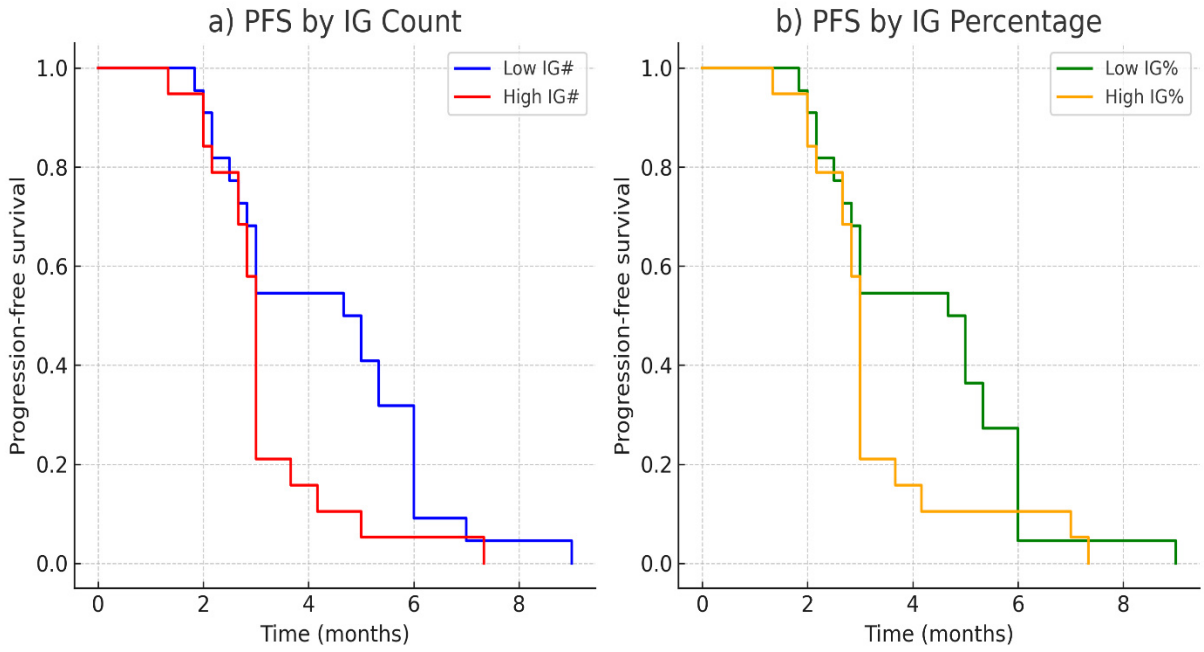

FIGURE 2. Kaplan–Meier curves of progression-free survival (PFS) according to immature granulocyte (IG) levels. a) PFS by IG count (low vs. high, median cut-off). b) PFS by IG percentage (low vs. high, median cut-off). The log-rank test was used to compare survival distributions between groups.

TABLE 5. Regression Coefficients for Immature Granulocyte Metrics Predicting Progression-Free Survival

Variable	b	SE	95% CI (LL, UL)	t	P-value
Constant	129.47	9.81	109.60, 149.33	13.19	**<0.001**
IG Count	–54.69	81.46	–219.60, 110.23	–0.67	0.506
IG Percentage	–4.44	9.88	–24.44, 15.57	–0.45	0.656

IG, immature granulocyte; b, unstandardized regression coefficient; SE, standard error; CI, confidence interval; LL, lower limit; UL, upper limit. R^2= 0.103, F(2, 38)= 2.47, P=0.098. Statistically significant P-values are shown in bold.

of T-cell receptors, thereby inhibiting cytotoxic T lymphocyte activation [19]. They also suppress natural killer cell activity, induce regulatory T cells, and secrete pro-tumorigenic and immunosuppressive cytokines such as IL-10, TGF-β, and VEGF [20]. These cytokines further reinforce the suppressive milieu, enabling tumor cells to evade immune surveillance, resist apoptosis, and develop tolerance to chemotherapeutic agents. In addition, chronic inflammation and MDSC accumulation are linked to activation of signaling pathways such as STAT3, NF-κB, and PI3K/AKT, which not only enhance tumor cell survival and proliferation but also upregulate multidrug resistance proteins, thereby directly contributing to chemotherapy resistance [21]. This may explain why, in our study, higher IG levels were associated with progressive disease and shorter PFS. These observations support the hypothesis that increased IGs are not merely a byproduct of inflammation, but may actively participate in creating a tumor-promoting, therapy-resistant environment. Previous research across different malignancies has highlighted the prognostic and predictive significance of inflammatory markers, including IGs, as surrogates for tumor burden, rapid progression, or therapeutic resistance [22]. The integration of IG monitoring into clinical practice could therefore aid in early identification of patients less likely to respond to standard chemotherapy, particularly in aggressive and treatment-refractory cancers such as advanced HER2-negative breast cancer, who have undergone multiple lines of chemotherapy, where chemotherapy response and associated toxicities can be highly variable. By enabling timely therapeutic adjustments, such as the introduction of immunomodulatory agents, anti-MDSC strategies, or novel targeted therapies, this approach could contribute to more personalized and effective treatment strategies, ultimately improving patient outcomes.

The observation of Grade 3-4 toxicity in 43.9% of cases in the study underscores the significant side effects of chemotherapy administered to patients with advanced breast cancer. This high toxicity rate increases the potential for using biomarkers like IGs to protect patients from unnecessary overtreatment and to identify those who will derive the least benefit from treatment or are at the highest risk of toxicity. While our study did not find a statistically significant difference in IG levels between patients with and without adverse events, aligning with findings that initial IG metrics may not reliably predict high-grade toxicities in all cohorts, the broader literature emphasizes the link between elevated IGs and systemic inflammation, tumor progression, and

TABLE 6. Immature Granulocyte Metrics by Grade 3–4 Adverse Event Status

Metric	Mean rank Grade 3–4 AE Absent (n = 23)	Mean rank Grade 3–4 AE Present (n = 18)	U	Z	P-value
IG Count	18.59	24.08	151.5	-1.538	0.12
IG Percentage	19.09	23.44	163.0	-1.183	0.24

AE, adverse event. Comparisons were performed using the Mann-Whitney U test due to non-normal data distribution.

treatment resistance [23]. Given the association of IGs with treatment efficacy, their role in predicting poor response, as observed in our study with progressive disease, could be critical. This predictive capacity, even without a direct link to toxicity incidence, might allow for the identification of patients unlikely to benefit, thereby potentially preventing their exposure to ineffective yet highly toxic therapies. Such an approach could significantly mitigate severe adverse effects that profoundly impact patients' quality of life in subsequent lines of therapy, potentially supporting the imperative for optimizing patient selection and preventing overtreatment in challenging clinical scenarios.

Strengths and Limitations

A major strength of this study is its focus on a clinically challenging and underexplored population, namely patients with HER2-negative breast cancer receiving third-line or later chemotherapy, a setting in which treatment decisions are often made with limited predictive guidance. By evaluating IG# and IG%—parameters that are routinely available from standard complete blood counts—this study explores a low-cost, widely accessible, and easily implementable biomarker without additional laboratory burden. Another important strength is the real-world nature of the cohort, reflecting daily clinical practice across multiple chemotherapy regimens and treatment lines, thereby enhancing the clinical relevance of the findings. Furthermore, the simultaneous assessment of treatment response, PFS, and toxicity allows for a comprehensive evaluation of the potential clinical utility of IGs, not only as prognostic indicators but also as tools that may help identify patients unlikely to benefit from further chemotherapy. This integrated approach aligns with the growing need to prevent overtreatment and to optimize patient selection in advanced-line breast cancer management.

The limitations of our study are multifaceted, primarily stemming from its modest sample size, which inherently restricts the statistical power and the ability to detect more subtle yet clinically meaningful associations. Although the correlation analyses between immature granulocyte parameters and progression-free survival yielded statistically significant results, the effect sizes were relatively modest, and the limited sample size weakens the power of these findings, indicating that they should be interpreted as exploratory rather than confirmatory. This constraint also heightens the risk of Type II errors, potentially masking true effects. Concurrently, the retrospective nature of the study design introduces inherent challenges, including potential for selection bias, the absence of standardized data collection protocols, and the difficulty in establishing definitive causal relationships due to uncontrolled confounding variables. These methodological limitations collectively impact the reliability and completeness of the gathered patient data and outcomes. Consequently, the generalizability of our findings to a broader patient population, including those with different breast cancer subtypes or varying clinical contexts, is limited. Future prospective, multi-center, and larger cohort studies are essential to validate these preliminary observations, more robustly define the role of IGs in predicting chemotherapy response and prognosis in HER2-negative breast cancer, and provide the robust evidence necessary for their confident integration into clinical practice for optimizing treatment strategies and reducing overtreatment.

CONCLUSION

Measuring IGs could represent a cost-effective and readily implementable method, potentially seamlessly integrating into routine complete blood count analyses without imposing additional workload or financial burden. The findings of our study tend to suggest that IG levels could serve as crucial predictive biomarkers. This could be particularly relevant for guiding treatment decisions in breast cancer, especially concerning third-line and subsequent chemotherapy regimens, which are frequently administered in daily clinical practice. Given the current lack of robust predictive biomarkers in these advanced settings, the potential role of IGs appears significant. Such insights could enable a more personalized approach to patient management, potentially allowing clinicians to prioritize tailored therapies or even best supportive care for patients who are already burdened by extensive treatments and for whom a curative outcome is no longer feasible. Therefore, there is a critical need for larger-scale, prospective studies to further validate these preliminary

observations and establish the utility of IGs in optimizing therapeutic strategies and improving patient outcomes in this challenging patient population.

Ethics Approval and Consent to Participate

This study was approved by the Bezmiâlem Vakıf University Non-Interventional Research Ethics Committee (Decision No.: 2025/31-03; date: 05.02.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author (Dr. Mustafa Ersoy), upon reasonable request.

Authors' Contribution

Study Conception: ME; Study Design: ME; Supervision: ME; Funding: ME; Materials: ME; Data Collection and/or Processing: ME; Statistical Analysis and/or Data Interpretation: ME; Literature Review: ME; Manuscript Preparation: ME; and Critical Review: ME.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Zhang C, Li N, Zhang P, et al. Advancing precision and personalized breast cancer treatment through multi-omics technologies. Am J Cancer Res. 2024;14(12):5614-5627. doi: 10.62347/MWNZ5609.
2. Ramón Y Cajal S, Sesé M, Capdevila C, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. J Mol Med (Berl). 2020;98(2):161-177. doi: 10.1007/s00109-020-01874-2.
3. Anand U, Dey A, Chandel AKS, et al. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2022;10(4):1367-1401. doi: 10.1016/j.gendis.2022.02.007.
4. Zafar A, Khatoon S, Khan MJ, Abu J, Naeem A. Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy. Discov Oncol. 2025 Apr 24;16(1):607. doi: 10.1007/s12672-025-02198-8.
5. Lustberg MB, Kuderer NM, Desai A, Bergerot C, Lyman GH. Mitigating long-term and delayed adverse events associated with cancer treatment: implications for survivorship. Nat Rev Clin Oncol. 2023;20(8):527-542. doi: 10.1038/s41571-023-00776-9.
6. Zhang F, Xia Y, Su J, et al. Neutrophil diversity and function in health and disease. Signal Transduct Target Ther. 2024;9(1):343. doi: 10.1038/s41392-024-02049-y.
7. Kaczmarek F, Marcinkowska-Gapińska A, Bartkowiak-Wieczorek J, et al. Blood-Based Biomarkers as Predictive and Prognostic Factors in Immunotherapy-Treated Patients with Solid Tumors-Currents and Perspectives. Cancers (Basel). 2025;17(12):2001. doi: 10.3390/cancers17122001.
8. Ndirangu K, Chabot I, Lewis K, et al. Disease landscape of advanced HER2-breast cancer patients by treatment line in three EU countries and USA. Future Oncol. 2024;20(25):1807-1824. doi: 10.2217/fon-2022-1228.
9. Park IH, Lee KS, Ro J. Effects of second and subsequent lines of chemotherapy for metastatic breast cancer. Clin Breast Cancer. 2015;15(1):e55-62. doi: 10.1016/j.clbc.2014.09.001.
10. Aguilar-Cazares D, Chavez-Dominguez R, Marroquin-Muciño M, et al. The systemic-level repercussions of cancer-associated inflammation mediators produced in the

tumor microenvironment. Front Endocrinol (Lausanne). 2022;13:929572. doi: 10.3389/fendo.2022.929572.
11. Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: from tumor initiation to metastatic progression. Genes Dev. 2018;32(19-20):1267-1284. doi: 10.1101/gad.314617.118.
12. Barisas DAG, Choi K. Extramedullary hematopoiesis in cancer. Exp Mol Med. 2024;56(3):549-558. doi: 10.1038/s12276-024-01192-4.
13. Nishida A, Andoh A. The Role of Inflammation in Cancer: Mechanisms of Tumor Initiation, Progression, and Metastasis. Cells. 2025;14(7):488. doi: 10.3390/cells14070488.
14. Pęczek P, Gajda M, Rutkowski K, Fudalej M, Deptała A, Badowska-Kozakiewicz AM. Cancer-associated inflammation: pathophysiology and clinical significance. J Cancer Res Clin Oncol. 2023;149(6):2657-2672. doi: 10.1007/s00432-022-04399-y.
15. de Visser KE, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell. 2023;41(3):374-403. doi: 10.1016/j.ccell.2023.02.016.
16. Zhao Y, Du J, Shen X. Targeting myeloid-derived suppressor cells in tumor immunotherapy: Current, future and beyond. Front Immunol. 2023;14:1157537. doi: 10.3389/fimmu.2023.1157537.
17. Li K, Shi H, Zhang B, et al. Myeloid-derived suppressor cells as immunosuppressive regulators and therapeutic targets in cancer. Signal Transduct Target Ther. 2021;6(1):362. doi: 10.1038/s41392-021-00670-9.
18. Yang Y, Li C, Liu T, Dai X, Bazhin AV. Myeloid-Derived Suppressor Cells in Tumors: From Mechanisms to Antigen Specificity and Microenvironmental Regulation. Front Immunol. 2020;11:1371. doi: 10.3389/fimmu.2020.01371.
19. Huang J, Zhao Y, Zhao K, Yin K, Wang S. Function of reactive oxygen species in myeloid-derived suppressor cells. Front Immunol. 2023;14:1226443. doi: 10.3389/fimmu.2023.1226443.
20. Joshi S, Sharabi A. Targeting myeloid-derived suppressor cells to enhance natural killer cell-based immunotherapy. Pharmacol Ther. 2022;235:108114. doi: 10.1016/j.pharmthera.2022.108114.
21. Sabit H, Adel A, Abdelfattah MM, et al. The role of tumor microenvironment and immune cell crosstalk in triple-negative breast cancer (TNBC): Emerging therapeutic opportunities. Cancer Lett. 2025;628:217865. doi: 10.1016/j.canlet.2025.217865.
22. Li S, Zhang C, Wang X, Wang J, Li K. Correlation of inflammatory markers with progression-free survival in advanced lung cancer patients treated with immune checkpoint inhibitors in combination with chemotherapy: a real-world study. J Thorac Dis. 2025;17(5):3456-3463. doi: 10.21037/jtd-2024-2198.
23. Bennett JM, Reeves G, Billman GE, Sturmberg JP. Inflammation-Nature's Way to Efficiently Respond to All Types of Challenges: Implications for Understanding and Managing "the Epidemic" of Chronic Diseases. Front Med (Lausanne). 2018;5:316. doi: 10.3389/fmed.2018.00316.

The European Research Journal 2026;12(1):97-107 Original Article

DOI: https://doi.org/10.18621/eurj.1798798 *Clinical Immunology and Allergy*

Risk Assessment and Skin Test Outcomes in Contrast Media Hypersensitivity: A Single-Center Study

Filiz Sadi Aykan[1], Fatih Çölkesen[2], Mehmet Kılınç[3], Recep Evcen[4], Eray Yıldız[5], Ferhat Sağun[2], Şevket Arslan[2]

[1]Department of Internal Medicine, Division of Clinical Immunology and Allergy, University of Health Sciences, Gülhane Training and Research Hospital, Ankara, Türkiye; [2]Department of Internal Medicine, Division of Clinical Immunology and Allergy, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye; [3]Department of Internal Medicine, Division of Clinical Immunology and Allergy, Batman Training and Research Hospital, Batman, Türkiye; [4]Department of Internal Medicine, Division of Clinical Immunology and Allergy, Recep Tayyip Erdoğan University Training and Research Hospital, Rize, Türkiye; [5]Department of Internal Medicine, Division of Clinical Immunology and Allergy, Necip Fazıl City Hospital, Kahramanmaraş, Türkiye

ABSTRACT

Objectives: Contrast media can cause adverse reactions ranging from mild skin symptoms to anaphylaxis. Although the pathophysiology is not fully understood, various risk factors have been reported. This study aimed to evaluate the risk factors and skin tests in patients experiencing hypersensitivity reactions to contrast media.

Methods: A total of 52 patients were included in the study. Patients experiencing contrast media-induced hypersensitivity reactions were evaluated for demographic and clinical characteristics, the type of contrast media used, and skin tests. They were compared with patients without a history of reactions. The patient group experiencing reactions was further divided into two groups: those with and without atopic disease.

Results: The patient group experiencing reactions was older. Comorbidity was high, and cardiovascular disease was the most common. Reactions were most frequently immediate and grade 1 and 2 in severity. The skin was most commonly affected, and anaphylaxis was detected in 28.6%. Approximately one-third had comorbid atopic disease, and respiratory system diseases and Hashimoto's thyroiditis were significantly more common in this group. In the group without comorbid atopic disease, malignancy and psychiatric disorders were more common, but not significantly so. Skin tests were negative in all groups.

Conclusions: Hypersensitivity reactions related to contrast medium use are increasing. Unnecessary examinations and tests cause delays in patient follow-up and treatment. All physicians must recognize and appropriately manage patients and reactions at high risk for contrast medium-related hypersensitivity reactions. We believe our study will contribute to the literature in managing and raising awareness of these reactions.

Keywords: Contrast Media, Hypersensitivity, Atopy, Skin Test

In modern medicine, iodinated contrast media (ICM) are increasingly used for computed tomography scans, and gadolinium-based contrast media (GBCM) are used for magnetic resonance imaging scans, in the diagnosis and follow-up of diseases [1, 2]. Although the frequency of radiocontrast medium-induced hypersensitivity reactions (HR) has decreased with the replacement of

Received: October 7, 2025 **Accepted:** November 8, 2025 **Available Online:** December 2, 2025 **Published:** January 4, 2026

How to cite this article: Sadi Aykan F, Çölkesen F, Kılınç M, et al. Risk Assessment and Skin Test Outcomes in Contrast Media Hypersensitivity: A Single-Center Study. Eur Res J. 2026;12(1):97-107. doi: 10.18621/eurj.1798798

Corresponding author: Filiz Sadi Aykan, MD., Assist. Prof., Phone: +90 312 304 20 00, E-mail: drfilizsadi@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

high-osmolar contrast media (CM) by low- or iso-osmolar agents, the increasing number of diagnostic tests using radiocontrast media makes HRs a significant problem [3]. In the 21st century, the general prevalence of immediate-type HR (IHR) with non-ionic radiocontrast media has been reported as 0.16-2.21%, while delayed (non-immediate) type HR (NIHR) has a general prevalence of 0.03-1.95% [4].

Adverse reactions can occur with any type of CM and can lead to fatal outcomes [4]. Three distinct categories of adverse reactions should be distinguished: HRs, toxic reactions, and events unrelated to CM exposure [5]. HRs can be classified into IHR and NIHR based on the time of reaction onset. In IHRs, symptoms typically begin within 1 hour immediately after drug administration [6]. Clinical manifestations can range from mild skin symptoms, such as urticaria, to severe reactions like anaphylactic shock, which can be fatal [7]. NIHRs, on the other hand, start more than 1 hour after the administration of the causative agent and are most commonly characterized by maculopapular exanthema [6].

The pathophysiology of HR with CM is not yet fully understood. Histamine or tryptase release has been demonstrated in many cases. This only indicates a mast cell and/or basophil activation mechanism but does not necessarily imply an immunoglobulin E-mediated allergic reaction [8]. It is emphasized that an allergic mechanism is demonstrated in only a minority of patients with IHR, but it appears more likely in patients with severe anaphylaxis [9]. In NIHRs, however, there is strong evidence that T-cell-mediated mechanisms play a role [6, 10, 11].

Various risk factors associated with CM-HRs have been reported. These include both patient-related factors, such as age, gender, atopy, and co-existing atopic or non-atopic diseases, and drug-related factors, such as dose, group, or administration route [4, 12, 13]. Skin tests and changing the risky CM for re-exposure are important in the evaluation of IHRs to non-ICMs. Recently, skin testing has been increasingly recommended, especially in severe IHRs [14]. Although less data are available for GBCMs, skin testing is similarly recommended, particularly in severe reactions [8]. However, the role of skin tests in diagnosis is limited due to low sensitivity in mild to moderate reactions with CMs [13].

With the increasing use of CM, the identification and management of adverse reactions, which may lead to serious outcomes, as well as the recognition of high-risk patients, have become critically important. Although clinical approaches may vary among institutions, it is essential to minimize the risk of CM-HR and to avoid unnecessary tests and interventions. In this study, in order to estimate the risk of CM-HR and contribute to optimizing the approach to high-risk patients, the general characteristics, comorbidities, risk factors, association with atopic diseases, and skin test results of patients with confirmed or suspected CM-HR were evaluated.

METHODS

Study Design

This retrospective study, involving human subjects, was conducted at the Adult Immunology and Allergy Clinic. It was carried out in accordance with the principles of the Declaration of Helsinki and approved by the local ethics committee of Necmettin Erbakan University Faculty of Medicine Hospital (decision no. 2023/4679). A total of 52 patients aged 18 years or older, who were either diagnosed with CM-HR or referred to the allergy and immunology clinic due to a history of other drug allergies before CM administration, and who underwent CM skin testing and evaluation by an allergy and immunology specialist, were included in the study. Patients who did not accept skin tests with CM and those for whom skin tests could not be performed due to dermographism were excluded from the study. A total of 52 patients were divided into two groups: those with and without CM-HR. The demographic characteristics, comorbidities, beta-blocker use, presence of atopic disease, characteristics of the CM causing the allergy, type and severity of the reaction, applied treatments and interventions, and skin tests of the 42 patients in the CM-HR group were evaluated. These were compared with patients without CM-HR. Additionally, the CM-HR patient group was further divided into two groups based on the presence of atopic disease for a comparative evaluation (Figure 1).

Data Collection

Patient data were retrieved from the hospital's electronic medical record and archive system, as well

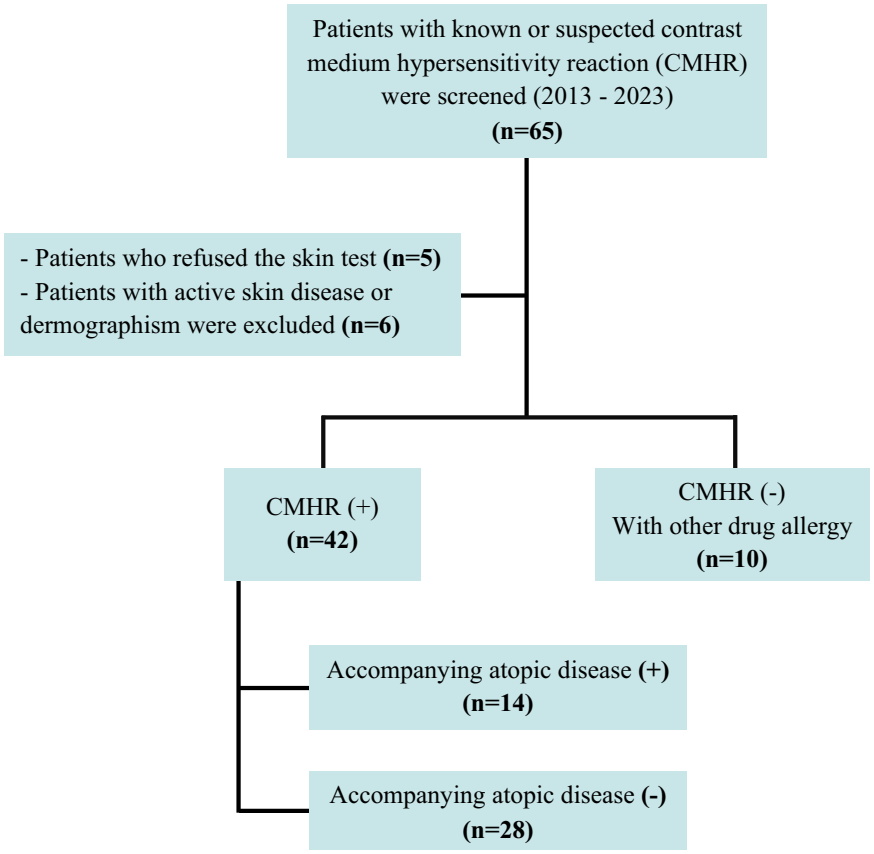

FIGURE 1. Flow chart of the patients included in the study.

as from the outpatient clinic forms specifically designed for drug allergy evaluations in the allergy clinic. Demographic data, comorbidities, characteristics of clinical findings, the CM where HR developed, the type and severity of HR, post-reaction treatment and interventions, and the results of skin tests performed with CM were obtained. This information was recorded in data forms designed for the study.

Protocols

1.Reaction Type

Reactions observed within the first hour after CM administration were classified as IHR type, while reactions occurring from one hour up to several days were considered NIHR type [15].

The Ring and Messmer scale was utilized to assess the severity of IHR type reactions [16]:

•*Grade 1 reaction:* Generalized cutaneous and/or mucocutaneous symptoms.

•*Grade 2 reaction:* Mild systemic reactions.

•*Grade 3 reaction:* Severe, life-threatening systemic involvement, including conditions like bronchospasm, laryngeal edema, and cardiovascular collapse.

•*Grade 4 reaction:* Cardiac and/or respiratory arrest.

NIHRs were evaluated according to the scales recommended by the European Academy of Allergy and Clinical Immunology (EAACI) interest group on drug hypersensitivity and the European Network of Drug Allergy (ENDA) [5, 17]:

•*Mild reaction:* Requiring no treatment

•*Moderate reaction:* Easily responsive to appropriate treatment and not requiring hospitalization

•*Severe reaction:* Requiring hospitalization or life-threatening conditions.

2. Skin Tests

Skin tests were performed 8-16 weeks after the patient's last reaction to CM or a different drug. Skin prick testing (SPT) was performed by an allergy and

immunology specialist on the volar forearm using non-ionic CM (iohexol, iopromide, iodixanol) or GBCM (gadoteric acid, gadobutrol, gadoxetate disodium). Simultaneously, negative (0.9% of physiologic saline solution) and positive (histamine intracutaneous, 1 mg/ml) controls were also applied. After 20 minutes, a wheal with an average diameter greater than 3 mm compared to the negative control was considered positive. Subsequently, an intradermal skin test (IDT) was performed with a tenfold diluted CM. 0.02-0.05 ml of sterile allergen solution was intradermally injected to create a bleb with a diameter of 3 mm. The borders of the bleb area formed by the injection were immediately outlined, and its diameter was measured and recorded. An increase in bleb diameter of 3 mm or more after 20 minutes was considered positive [17]. For patients with a severe history of previous reactions, an IDT was performed with CM diluted at ratios of 1:1000, 1:100, and 1:10. For those with NIHR, a delayed IDT protocol was applied, with readings taken at 24, 48, and 72 hours [18]. The results of the tests were interpreted and evaluated by the same allergy and immunology specialist who performed the procedures.

Statistical Analysis

Statistical analyses of the study were performed using SPSS (Statistical Package for the Social Sciences) version 23.0. Descriptive statistics included mean ± standard deviation for continuous variables, and frequencies and percentages for categorical variables. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. The Chi-square (χ^2) test was applied to examine the relationship between categorical variables. A P-value of < 0.05 was considered statistically significant.

RESULTS

Descriptive Statistics

Fifty-two patients who underwent skin tests with CM were included in the study. Ten patients were referred by a radiologist due to not experiencing CM-HR but having other drug allergies. CM-HR was present in 42 (80.76%) patients. The mean age of the CM-HR patient group was 50.3 years, and 57.1% were female. One or more comorbidities were present in

TABLE 1. General Characteristics of Patients with Contrast Media Hypersensitivity, Reactions, and Contrast Agents

Age (year), (Mean±SD)	50.3±15.2
Gender, n (%)	
Female	24 (57.1)
Male	18 (42.9)
Comorbidity, n (%)	
None	7 (16.7)
One comorbidity	9 (21.4)
More than one comorbidity	26 (61.9)
Comorbidity type, n (%)	
Cardiovascular	25 (59.5)
Respiratory	17 (40.5)
Diabetes mellitus	9 (21.4)
Hashimoto thyroiditis	4 (9.5)
Malignancy	5 (11.9)
Psychiatry	7 (16.7)
Other	6 (14.3)
Beta-blocker use, n (%)	8 (19)
Atopic disease, n (%)	
Asthma	6 (14.3)
Allergic rhinitis	5 (11.9)
Urticaria	2 (4.8)
Atopic dermatitis	2 (4.8)
History of other drug allergies, n (%)	10 (23.8)
Reaction site, n (%)	
Skin	32 (76.2)
Respiratory	18 (42.9)
Cardiovascular	6 (14.3)
Gastrointestinal	2 (4.8)
Neurological	2 (4.8)
Reaction type, n (%)	
Immediate	36 (85.7)
Non-immediate	6 (14.3)
Immediate reaction severity, n (%)	
Grade 1	14 (33.3)
Grade 2	10 (23.8)
Grade 3	8 (19.0)
Grade 4	4 (9.5)
Non-immediate reaction severity n (%)	
Mild	2 (4,8)
Moderate	4 (9.5)
Severe	0 (0)
Development of anaphylaxis, n (%)	12 (28.6)
Adrenaline administration, n (%)	11 (26.2)
Allergic contrast agent type, n (%)	
Gadolinium-based contrast agent	
Gadobutrol	8 (19)
Non-iyonik contrast agent	
Iohexol	20 (47.6)
Iopromid	14 (33.3)
Diagnostic skin tests, n (%)	2 (4.8)
Iohexol	2 (4.8)
Alternative skin tests, n (%)	40 (95.23)
Iohexol	16 (38)
Iopromide	4 (9.5)
Iodixanol	2 (4.8)
Gadoteric acit	10 (28.8)
Gadosectate disodium	4 (9.5)
Gadobutyrol	4 (9.5)

SD, standard deviation.

83.3% (n=35) of these patients. Cardiovascular comorbidities were the most common (59.5%), followed by respiratory system diseases. A history of atopic disease was present in 33.3% of patients. IHR occurred in 85.7% (n=36) of patients, with the majority being grade 1 and 2 reactions. The skin was the most commonly affected area (76.2%). Anaphylaxis developed in 12 (28.6%) patients. HR developed with GBCA (Gadobutrol) in 8 (19%) patients and with non-ionic CM (iohexol ve iopromid) in 34 (80.9%) patients. Due to subjective complaints, descriptive skin tests were performed with the CM that caused a reaction in 2 patients. Skin tests with alternative CM were performed in 40 (95.23%) patients. All skin tests in the CM-HR group were found to be negative (Table 1). In the group without CM-HR, skin tests were performed with CM determined by the radiologist and were found to be negative.

Comparison of Patients with and Without Contrast Media Allergy

The demographic characteristics and comorbidities of patients with CM-HR (n=42) and without (n=10) were compared. No difference was observed between the two groups in terms of gender. The CM-HR group was found to be older, and this difference was statistically significant (P<0.01). In this group, the presence of comorbidity and cardiovascular comorbidity was significantly higher (P=0.03 and P<0.01, respectively) (Table 2).

Comparison of Patients with and without Contrast Media Allergy Based on the Presence of Atopy

Patients with CM-HR were divided into two groups based on the presence (n=14) or absence (n=28) of atopic disease. The demographic characteristics, comorbidities, reaction features, severity, and properties of the contrast media in the two groups were compared. No difference was observed between the two groups in terms of age and gender. Respiratory system comorbidity and Hashimoto's thyroiditis were significantly more prevalent in the group with accompanying atopic disease (P<0.01 and P<0.01, respectively). In the group without atopic disease, malignancy and psychiatric disorders were more frequent, but this difference was not statistically significant. No significant difference was observed in terms of reaction type, anaphylaxis development, and CM type (Table 3).

TABLE 2. Comparison of Patients with and Without Contrast Medium Hypersensitivity

	Those with hypersensitivity reactions to contrast media (n=42)	Those without contrast medium hypersensitivity (n=10)	P-value
Age (years), (mean±SD)	50.3±15.2	29.5±5.1	**<0.01**
Gender, n (%)			0.28
Female	24 (57.1)	8 (80)	
Male	18 (42.9)	2 (20)	
Comorbidity, n (%)	35 (83.3)	5 (50)	**0.03**
Comorbidity type, n (%)			
Cardiovascular	25 (59.5)	1 (10)	**<0.01**
Respiratory	17 (40.5)	2 (20)	0.29
Diabetes mellitus	9 (21.4)	0 (0)	0.17
Hashimoto thyroiditis	4 (9.5)	1 (10)	1
Malignancy	5 (11.9)	0 (0,0)	0.56
Psychiatry	7 (16.7)	0 (0)	0.32
Other	6 (14.3)	0 (0)	0.58
Beta-blocker use, n (%)	8 (19)	0 (0)	0.32

SD, standard deviation. Statistically significant P-values are shown in bold.

TABLE 3. Comparison of Patients with Contrast Medium Hypersensitivity According to the Presence of Atopic Disease

	Atopic disease group (n=14)	Group without atopic disease (n=28)	P-value
Age (years), (mean±SD)	44.2±13.7	53.3±15.2	0.06
Gender, n (%)			0.18
Female	10 (71.4)	14 (50)	
Male	4 (28.6)	14 (50)	
Comorbidity, n (%)	14 (100)	21 (75)	0.07
Cardiovascular	7 (50)	18 (64.3)	0.37
Respiratory	10 (71.4)	7 (25)	**<0.01**
Diabetes mellitus	3 (21.4)	6 (21.4)	1
Hashimoto thyroiditis	4 (28.6)	0 (0.0)	**<0.01**
Malignancy	0 (0)	5 (17.9)	0.15
Psychiatry	2 (14.3)	5 (17.9)	1
Other	4 (28.6)	2 (7.1)	0.15
Beta-blocker use, n (%)	2 (14.3)	6 (21.4)	0.69
Reaction site, n (%)			
Skin	10 (71,4)	22 (78.6)	0.70
Respiratory	6 (42.9)	12 (42.9)	1
Cardiovascular	0 (0)	6 (21.4)	0.08
Gastrointestinal	0 (0)	2 (7.1)	0.54
Neurological	0 (0)	2 (7.1)	0.54
Reaction type, n (%)			1.00
Immediate	12 (85.7)	24 (85.7)	
Non-immediate	2 (14.3)	4 (14.3)	
Reaction severity, n (%)			0.65
Mild to moderate	8 (57.1)	18 (64.3)	
Moderate to severe	6 (42.9)	10 (35.7)	
Patients who developed anaphylaxis, n (%)	5 (35.7)	7 (25)	1
Type of allergic contrast agent, n (%)			
Gadolinium-based contrast agent	2 (14.3)	6 (21.4)	0.69
Non-ionic contrast agent	12 (85.7)	22 (78.6)	

SD, standard deviation. Statistically significant P-values are shown in bold.

DISCUSSION

In this study, 52 patients who underwent skin tests with CM were evaluated. Of these, 42 patients had a history of CM-HR, while 10 patients had a history of HR to other drugs (antibiotics, NSAIDs, and vitamin preparations) but no history of CM-HR. The majority of the CM-HR group had one or more comorbidities, with cardiovascular comorbidity being the most common. Atopic disease was detected in approximately one-third of the patients. The most frequent reactions were IHR, predominantly Grade 1 and 2, and the skin was the most commonly affected organ. Anaphylaxis developed in 28.6% of the patients. The CM-HR group was found to be older, with a higher prevalence of comorbidities, especially cardiovascular diseases, and this difference was statistically significant. When the CM-HR group was compared based on the presence of atopic disease, respiratory system diseases, and Hashimoto's

thyroiditis were significantly more common in the group with concomitant atopic disease. However, although no significant difference was observed in the group without atopic disease, malignancy and psychiatric disorders were found to be more prevalent. No difference was observed between the groups in terms of reaction type, anaphylaxis development, and CM type. Most patients underwent skin tests with alternative CM, and all results were negative.

In both IHR and NIHR, the most recognized and prevalent risk factor for CM is a history of prior CM-HR. The risk of IHR in these patients during repeated CM administrations ranges from 21% to 60% [4, 19, 20]. Additionally, previous studies have identified a history of other drug allergies, being aged 20-29 or over 55, and the presence of atopy or atopic diseases as other risk factors increasing the likelihood of CM-HR [4, 12, 13, 21-25]. In our study, the group with CM-HR did not have a history of recurrence, as it was their first reaction. However, 23.8% of patients in this group had a history of HR with other drugs. Conversely, all patients in the group without CM-HR were referred due to a history of HR with other drugs. Similar to previous studies, a high incidence of other drug allergies was observed in both groups. Studies regarding the gender factor, however, present conflicting findings. Some studies reported no significant difference between genders in CM-HR cases [22, 23, 26]. Subsequent studies, however, indicated that female gender is a significant risk factor [13, 25]. Although this topic remains controversial, our study did not reveal any gender differences. Nevertheless, our CM-HR patient group was significantly older. This finding supports studies suggesting that advanced age, rather than young age, poses a higher risk. Although the literature on this topic is conflicting, no gender difference was observed in our study [4].

Although the underlying mechanisms of CM-HR are not yet fully understood, immunoglobulin E (IgE) is thought to play a partial role in some subgroups. This may explain why atopic diseases and asthma are considered among the potential risk factors for CM-HR [27, 28]. However, CM-HRs cannot be explained solely by IgE-mediated pathways. Reactions may occur via immunological (including allergic reactions) or non-immunological mechanisms [29]. Hypersensitivity reactions occurring upon first exposure may be associated with direct membrane-mediated activation of mast cells and basophils, leading to histamine release [30]. This suggests that non–IgE-mediated reactions may also occur [29]. In our study, approximately one-third of the patients with CM-HR had atopic diseases, primarily allergic asthma, supporting the potential contribution of both immunological and non-immunological mechanisms. Although this finding strengthens the possibility that IgE-mediated sensitization may increase risk, it also highlights the relevance of non-immunological pathways. Moreover, a previous study demonstrated that asthma significantly increases the risk of CM-HR and is an independent risk factor [31]. Another study reported that asthma, food allergy, and non-allergic drug hypersensitivity were significantly more common among patients with GBCM reactions compared with controls [32]. Conversely, some studies have not demonstrated a statistically significant correlation between atopy and CM-HR, and therefore did not support its inclusion as a risk factor [33]. These conflicting findings indicate that the role of atopy may vary depending on the studied population. Although the prevalence of asthma and atopic disease was high in our cohort, the presence of atopy was not found to be independently associated with contrast media hypersensitivity (P=0.996). Because patients who already experienced reactions (n=42) were retrospectively evaluated and the sample size was limited without a control group, the statistical power was insufficient to draw strong conclusions. Larger, well-designed prospective studies are needed to better determine independent risk factors.

Asthma and other drug allergies are among the most well-known risk factors, and our study observed similar results [31]. However, in our CM-HR patient group, comorbidities, especially cardiovascular ones, were significantly more prevalent. Beta-blocker use did not differ. Previous studies have also shown that beta-blockers do not directly pose a risk, and the presence of cardiovascular disease may be associated with a more severe reaction [25, 34]. In the CM-HR group with coexisting atopic disease, respiratory system comorbidity and Hashimoto's thyroiditis were found to be significantly higher. Both allergy and autoimmunity stem from inappropriate immune system responses. The significant finding of an autoimmune disease like Hashimoto's thyroiditis in

patients experiencing CM-HR in our study may indicate a general dysregulation of the immune system [35]. Impaired immune tolerance in patients with an autoimmune background may increase the tendency to develop an abnormal or exaggerated response to potentially immunogenic agents like CMs [36, 37]. Furthermore, evidence suggesting that excessive iodine intake may play a role in the pathogenesis of autoimmune thyroid diseases also offers an important perspective on how CM exposure might modulate the immune response in this patient group [38]. In light of these data, our study is considered to provide valuable insights into the risk of CM-HR in patients with autoimmune disease.

In the group with CM-HR not accompanied by atopic disease, malignancy, and psychiatric disorders were observed to be more prevalent. A previous study identified the presence of psychiatric disorders as a significant risk factor [13]. An older study also indicated anxiety as a risk factor for CM-HR [39]. Although our study did not find a significant difference, future studies with larger patient numbers may shed more light on this issue.

CM-related immediate hypersensitivity reactions constitute approximately 70% of all reactions [5]. Non-immediate hypersensitivity reactions, however, are relatively difficult to identify because they can occur several days after injection and may be confused with symptoms caused by other factors. One study reported a prevalence of NIHRs as 12.4%, with a higher proportion of moderate reactions [40, 41]. In our study, IHRs were significantly higher, (%85.7) with the majority being Grade 1 and 2 reactions, which is consistent with the existing literature. NIHRs accounted for 14.3%, and as supported by previous studies, moderate reactions were more common. All anaphylactic reactions developed during IHRs. In most studies, the skin has been reported as the most frequently affected organ in both IHRs and NIHRs [5, 13, 42]. Our study also found the skin to be the most affected organ, consistent with previous research (%76.2).

In CM-HRs, skin testing is not recommended as a screening tool for the condition. Positive reactions to SPTs are rare, but sensitivities up to 25% have been reported for IDTs [43]. Similarly, another study found no SPT positivity in patients with IHRs, but the sensitivity of early readings of IDTs was 20%. [13] The low sensitivity of skin testing was attributed to the patients having Grade 1 and 2 IHRs. In a study involving 1048 patients, only two patients showed a positive response in hypersensitivity screening performed before CM administration [44]. Another study reported negative skin tests in high-risk patients and those with mild reactions [41]. In our study, skin tests were negative in all IHRs, supporting the literature. Although the tests were performed with an alternative CM different from the previous reaction, the majority consisted of Grade 1-2 severity reactions. However, skin tests were also performed in the non-CM-HR group before the first CM exposure and were found to be negative, similar to the literature. The low rates of positive skin tests in mild reactions also support the negative results in our patient group, which predominantly consisted of mild reactions. In NIHRs, diagnosis is usually made with patch tests and late readings of IDTs. One study reported 10% (1 patient) positivity in skin tests performed on 10 NIHR patients [13]. In our study, delayed reactions occurred in 6 patients, and skin tests (SPT+IDT early and late reading) were negative. Although the number of NIHR patients was small, as in previous studies, the findings supported the low positivity rates in the literature. The specificity of skin testing for CMs is high, but its role in diagnosis is limited due to its low sensitivity [13]. In this situation, skin tests for CM-HRs appear ineffective, unnecessary, and time-consuming in patients with risk factors or a history of mild reactions to CMs. In patients who have previously experienced NIHR or IHR, especially moderate to severe IHR, and require re-administration of CMs, skin tests should be performed to identify alternative agents for future procedures. For mild reactions, changing the CM along with premedication is usually sufficient [19]. However, its value as a pre-screening tool remains questionable. The best approach for a patient with CM-HR is to avoid the responsible agent and seek alternatives. However, physicians should be aware of immediate reactions in all cases.

Strengths and Limitations

This study had several limitations. Firstly, it was retrospective, requiring reliance on patient statements. Secondly, our patient number was small, and there was no healthy control group. However, a significant advantage was that negative skin tests

with CM in patients presenting with other drug allergies supported the notion that skin tests are unnecessary if there is no history of CM-HR, even if risk factors are present.

CONCLUSION

The increasing use of contrast media in modern medicine also increases the incidence of hypersensitivity reactions, which, although rare, can be fatal. Therefore, identifying patients at high risk for CM-HRs, recognizing and managing both immediate and non-immediate HRs, avoiding unnecessary tests, and ensuring training and experience in anaphylaxis are essential for all medical specialties. There is a need for prospective and more comprehensive studies on this topic, and we believe our study will contribute to identifying risk factors in CM-HRs and inform the approach to skin tests.

Ethics Approval and Consent to Participate

This study was approved by the Necmettin Erbakan University Non-Drugs and Medical Device Research Ethics Committee (Decision No: 2023/4679-188; date: 15.12.2023). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: FSA, FÇ, MK; Study Design: FSA, FÇ, RE; Supervision: FSA, MK, RE; Funding: FSA; Materials: FSA; Data Collection and/or Processing: FSA, MK, FS; Statistical Analysis and/or Data Interpretation: FSA, RE, EY; Literature Review: FSA, EY, FS; Manuscript Preparation: FSA, FS, ŞA; and Critical Review: FSA,EY, ŞA.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Christiansen C. X-ray contrast media--an overview. Toxicology. 2005;209(2):185-187. doi: 10.1016/j.tox.2004.12.020.
2. Heshmatzadeh Behzadi A, Prince MR. Preventing Allergic Reactions to Gadolinium-Based Contrast Agents. Top Magn Reson Imaging. 2016;25(6):275-279. doi: 10.1097/RMR.0000000000000106.
3. Kim MH, Park CH, Kim DI, et al. Surveillance of contrast-media-induced hypersensitivity reactions using signals from an electronic medical recording system. Ann Allergy Asthma Immunol. 2012;108(3):167-171. doi: 10.1016/j.anai.2012.01.012.
4. Lee SY, Lim KW, Chang YS. Radiocontrast media hypersensitivity in the Asia Pacific region. Asia Pac Allergy. 2014;4(2):119-125. doi: 10.5415/apallergy.2014.4.2.119.
5. Brockow K, Christiansen C, Kanny G, et al; ENDA; EAACI interest group on drug hypersensitivity. Management of hypersensitivity reactions to iodinated contrast media. Allergy. 2005;60(2):150-158. doi: 10.1111/j.1398-9995.2005.00745.x.
6. Brockow K, Sánchez-Borges M. Hypersensitivity to contrast media and dyes. Immunol Allergy Clin North Am.

2014;34(3):547-564, viii. doi: 10.1016/j.iac.2014.04.002.
7. Muraro A, Roberts G, Worm M, et al; EAACI Food Allergy and Anaphylaxis Guidelines Group. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy. 2014;69(8):1026-1045. doi: 10.1111/all.12437.
8. Clement O, Dewachter P, Mouton-Faivre C, et al. Immediate Hypersensitivity to Contrast Agents: The French 5-year CIRTACI Study. EClinicalMedicine. 2018;1:51-61. doi: 10.1016/j.eclinm.2018.07.002.
9. Yoon SH, Lee SY, Kang HR, et al. Skin tests in patients with hypersensitivity reaction to iodinated contrast media: a meta-analysis. Allergy. 2015;70(6):625-637. doi: 10.1111/all.12589.
10. Fok JS, Smith WB. Hypersensitivity reactions to gadolinium-based contrast agents. Curr Opin Allergy Clin Immunol. 2017;17(4):241-246. doi: 10.1097/ACI.0000000000000371.
11. Torres MJ, Gomez F, Doña I, et al. Diagnostic evaluation of patients with nonimmediate cutaneous hypersensitivity reactions to iodinated contrast media. Allergy. 2012;67(7):929-935. doi: 10.1111/j.1398-9995.2012.02840.x.
12. Bumbăcea RS, Petruţescu B, Bumbăcea D, Strâmbu I. Immediate and delayed hypersensitivity reactions to intravascular iodine based radiocontrast media -- an update. Pneumologia. 2013;62(1):47-51.
13. Goksel O, Aydın O, Atasoy C, et al. Hypersensitivity reactions to contrast media: prevalence, risk factors and the role of skin tests in diagnosis--a cross-sectional survey. Int Arch Allergy Immunol. 2011;155(3):297-305. doi: 10.1159/000320760.
14. Sánchez-Borges M, Aberer W, Brockow K, et al. Controversies in Drug Allergy: Radiographic Contrast Media. J Allergy Clin Immunol Pract. 2019;7(1):61-65. doi: 10.1016/j.jaip.2018.06.030.
15. Naisbitt DJ, Gordon SF, Pirmohamed M, Park BK. Immunological principles of adverse drug reactions: the initiation and propagation of immune responses elicited by drug treatment. Drug Saf. 2000;23(6):483-507. doi: 10.2165/00002018-200023060-00002.
16. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet. 1977;1(8009):466-469. doi: 10.1016/s0140-6736(77)91953-5.
17. Aberer W, Bircher A, Romano A, et al; European Network for Drug Allergy (ENDA); EAACI interest group on drug hypersensitivity. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy. 2003;58(9):854-863. doi: 10.1034/j.1398-9995.2003.00279.x.
18. Prieto-García A, Tomás M, Pineda R, et al. Skin test-positive immediate hypersensitivity reaction to iodinated contrast media: the role of controlled challenge testing. J Investig Allergol Clin Immunol. 2013;23(3):183-189.
19. Schönmann C, Brockow K. Adverse reactions during procedures: Hypersensitivity to contrast agents and dyes. Ann Allergy Asthma Immunol. 2020;124(2):156-164. doi: 10.1016/j.anai.2019.11.022.
20. Webb JA, Stacul F, Thomsen HS, Morcos SK; Members of The Contrast Media Safety Committee Of The European Society of Urogenital Radiology. Late adverse reactions to intravascular iodinated contrast media. Eur Radiol. 2003;13(1):18118-4. doi: 10.1007/s00330-002-1650-5.
21. Newmark JL, Mehra A, Singla AK. Radiocontrast media allergic reactions and interventional pain practice--a review. Pain Physician. 2012;15(5):E665-675.
22. Katayama H, Yamaguchi K, Kozuka T, Takashima T, Seez P, Matsuura K. Adverse reactions to ionic and nonionic contrast media. A report from the Japanese Committee on the Safety of Contrast Media. Radiology. 1990;175(3):621-628. doi: 10.1148/radiology.175.3.2343107.
23. Katayama H, Kozuka T, Ebata K. Assessment of the side-effects of iomeprol. Eur J Radiol. 1994;18 Suppl 1:S115-9. doi: 10.1016/0720-048x(94)90105-8.
24. Katayama H, Tanaka T. Clinical survey of adverse reactions to contrast media. Invest Radiol. 1988;23 Suppl 1:S88-9. doi: 10.1097/00004424-198809001-00005.
25. Pradubpongsa P, Dhana N, Jongjarearnprasert K, Janpanich S, Thongngarm T. Adverse reactions to iodinated contrast media: prevalence, risk factors and outcome-the results of a 3-year period. Asian Pac J Allergy Immunol. 2013;31(4):299-306. doi: 10.12932/AP0297.31.4.2013.
26. Thomas M, Peedicayil J, Koshi T, Korah I. Adverse reactions to radiocontrast media in an Indian population. Br J Radiol. 1999;72(859):648-652. doi: 10.1259/bjr.72.859.10624321.
27. Lee SY, Kang DY, Kim JY, et al. Incidence and Risk Factors of Immediate Hypersensitivity Reactions Associated With Low-Osmolar Iodinated Contrast Media: A Longitudinal Study Based on a Real-Time Monitoring System. J Investig Allergol Clin Immunol. 2019;29(6):444-450. doi: 10.18176/jiaci.0374.
28. Trcka J, Schmidt C, Seitz CS, Bröcker EB, Gross GE, Trautmann A. Anaphylaxis to iodinated contrast material: nonallergic hypersensitivity or IgE-mediated allergy? AJR Am J Roentgenol. 2008;190(3):666-670. doi: 10.2214/AJR.07.2872.
29. Costantino MT, Romanini L, Gaeta F, et al. SIRM-SIAAIC consensus, an Italian document on management of patients at risk of hypersensitivity reactions to contrast media. Clin Mol Allergy. 2020;18:13. doi: 10.1186/s12948-020-00128-3.
30. Isola S, Furci F, Gangemi S. The involvement of osmolarity in the safety of contrast media. Clin Mol Allergy. 2018;16:19. doi: 10.1186/s12948-018-0097-4.
31. Chatani R, Kondo S, Kamimura T, et al. Exploring Factors Affecting the Occurrence of Hypersensitivity Reactions Induced by Nonionic Iodine Contrast Media. J Clin Pharmacol. 2023;63(9):1002-1008. doi: 10.1002/jcph.2256.
32. Nucera E, Parrinello G, Gangemi S, et al. Contrast Medium Hypersensitivity: A Large Italian Study with Long-Term Follow-Up. Biomedicines. 2022;10(4):759. doi: 10.3390/biomedicines10040759.
33. Torres MJ, Trautmann A, Böhm I, et al. Practice parameters for diagnosing and managing iodinated contrast media hypersensitivity. Allergy. 2021;76(5):1325-1339. doi: 10.1111/all.14656.
34. Lang DM, Alpern MB, Visintainer PF, Smith ST. Increased risk for anaphylactoid reaction from contrast media in patients on beta-adrenergic blockers or with asthma. Ann Intern Med. 1991;115(4):270-276. doi: 10.7326/0003-4819-115-4-270.
35. Rottem M, Gershwin ME, Shoenfeld Y. Allergic disease and autoimmune effectors pathways. Dev Immunol. 2002;9(3):161-167. doi: 10.1080/1044667031000137638.
36. Sakamoto E, Katahira Y, Mizoguchi I, et al. Chemical- and

Drug-Induced Allergic, Inflammatory, and Autoimmune Diseases Via Haptenation. Biology (Basel). 2023;12(1):123. doi: 10.3390/biology12010123.
37. Watanabe Y, Yamaguchi Y. Drug allergy and autoimmune diseases. Allergol Int. 2022;71(2):179-184. doi: 10.1016/j.alit.2022.02.001.
38. Khudair A, Khudair A, Niinuma SA, Habib H, Butler AE. Beyond thyroid dysfunction: the systemic impact of iodine excess. Front Endocrinol (Lausanne). 2025;16:1568807. doi: 10.3389/fendo.2025.1568807.
39. Lalli AF. Urographic contrast media reactions and anxiety. Radiology. 1974;112(2):267-271. doi: 10.1148/112.2.267.
40. Yasuda R, Munechika H. Delayed adverse reactions to nonionic monomeric contrast-enhanced media. Invest Radiol. 1998;33(1):1-5. doi: 10.1097/00004424-199801000-00001.
41. Tepetam FM, Çiftaslan N, Oruç Ö, et al. Should patients with risk factors be tested for hypersensitivity to contrast media: a prospective study. Radiol Med. 2016;121(8):660-666. doi: 10.1007/s11547-016-0646-1.
42. Brockow K. Contrast media hypersensitivity--scope of the problem. Toxicology. 2005;209(2):189-192. doi: 10.1016/j.tox.2004.12.032.
43. Brockow K, Romano A, Aberer W, et al; European Network of Drug Allergy and the EAACI interest group on drug hypersensitivity. Skin testing in patients with hypersensitivity reactions to iodinated contrast media - a European multicenter study. Allergy. 2009;64(2):234-241. doi: 10.1111/j.1398-9995.2008.01832.x.
44. Kim SH, Jo EJ, Kim MY, et al. Clinical value of radiocontrast media skin tests as a prescreening and diagnostic tool in hypersensitivity reactions. Ann Allergy Asthma Immunol. 2013;110(4):258-262. doi: 10.1016/j.anai.2013.01.004.

The European Research Journal 2026;12(1):108-118 Original Article

DOI: https://doi.org/10.18621/eurj.1802723 *Emergency Medicine*

Comparison of the San Francisco Syncope Rule, Canadian Syncope Risk Score and Anatolian Syncope Rule in Patients Presenting to the Emergency Department with Syncope

Burcu Doğan[1], Seval Komut[2], Hasan Sultanoğlu[3], Cemal Yasin Duran[4], Merve Yazla[1], Handan Günsay[1]

[1]Department of Emergency Medicine, University of Health Sciences, Ankara Etlik City Hospital, Ankara, Türkiye; [2]Department of Emergency Medicine, Hitit University, Erol Olçok Training and Research Hospital, Çorum, Türkiye; [3]Department of Emergency Medicine, Faculty of Medicine, Düzce University, Düzce, Türkiye; [4]Department of Emergency Medicine, Samsun Training and Research Hospital, Samsun, Türkiye

ABSTRACT

Objectives: Syncope is a common cause of emergency department admissions and poses challenges in patient management due to its broad etiology and risk of short-term adverse events. This study aimed to comparatively evaluate the ability of the San Francisco Syncope Rule, Canadian Syncope Risk Score and Anatolian Syncope Rule to predict short-term (1-week and 1-month) adverse events in patients presenting to the emergency department with syncope.

Methods: This multicenter, prospective, observational study included 108 patients who presented to the emergency departments of two tertiary-level hospitals with syncope. Patients aged <18 years, pregnant women, those with non-syncopal causes of transient loss of consciousness, and those requiring hospitalization at presentation were excluded. Patients were contacted by phone on the 7th and 30th days after discharge to assess adverse events, including death, life-threatening arrhythmias, myocardial infarction, aortic dissection, pulmonary embolism, major bleeding, and subarachnoid hemorrhage.

Results: The mean age was 45.9±18.9 years, and 57.4% were female. The most common comorbidity was hypertension (34.3%), and vasovagal syncope (47.2%) was the leading etiology. Within 1 week, 8 (7.4%) patients experienced adverse events; none occurred at 1 month. Median scores were 0 for the San Francisco Syncope Rule, 1 for the Anatolian Syncope Rule, and 0 for the Canadian Syncope Risk Score; among those with events: 0.5, 2, 3, respectively.

Conclusions: The San Francisco Syncope Rule showed limited early discrimination, the Canadian Syncope Risk Score identified high-risk patients, and the Anatolian Syncope Rule better stratified low–moderate risk groups. Risk scores should complement, not replace, clinical judgment.

Keywords: Emergency Department, Syncope,Risk Assessment

Received: October 13, 2025 **Accepted:** November 20, 2025 **Available Online:** December 5, 2025 **Published:** January 4, 2026

How to cite this article: Doğan B, Komut S, Sultanoğlu H, Duran CY, Yazla M, Günsay H. Comparison of the San Francisco Syncope Rule, Canadian Syncope Risk Score and Anatolian Syncope Rule in Patients Presenting to the Emergency Department with Syncope. Eur Res J. 2026;12(1):108-118. doi: 10.18621/eurj.1802723

Corresponding author: Burcu Doğan, MD., Phone: +90 312 797 00 00, E-mail: burcudogan.dr@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

Syncope is defined as a transient loss of consciousness accompanied by loss of postural tone, which develops as a result of decreased cerebral perfusion and resolves completely within a short period of time. Syncope, which occurs at least once in a lifetime in approximately one-third of the population (18–47%), accounts for 0.6–3% of all emergency department (ED) visits [1, 2]. Developing secondary to a wide range of underlying pathologies, syncope represents a clinical spectrum rather than a single diagnosis. Although it is often due to benign causes, cardiac syncope carries a significant risk of morbidity and mortality; in this group, one-year mortality can reach 20–30%, which is approximately twice as high as mortality due to other causes [3]. Nevertheless, non-cardiac syncope may also lead to major complications such as falls, fractures, and head trauma, particularly in elderly individuals [4].

The differential diagnosis of syncope in the ED is challenging because of its broad etiological spectrum and the fact that most patients are asymptomatic at the time of presentation [5]. The etiology cannot be determined in approximately half of the patients presenting with syncope [2]. These diagnostic difficulties have led clinicians to develop risk stratification tools that can assist in patient management and help predict high-risk groups. For this purpose, several risk scoring systems have been proposed, including the San Francisco Syncope Rule (SFSR), the Osservatorio Epidemiologico sulla Sincope nel Lazio (OESIL), the Risk Stratification of Syncope in the ED (ROSE), the Canadian Syncope Risk Score (CSRS), and the Anatolian Syncope Rule (ASR). However, each of these scoring systems has its own advantages and disadvantages; some are based on complex parameters or have limited applicability in clinical practice [3].

This study aimed to compare the predictive ability of the SFSR, ASR, and CSRS in estimating adverse events that may occur within the first week and first month in patients presenting to the ED with syncope. In this way, we aimed to help emergency physicians identify which patients can be safely discharged and which require close follow-up or further evaluation.

METHODS

This study was a multicenter, prospective, cohort study conducted in the EDs of two tertiary-level hospitals between April 1, 2023, and March 31, 2024. Ethical approval was obtained from the Hitit University Non-Interventional Clinical Research Ethics Committee (Date: March 29, 2023; Decision No: 2023-03).

A total of 108 patients aged over 18 years, who presented to the ED with syncope, were included in the study. Patients younger than 18 years, pregnant women, those who refused or were unable to provide consent for participation, individuals with transient loss of consciousness due to non-syncopal causes (such as seizure, hypoglycemia, head trauma, etc.), patients who were still unconscious at the time of ED admission (due to shock, coma, alcohol/substance/drug use, etc.), those who could not be followed up, and patients with diagnoses requiring hospitalization at the time of presentation -- such as acute coronary syndrome, pulmonary thromboembolism, and hemorrhagic or ischemic cerebrovascular events -- were excluded from the study.

Each patient who agreed to participate in the study was evaluated by the attending physician, who completed a standardized data collection form. The investigators did not intervene in the management of the patients. Patients who were discharged from the ED were contacted by telephone at 1 week and at the end of the first month after discharge to determine whether any adverse events had occurred. These adverse events included death (exitus), life-threatening arrhythmias, myocardial infarction, aortic dissection, pulmonary thromboembolism, severe pulmonary hypertension, major bleeding, subarachnoid hemorrhage, and syncope requiring interventional treatment [6-8].

The SFSR consists of five parameters: a history of congestive heart failure (C), hematocrit <30% (H), abnormal electrocardiogram (ECG) findings (E), shortness of breath (S), and systolic blood pressure <90 mmHg (S). Each parameter is assigned one point, and the presence of any one of these factors classifies the patient as high risk. These criteria are easily remembered by the acronym “CHESS”.

The ASR consists of six parameters: dyspnea, orthostatic hypotension, predisposing factors for syncope, age >58 years, history of heart failure, and abnormal ECG findings. Each of the first five parameters is assigned one point, while abnormal ECG findings are assigned two points. A total score of 0–1 indicates low

risk, whereas a score of ≥2 indicates high risk.

The CSRS consists of ten parameters: the presence of vasovagal symptoms is assigned −1 point; history of heart disease, abnormal QRS axis, and QRS duration >130 ms are assigned 1 point each; systolic blood pressure <90 mmHg or >180 mmHg, elevated troponin, QTc >480 ms, and a presumptive diagnosis of cardiac syncope are each assigned 2 points; a presumptive diagnosis of vasovagal syncope is assigned −2 points, and other types of syncope are assigned 0 points. A total score of −3 to −2 indicates very low risk, −1 to 0 indicates low risk, 1 to 3 indicates medium risk, 4 to 5 indicates high risk, and >6 indicates very high risk.

The SFSR, ASR, and CSRS scores were calculated by the investigators based on the information recorded in the data collection forms.

Sample Size Calculation

Based on the sensitivity and specificity values reported in the reference study (Kessler C, Tristano JM, De Lorenzo R. The emergency department approach to syncope: evidence-based guidelines and prediction rules. Emerg Med Clin North Am. 2010;28(3):487-500), the required sample size was calculated by a biostatistician with a confidence level of 95% (1–α) and a test power of 95% (1–β). The analysis indicated that at least 90 patients were required to achieve adequate statistical power. Considering a potential data loss of 10–20%, a total of 108 patients were included in the study.

Statistical Analysis

Data analysis was performed using the SPSS 23.0 software package (IBM Corp., Armonk, NY, USA). For continuous variables, mean±standard deviation (SD) and median (minimum–maximum) values were reported; for categorical variables, numbers and percentages (%) were presented. The distribution characteristics of continuous variables were evaluated for normality using both visual methods (histograms, Q–Q plots) and analytical tests (Kolmogorov–Smirnov and Shapiro–Wilk tests). Inferential statistical analyses, including sensitivity, specificity, predictive values, and ROC curve estimations, were not performed because the number of adverse events (n=8) was too small to yield statistically meaningful or reliable results. Conducting such analyses with a very limited event count could produce unstable and potentially

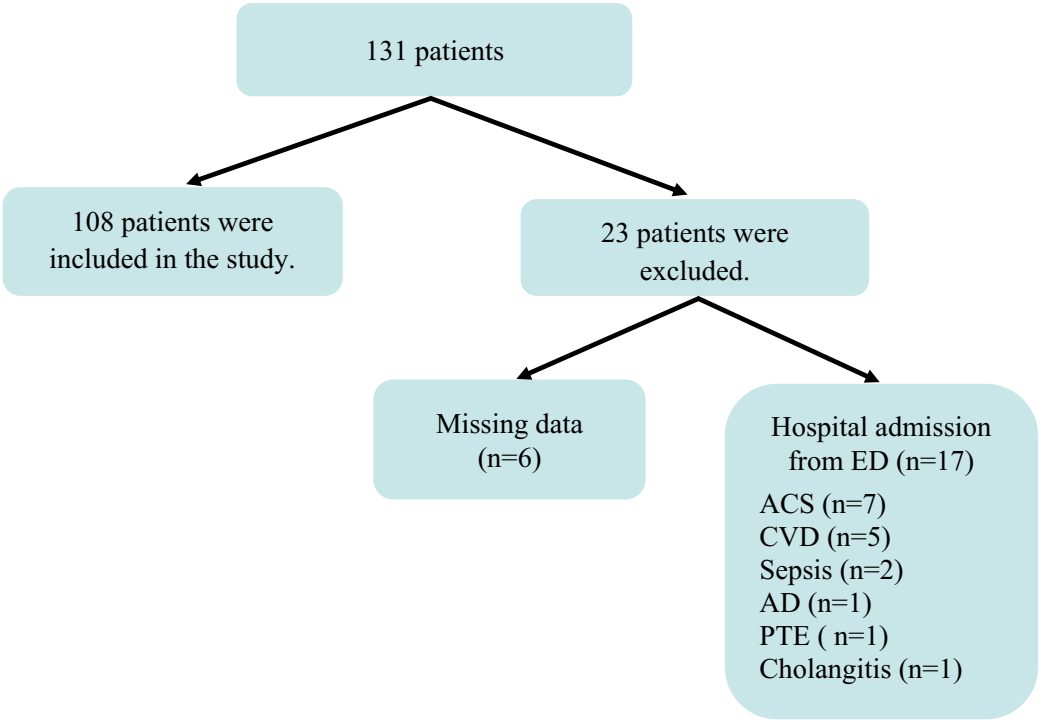

FIGURE 1. Study flow diagram showing patient inclusion and exclusion. ED, emergency department; ACS, acute coronary syndrome; CVD, cerebrovascular disease; AD, aortic dissection; PTE, pulmonary thromboembolism.

misleading estimates. Therefore, only descriptive statistics were used to present the distributions and trends of the three risk scores. A P-value <0.05 was considered statistically significant.

RESULTS

Between April and December 2023, a total of 131 patients who presented to the ED with syncope were assessed for eligibility, and 108 patients who met the inclusion criteria were enrolled in the study. Seventeen patients were excluded because they required hospitalization due to their diagnoses, and six patients were excluded due to missing data (Figure 1).

The mean age of the 108 included patients was 45.9±18.9 years (range: 18–88 years), and 57.4% (n=62) were female. When the distribution of comorbidities was examined, hypertension (HT) was the most common condition, present in 34.3% (n=37) of patients. This was followed by arrhythmias in 21.3% (n=23), diabetes mellitus (DM) in 18.5% (n=20), coronary artery disease in 17.6% (n=19), heart failure in 8.3% (n=9), neurological diseases in 4.6% (n=5), and valvular heart disease in 2.8% (n=3). In addition, 29.6% (n=32) of patients had other comorbidities.

TABLE 1. Baseline Clinical and Demographic Characteristics

Gender, n (%)		
Female	62 (57.4%)	
Male	46 (42.6%)	
Comorbidities, n (%)	**Present**	**Absent**
Hypertension	37 (34.3%)	71 (65.7%)
Diabetes mellitus	20 (18.5%)	88 (81.5%)
Coronary artery disease	19 (17.6%)	89 (82.4%)
Heart failure	9 (8.3%)	99 (91.7%)
Arrhythmia	23 (21.3%)	85 (78.7%)
Valvular heart disease	3 (2.8%)	105 (97.2%)
Neurological disorders	5 (4.6%)	103 (95.4%)
Other	32 (29.6%)	76 (70.4%)
Symptoms, n (%)	**Present**	**Absent**
Dizziness	31 (28.7%)	77 (71.3%)
Nausea	27 (25%)	81 (75%)
Palpitation	27 (25%)	81 (75%)
Dyspnea	14 (13%)	94 (87%)
Vomiting	11 (10.2%)	97 (89.8%)
Chest pain	7 (6.5%)	101 (93.5%)
Other	10 (9.3%)	98 (90.7%)
Vital signs	**Mean±SD**	**Min-Max**
Systolic blood pressure (mmHg)	123.7±21.2	65-187
Diastolic blood pressure (mmHg)	75.5±11.5	46-105
Heart rate (bpm)	86.1±21.7	48-148
Saturation (%)	96.0±3.8	80-100
Temperature (°C)	36.6 ±0.5	35.9-38.1

Min, minimum; Max, maximum; SD, standard deviation.

TABLE 2. Distribution of Patients by Risk Scores with Median (Min–Max) Values

Score	Point	Patient count (n)	Percentage (%)	Median	Min-Max
SFSR	0	79	73.1	0	0 - 3
	1	24	22.2		
	2	4	3.7		
	3	1	0.9		
ASR	0	16	14.8	1	0 - 5
	1	39	36.1		
	2	22	20.4		
	3	13	12		
	4	14	13		
	5	4	3.7		
CSRS	-3	18	16.7	0	(-3) - (4)
	-2	15	13.9		
	-1	15	13.9		
	0	26	24.1		
	1	11	10.2		
	2	8	7.4		
	3	12	11.1		
	4	3	2.8		

ASR, Anatolian syncope rule; CSRS, Canadian syncope risk score; SFSR, San Francisco syncope rule; Min, minimum; Max, maximum.

When the presenting symptoms were examined, the most common symptom was dizziness, observed in 31 (28.7%) patients. Nausea was present in 27 (25%) patients, palpitations in 27 (25%) patients, dyspnea in 14 (13%) patients, vomiting in 11 (10.2%) patients, and chest pain in 7 (6.5%) patients. Additionally, 10 (9.3%) patients had various other symptoms categorized under the heading "others."

In the evaluation of vital signs, the mean systolic blood pressure was 123.7±21.2 mmHg (range: 65–187 mmHg), and the mean diastolic blood pressure was 75.5±11.5 mmHg (range: 46–105 mmHg). The mean pulse rate was 86.1±21.7 beats per minute (range: 48–148), the oxygen saturation was 96.0±3.8% (range: 80–100), and the body temperature was 36.6±0.5°C (range: 35.9–38.1). The demographic and clinical characteristics of the patients are presented in Table 1.

When the distribution of predisposing factors for vasovagal syncope was examined, emotional distress was the most frequently observed factor (25%, n=27), followed by being in a crowded environment (17.6%, n=19), prolonged standing (16.7%, n=18), pain (8.3%,

TABLE 3. Distribution of Adverse Events at 1-Week and 1-Month Follow-Up

Adverse events	1-week			1-month	
	Absent	Present	Diagnosis	Absent	Present
	100 (92.6%)	8 (7.4%)		108 (100%)	0 (0%)
		5 (4.6%)	Arrhythmia		
		3 (2.8%)	ACS		

ACS, acute coronary syndrome.

TABLE 4. Demographic and Clinical Characteristics of Patients with Adverse Events

Gender, n (%)		
Male	5 (62.5%)	
Female	3 (37.5%)	
Comorbidities, n (%)	**Present**	**Absent**
Arrhythmia	4 (50%)	4 (50%)
Hypertension	3 (37.5%)	5 (62.5%)
Coronary artery disease	3 (37.5%)	5 (62.5%)
Diabetes mellitus	2 (25%)	6 (75%)
Heart failure	1 (12.5%)	7 (87.5%)
Valvular heart disease	1 (12.5%)	7 (87.5%)
Symptoms, n (%)	**Present**	**Absent**
Palpitation	4 (50%)	4 (50%)
Chest pain	3 (37.5%)	5 (62.5%)
Dyspnea	2 (25%)	6 (75%)
Other	1 (12.5%)	7 (87.5%)
ECG findings, n (%)		
Pathological findings	7 (87.5%)	1 (12.5%)
Atrial fibrillation	3 (37.5%)	
T wave inversion	1 (12.5%)	
Other	3 (37.5%)	

ECG, electrocardiogram.

n=9), fear (8.3%, n=9), fever (7.4%, n=8), and dehydration (4.6%, n=5). Other factors categorized under the heading "others" were present in 9.3% (n=10) of the patients.

When the etiological distribution of syncope was evaluated, vasovagal syncope was the most common type (47.2%, n=51), followed by cases in which the etiology could not be determined (32.4%, n=35). Less frequent causes included orthostatic hypotension (8.3%, n=9), arrhythmia (6.5%, n=7), and psychogenic causes (3.7%, n=4). Carotid sinus hypersensitivity (0.9%, n=1) and pregnancy (0.9%, n=1) were also among the observed causes.

When the ECG findings were evaluated, no abnormalities were detected in 62% (n=67) of the cases. Among the detected pathologies, atrial fibrillation was the most common (5.6%, n=6), followed by T-wave inversion (4.6%, n=5), right bundle branch block (3.7%, n=4), left bundle branch block (2.8%, n=3), ST-segment depression (2.8%, n=3), atrioventricular block (0.9%, n=1), and benign early repolarization (0.9%, n=1). Less common ECG abnormalities observed in 16.7% (n=18) of the patients were grouped under the heading "others."

In terms of ECG axis distribution, a normal axis was observed in the majority of patients (77.8%, n=84), while left axis deviation was found in 14.8% (n=16), right axis deviation in 6.5% (n=7), and marked axis deviation in only one patient (0.9%).

When the distributions of the risk scores used in the study were examined, the SFSR score ranged from 0 to 3, with a median value of 0. The low median value and right-skewed distribution suggested that the majority of patients were in the low-risk group.

The ASR score ranged from 0 to 5, with a median value of 1, indicating that the patient distribution was concentrated in the low-to-moderate risk category, although a small number of patients had higher scores. The CSRS score ranged from −3 to 4, with a median value of 0, indicating that most cases had a neutral or low-risk profile (Table 2).

As a result of the study, no adverse events were observed in 92.6% (n=100) of the patients during the first week following discharge from the ED. Among the adverse events detected, arrhythmia occurred in 4.6% (n=5) of the patients and acute coronary syndrome in 2.8% (n=3). At the end of the first month, no

TABLE 5. Distribution of Risk Scores Among Patients with Adverse Events

Number	SFSR	ASR	CSRS
1	0	2	3
2	0	1	-2
3	0	0	3
4	1	2	3
5	3	5	3
6	0	0	3
7	1	3	3
8	2	5	2
Score	**Median**	**Minimum**	**Maximum**
SFSR	0.5	0	3
ASR	2	0	5
CSRS	3	-2	3

ASR, Anatolian syncope rule; CSRS, Canadian syncope risk score; SFSR, San Francisco syncope rule.

adverse events were observed in any patient (Table 3).

When the demographic and clinical characteristics of the eight patients who experienced adverse events were examined, 62.5% (n=5) were male. The three most common comorbidities were arrhythmia (50%, n=4), HT (37.5%, n=3), and coronary artery disease (37.5%, n=3). The most frequent presenting symptoms were palpitations (50%, n=4) and dyspnea (25%, n=2). Abnormal ECG findings were observed in seven patients (87.5%), with atrial fibrillation (37.5%, n=3) being the most common abnormality (Table 4).

When the distribution of scores of the eight patients who experienced adverse events was examined, the SFSR score ranged from 0 to 3, with a median value of 0.5, and was clustered within the low-to-moderate risk range. The ASR score ranged from 0 to 5, with a median value of 2, and it was observed that some patients had higher risk scores. The CSRS score ranged from −2 to 3, with a median value of 3, demonstrating a higher median value compared to the other scoring systems (Table 5).

DISCUSSION

Syncope is a common cause of ED visits. It poses both diagnostic uncertainty and a risk of short-term adverse events, making its management particularly challenging for emergency physicians. Therefore, the use of reliable and easily applicable early risk stratification tools plays a critical role in guiding appropriate clinical decision-making.

In this study, the relationships between the SFSR, ASR, and CSRS scores and adverse events occurring at 1-week and at the end of the first month were compared descriptively among patients presenting with syncope. Although direct comparison between the scores is not feasible because their absolute values are based on different scoring systems, an examination of their distributions revealed important patterns. The SFSR scores clustered at lower values. The CSRS scores were higher in patients who developed adverse events and might provide an advantage in identifying high-risk groups. The ASR, with its broader distribution, appeared to enhance discrimination among low- to moderate-risk patients.

In our study, the mean age of patients evaluated for syncope was 45.9±18.9 years (18–88 years), and 57.4% (n=62) of the participants were female. The most frequently detected comorbidities were cardiac pathologies, followed by DM. These findings are consistent with the literature. Bernier *et al*. [9] reported that 53.7% of patients in their large population-based cohort were female, while Ling *et al*. [10] identified HT as the most common comorbidity among patients with syncope, followed by DM. Therefore, the demographic characteristics and comorbidity distribution observed in our study are in parallel with previous reports, suggesting that our patient population is representative of the general syncope population.

In our study, the incidence of adverse events was 7.4% (n=8) within the first week, and no adverse events were observed at the end of the first month. The majority of the eight patients who experienced adverse events had cardiac comorbidities such as arrhythmia and HT. When compared with the literature, the SAEM-GRACE meta-analysis published in 2025 reported that the rate of adverse events among patients discharged from the ED ranged between 0% and 3.7% [11]. Furthermore, another meta-analysis published in 2018 demonstrated that the presence of cardiac comorbidities was significantly associated with an increased risk of short-term adverse outcomes [12]. When evaluated together with these data, our findings suggest that cardiac comorbidities are a critical determinant of short-term prognosis in ED and should be carefully considered in risk stratification.

In our study, the median SFSR value was found to be 0. Even among patients who experienced adverse events, the median score was only 0.5. This finding indicates that serious events can occur even at low scores, suggesting that the SFSR has limited discriminatory power for risk stratification. In the derivation study conducted by Quinn *et al*. [6], the SFSR demonstrated high performance in predicting 1-week adverse events, with 96% sensitivity and 62% specificity. Similarly, in the validation study by the same group in 2006, 98% sensitivity and 56% specificity were reported [13]. However, these results have not been consistently reproduced in subsequent external validation studies. For example, Safari *et al*. [14], in their analysis of 187 syncope patients, reported that the SFSR had limited effectiveness in predicting short-term adverse outcomes, with AUC values of 0.57 for mortality, 0.67 for myocardial infarction, and 0.45 for cerebrovascular events. Sensitivity ranged between

44% and 83%, while specificity generally remained below 50%. Similarly, Bozorgi *et al*. [3] reported an AUC of 0.66 and a sensitivity of 42% for the SFSR during a 3-month follow-up, although the specificity was relatively high (90%). In light of these findings, the results of our study are consistent with the existing literature. They suggest that the SFSR has limited discriminatory ability in early risk stratification, as adverse events may still occur even at low score values. Therefore, it cannot be considered a reliable standalone tool.

In our study, the median CSRS value was 0 in the entire cohort, while it increased to 3 among patients who experienced adverse events. This finding suggests that an increase in the score is associated with a marked elevation in the short-term risk of adverse outcomes. The calculation of the CSRS based on ten parameters and the inclusion of both negative and positive points provide a wider distribution in risk stratification. This feature contributes particularly to the clearer differentiation of high-risk groups. Similarly, in the large-scale derivation study conducted by Thiruganasambandamoorthy *et al*. [8], the CSRS was reported to have strong discriminatory power, with the risk increasing gradually with higher scores. In a more recent small-sample study, the CSRS likewise demonstrated high accuracy in predicting cases that developed complications [15]. However, in both that study and ours, the relatively limited sample size represents an important limitation regarding the generalizability of the findings. Despite this, in our study, adverse events clustered predominantly in the moderate-to-high risk categories of the CSRS. This observation supports the literature indicating that the CSRS may be a more advantageous tool for distinguishing between low- and high-risk patients.

In our study, the ASR scores demonstrated a broader distribution compared to the SFSR and CSRS. The median value among patients who experienced adverse events was 2. This finding indicates that the ASR may have the potential to provide a more detailed risk stratification, particularly in the early period. The ASR is calculated based on six parameters, and since some parameters are assigned two points, the total scoring range is broader (0–5). This feature allows for clearer differentiation between low- and moderate-risk groups. Indeed, as shown in Table 2, the median ASR value in the overall cohort was 1, whereas in Table 5, among patients who developed adverse events, this value increased to 2. This demonstrates that the rise in score was associated with a clinically significant increase in risk.

In the derivation study conducted by Kayayurt *et al*. [7], the ASR was also reported to have greater discriminatory power in predicting short-term adverse events, particularly when compared with the SFSR. Our findings are consistent with these results and support that the ASR may serve as a clinically useful and applicable tool for risk stratification. However, the fact that the ASR was developed exclusively in the Turkish population and that its external validation in different populations remains limited represents an important limitation regarding the generalizability of this scoring system.

The variable results of the SFSR reported in different studies limit the use of this scoring system as a standalone tool. Therefore, it is suggested that combining it with clinical judgment or supporting it with an additional risk score may enhance its effectiveness in risk stratification.

Our findings revealed that the ASR demonstrated stronger performance, particularly in the classification of low- and moderate-risk groups, whereas the CSRS showed superior ability in identifying high-risk patients. In this context, the ASR may serve as a more practical tool for identifying low-risk patients who can be safely discharged from the ED. The fact that the ASR was developed in the Turkish population provides an advantage in reflecting local clinical characteristics. However, in our study, its broader score distribution and higher median values among patients who developed adverse events compared to the SFSR and CSRS may also be associated with this population-specific feature. Nevertheless, the limited external validation of the ASR in different populations remains an important limitation regarding the generalizability of these findings.

Risk scores are valuable tools that can assist clinicians in predicting the short-term prognosis of patients presenting with syncope. However, consistent evidence demonstrating that these scores outperform clinical judgment remains limited in the existing literature. Liang *et al*. [16] reported that syncope risk scores did not provide additional superiority over clinical judgment in predicting short-term adverse events.

Similarly, a systematic review and meta-analysis conducted by Wakai *et al*. [11] found that none of the 13 different risk stratification tools used in the ED outperformed clinical judgment, and that the overall quality of available evidence was low. Consistent with these findings, the 2018 ESC Guidelines for the Diagnosis and Management of Syncope also emphasize that risk stratification should not rely solely on risk scores [17]. Clinical judgment based on detailed history, physical examination, and ECG constitutes the essential foundation of patient assessment.

The comparative analysis of the three risk scores revealed distinct differences in their capacities to predict short-term adverse events. Our findings indicate that the SFSR has limited discriminatory power in predicting early adverse outcomes. The CSRS demonstrates stronger performance, particularly in identifying high-risk groups. The ASR, owing to its broader score distribution, allows for a more detailed classification among low- and moderate-risk patients. These results suggest that each scoring system has unique advantages in different clinical contexts. Incorporating these scores as complementary tools to support clinical judgment would be appropriate when developing risk stratification strategies in the ED.

Strengths and Limitations

Our study provides valuable insight by comparing three syncope risk scores within a prospective, multicenter cohort that closely reflects real-world ED practice. Despite the low number of adverse events, the detailed descriptive presentation of score distributions offers an important contribution to understanding early risk differentiation in discharged syncope patients. By focusing exclusively on individuals discharged from the ED, the study presents an important perspective on the safety and appropriateness of ED disposition decisions. Furthermore, the methodological transparency and clearly articulated limitations enhance the credibility and interpretability of the findings.

This study has several limitations. First, the very limited number of adverse events rendered inferential analyses statistically unreliable and potentially misleading; therefore, sensitivity, specificity, and predictive values were not calculated, as described in the Materials and Methods section. Second, hospitalized patients were excluded by design, as the study aimed to evaluate short-term adverse events among individuals discharged from the ED. This exclusion should not be considered a source of bias but rather a scope limitation, since the study specifically focused on evaluating the predictive performance of risk scores in patients who were discharged despite varying risk levels. Third, the ECGs were interpreted by the evaluating physician as part of the clinical process, which introduces a potential observer bias.

Finally, adverse events were assessed solely through telephone interviews and not verified by reviewing medical records; this may have increased the likelihood of underreporting or inaccurate reporting and introduced an additional source of bias.

CONCLUSIONS

In this multicenter prospective study, three different risk scores were evaluated in relation to short-term adverse events among patients with syncope. The findings indicate that the SFSR had limited discriminatory ability, the CSRS was more advantageous in identifying high-risk patients, and the ASR provided a more detailed classification within low-to-moderate risk groups. Since the study was based solely on descriptive statistics, performance metrics were not calculated, and the generalizability of the results remains limited.

In conclusion, in the management of patients with syncope, the use of risk scores as adjuncts to clinical evaluation is of great importance. Equally important is the selection of the most suitable scoring tool based on the demographic and clinical characteristics of the patient population.

Ethics Approval and Consent to Participate

This study was approved by the Hitit University Non-Interventional Clinical Research Ethics Committee (Decision No: 2023-03; date: 29.03.2023). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

Data Availability

All data generated or analyzed during this study are

included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: BD, SK; Study Design: BD, HS; Supervision: BD, HS, SK; Funding: BD, HS, SK; Materials: N/A; Data Collection and/or Processing: HS, BD, SK, CYD; Statistical Analysis and/or Data Interpretation: MY, BD, HG; Literature Review: HS, BD, SK, HG; Manuscript Preparation: BD, MY, HS; and Critical Review: BD, MY, HG, HS, SK.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Albassam OT, Redelmeier RJ, Shadowitz S, Husain AM, Simel D, Etchells EE. Did This Patient Have Cardiac Syncope?: The Rational Clinical Examination Systematic Review. JAMA. 2019;321(24):2448-2457. doi: 10.1001/jama.2019.8001.
2. Furlan L, Jacobitti Esposito G, Gianni F, Solbiati M, Mancusi C, Costantino G. Syncope in the Emergency Department: A Practical Approach. J Clin Med. 2024;13(11):3231. doi: 10.3390/jcm13113231.
3. Bozorgi A, Hosseini K, Jalali A, Tajdini M. A New Feasible Syncope Risk Score Appropriate for Emergency Department: A Prospective Cohort Study. Crit Pathw Cardiol. 2018;17(3):151-154. doi: 10.1097/HPC.0000000000000146.
4. Rivasi G, Ungar A, Moya A, Brignole M, Sutton R, Fedorowski A. Syncope: new solutions for an old problem. Kardiol Pol. 2021;79(10):1068-1078. doi: 10.33963/KP.a2021.0138.
5. Tannenbaum L, Keim SM, April MD, Long B, Koyfman A, Mattu A. Can I Send This Syncope Patient Home From the Emergency Department? J Emerg Med. 2021;61(6):801-809. doi: 10.1016/j.jemermed.2021.07.060.
6. Quinn JV, Stiell IG, McDermott DA, Sellers KL, Kohn MA, Wells GA. Derivation of the San Francisco Syncope Rule to predict patients with short-term serious outcomes. Ann Emerg Med. 2004;43(2):224-232. doi: 10.1016/s0196-0644(03)00823-0.
7. Kayayurt K, Akoglu H, Limon O, et al. Comparison of existing syncope rules and newly proposed Anatolian syncope rule to predict short-term serious outcomes after syncope in the Turkish population. Int J Emerg Med. 2012;5:17. doi: 10.1186/1865-1380-5-17.
8. Thiruganasambandamoorthy V, Kwong K, Wells GA, et al. Development of the Canadian Syncope Risk Score to predict serious adverse events after emergency department assessment of syncope. CMAJ. 2016;188(12):E289-E298. doi: 10.1503/cmaj.151469.
9. Bernier R, Tran DT, Sheldon RS, Kaul P, Sandhu RK. A Population-Based Study Evaluating Sex Differences in Patients Presenting to Emergency Departments With Syncope. JACC Clin Electrophysiol. 2020;6(3):341-347. doi: 10.1016/j.jacep.2019.11.002.
10. Ling L, Feng T, Xue X, Ling Z. Etiology, risk factors, and prognosis of patients with syncope: A single-center analysis. Ann Noninvasive Electrocardiol. 2021;26(6):e12891. doi: 10.1111/anec.12891.
11. Wakai A, Sinert R, Zehtabchi S, et al. Risk-stratification tools for emergency department patients with syncope: A systematic review and meta-analysis of direct evidence for SAEM GRACE. Acad Emerg Med. 2025;32(1):72-86. doi: 10.1111/acem.15041.
12. Gibson TA, Weiss RE, Sun BC. Predictors of Short-Term Outcomes after Syncope: A Systematic Review and Meta-Analysis. West J Emerg Med. 2018;19(3):517-523. doi: 10.5811/westjem.2018.2.37100.
13. Quinn J, McDermott D, Stiell I, Kohn M, Wells G. Prospective validation of the San Francisco Syncope Rule to predict patients with serious outcomes. Ann Emerg Med. 2006;47(5):448-454. doi: 10.1016/j.annemergmed.2005.11.019.
14. Safari S, Baratloo A, Hashemi B, et al. Comparison of different risk stratification systems in predicting short-term serious outcome of syncope patients. J Res Med Sci. 2016;21:57. doi: 10.4103/1735-1995.187305.
15. Moussa BS, Ali MA, Ali AAE, Abou Zeid AESM. Assessment of Canadian Syncope Risk Score in the prediction of outcomes of patients with syncope at the Emergency Department of Suez Canal University: STROBE compliant. Medicine (Baltimore). 2022;101(25):e29287. doi: 10.1097/MD.0000000000029287.
16. Liang Y, Li X, Tse G, et al. Syncope Prediction Scores in the

Emergency Department. Curr Cardiol Rev. 2022;18(5):1-7. doi: 10.2174/1573403X18666220321104129.
17. Brignole M, Moya A, de Lange FJ, et al; ESC Scientific Document Group. 2018 ESC Guidelines for the diagnosis and management of syncope. Eur Heart J. 2018;39(21):1883-1948. doi: 10.1093/eurheartj/ehy037.

The European Research Journal 2026;12(1):119-128 Original Article

DOI: https://doi.org/10.18621/eurj.1803088 *Otorhinolaryngology, Head and Neck Surgery*

In Vivo Evaluation of [Gly14]-Humanin's Protective Effect Against Cisplatin-Induced Ototoxicity

Murat Yaşar[1], Sedat Gökmen[2], Fatma Atalay[1], İrfan Çınar[3], Yusuf Aydin[1], Musa Tatar[4]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine, Kastamonu University, Kastamonu, Turkiye; [2]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kastamonu University, Kastamonu, Türkiye; [3]Department of Pharmacology, Faculty of Medicine, Kastamonu University, Kastamonu, Türkiye; [4]Department of Histology and Embryology, Faculty of Veterinary Medicine, Kastamonu University, Kastamonu, Türkiye

ABSTRACT

Objectives: This study aimed to evaluate in vivo the potential protective effects of [Gly14]-humanin against cisplatin-induced ototoxicity (CIO) and its modulatory effects against oxidative stress, inflammatory, and apoptotic markers

Methods: Thirty-five male Balb/c mice were randomly divided into five groups: control, cisplatin, cisplatin + [Gly14]-humanin (3 mg/kg), cisplatin + (6 mg/kg), and vitamin E. Cisplatin (10 mg/kg, b.w.) was administered as a single dose. [Gly14]-humanin was administered intraperitoneally for 14 days. Biochemical analyses (Superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA)), gene expression levels (TNF-α, IL-1β, IL-6, CASP-3, CASP-9, Bcl-2/Bax), and histopathological examinations (PARP1, PARP2 immunoreactivity) were performed in cochlear tissue samples to evaluate oxidative stress, inflammation, and apoptosis.

Results: Cisplatin decreased SOD and GSH levels and increased MDA levels in the cochlear tissue ($P<0.05$). With the application of [Gly14]-humanin, these parameters approached their normal levels. Cisplatin increased the values of pro-inflammatory and pro-apoptotic markers (TNF-α, IL-1β, IL-6, Caspase-3 (CAS-3), Caspase-9 (CAS-9)) significantly while reducing the Bcl-2/Bax ratio ($P<0.05$). [Gly14]-humanin administration reversed these effects in a dose-dependent manner. Histological analysis also revealed decreased PARP1 and PARP2 immunoreactivity in [Gly14]-humanin treated groups, comparable to the positive control (vitamin E).

Conclusions: The findings of this study show that [Gly14]-humanin exhibits important protective effects against CIO by attenuating oxidative stress, inflammation, and apoptosis in cochlear tissues. Further studies evaluating the clinical efficacy and reliability of [Gly14]-humanin are now needed.

Keywords: [Gly14]-Humanin, Cisplatin, Ototoxicity, Oxidative Stress, Cochlear Protection

Cisplatin is a widely used anticancer drug, particularly in the treatment of solid tumors such as cancers of the testis, lung, ovary, and head and neck [1, 2]. However, it also causes severe side effects, such as ototoxicity, nephrotoxicity, neurotoxicity, and myelosuppression. Permanent

Received: October 15, 2025 **Accepted:** November 24, 2025 **Available Online:** December 9, 2025 **Published:** January 4, 2026

How to cite this article: Yaşar M, Gökmen S, Atalay F, Çınar İ, Aydin Y, Tatar M.In Vivo Evaluation of [Gly14]-Humanin's Protective Effect Against Cisplatin-Induced Ototoxicity. Eur Res J. 2026;12(1):119-128. doi: 10.18621/eurj.1803088

Corresponding author: Yusuf Aydın, MD., Assist. Prof., Phone: +90 366 280 10 00, E-mail: yusufaydin@kastamonu.edu.tr

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

hearing loss develops in a significant proportion of patients treated with cisplatin (40-80% of adults and at least 40% of children). Hearing loss in cisplatin-induced ototoxicity (CIO) is typically bilateral, sensorineural, progressive, irreversible, and frequently accompanied by tinnitus and vertigo [1, 3, 4].

There are several mechanisms under the CIO. Studies have suggested that CIO may derive from several mechanisms, including oxidative stress, DNA damage, inflammation, apoptosis, and ferroptosis [5-8]. Another important mechanism is thought to be the accumulation of reactive oxygen species (ROS) induced by cisplatin [1, 2, 5, 9]. Excessive intracellular ROS accumulation can trigger cell death by damaging the cellular structure [10, 11]. Cisplatin's inhibition of antioxidant enzyme activity can result in an increase in ROS inside the cochlea. This causes apoptosis of hair cells and spiral ganglion neurons, thus resulting in sensorineural hearing loss [12]. Cisplatin is reported to exhibit an ototoxic effect not only by damaging hair cells in the organ of Corti, but also spiral ganglion neurons, support cells, and the stria vascularis [13].

Humanin is a 24-amino acid peptide synthesized in association with mitochondrial translocation. It is endogenous in origin and biologically active. It was first identified for its protective effects against neurodegenerative processes associated with Alzheimer's disease [14-17]. Humanin and its synthetic derivatives exhibit antiapoptotic, anti-inflammatory, and antioxidant activities against oxidative stress and hypoxic conditions [18-21]. [Gly14]-humanin is one humanin derivative. While the serine amino acid is present in position 14 in the humanin peptide chain, the amino acid glycine is present in [Gly14]-humanin. Research has identified [Gly14]-humanin as a potent humanin analog, particularly noteworthy for its neuroprotective effects [22, 23].

This study aimed to evaluate in vivo the potential protective effects of [Gly14]-humanin against CIO and its modulatory effects against oxidative stress, inflammatory, and apoptotic markers.

METHODS

Chemicals and Reagents

[Gly14]-humanin (99.86% purity), cisplatin (CAS number: 15663-27-1), and all other reagents were purchased from MedChemExpress. Superoxide dismutase (SOD) (Sunred, Cat: 201-02-0291), glutathione (GSH) (Sunred, Cat; 201-02-180), and malondialdehyde (MDA) (Sunred, Cat; 201-02-0626) were purchased from SunRed Biotechnology Company (SRB) Ltd. (Shanghai, China).

Animals

This study was conducted with 35 male Balb/c mice (6–8 weeks old, weighing 20–25 g) obtained from the Kastamonu University Experimental Animals Application and Research Center, Kastamonu. These were housed in standard cages under well-regulated conditions (relative humidity range: 45±5%, temperature: 24±1°C, in a 12-h light/12-h dark cycle). The animals were maintained on a standard pellet diet and allowed ad libitum access to water during the experimental period. All animal procedures described in this study were approved by the Animal Care and Use Committee at Kastamonu University, and all the methods used during the animal experiments were in accordance with the guidelines approved by the Animal Ethics Committee (25.10.2024/11/39).

Experimental Design

The animals were randomly divided into the following five groups:

I. Control group: The healthy control group did not receive any drug administration (n=7).

II. Cisplatin group: A single dose of cisplatin (10 mg/kg, b.w.) was injected intraperitoneally into the mice on the 10th day [24] (n=7).

III. Cisplatin + [Gly14]-humanin group: A single dose of cisplatin (10 mg/kg, b.w.) on the 10th day of the treatment schedule, along with daily administration of [Gly14]-humanin (3 mg/kg b.w./day) for 14 days (n=7).

IV. Cisplatin + [Gly14]-humanin group: A single dose of cisplatin (10 mg/kg, b.w.) on the 10th day of the treatment schedule, along with daily administration of [Gly14]-humanin (6 mg/kg b.w./day) for 14 days (n=7).

V. Vitamin E group: Vitamin E (100 mg/kg b.w./day) was administered by gavage to the mice orally for 14 days (n=7). A table (1) showing the dose schedule of the experiment is presented as a timeline.

TABLE 1. The Dose Schedule of the Experiment is Presented as a Timeline

Experimental day	Control group (0.5 mL)	Cisplatin group (10 mg/kg, b.w.)	[Gly-14] humanin group (3 mg/kg)	[Gly-14] humanin group (6 mg/kg)	Vitamin E group (100 mg/kg)
Day 1-9	Saline only	Saline only	Daily [Gly-14] humanin	Daily [Gly-14] humanin	Daily Vitamin E
Day 10	Saline only	Cisplatin injection	Daily [Gly-14] humanin +Cisplatin injection	Daily [Gly-14] humanin +Cisplatin injection	Daily Vitamin E +Cisplatin injection
Day 11-14	Saline only	Observation	Daily [Gly-14] humanin	Daily [Gly-14] humanin	Daily Vitamin E
Day 15	All animals in all groups were sacrificed under high-dose anesthesia				

In order to evaluate the preventive effects of [Gly14]-humanin against CIO, doses of 3 and 6 mg/kg were selected in the present investigation based on previous research. Vitamin E (100 mg/kg) was also used as a positive control [25].

At the end of the experimental period, 24 h after receiving the last treatment, the cochlea and vestibular system were collected from all the experimental group mice under deep anesthesia. All mice were sacrificed by removing blood from the left ventricle under deep anesthesia with ketamine (100 mg/kg) + xylazine (10 mg/kg). Animals under deep anesthesia were euthanized by cervical dislocation. The cochlea and vestibular system were used for biochemical, molecular, and histopathological analysis.

Biochemical Analysis

The ELISA method was employed for measuring SOD activity and GSH and MDA levels in cochlear tissue samples, using a previously described method. Tissues were homogenized by adding nine times their weight in iced phosphate-buffered saline (PBS; 0.01 M, pH=7.4) solution for the analysis of tissue samples. Centrifugation was performed at 5000 g for 5 minutes, and the resulting supernatant was used for analysis. Commercial kits (Elabscience, Wuhan, China) were used for the measurement of GSH, SOD, and MDA levels. GSH, MDA, and SOD levels were measured using a microtiter plate ELISA reader (Epoch Microplate Spectrophotometer, BioTek, USA).

Gene Expression Analysis TNF-α, IL-1β, IL-6, Caspase-3 (CAS-3), Caspase-9 (CAS-9), Bax, and Bcl-2 mRNA expression levels in tissue samples were determined by the real-time PCR method.

RNA Extraction

The tissue samples were homogenized in Tissue Lyser II (Qiagen) in the presence of liquid nitrogen. Total RNA was isolated from the cochlea and vestibular system of the experimental and control groups with a QIAcube Connect Qiagen kit for RNA isolation according to the manufacturer's instructions, and the recovery was estimated.

Reverse Transcriptase Reaction and cDNA Synthesis: cDNA was reverse transcribed from total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems), following the manufacturer's protocol. The recovery of cDNA was then determined using nanodrop spectrophotometry (EPOCH Take3 Plate, BioTek) before storage at -20 °C.

Real-Time Quantitative PCR

Total RNA extraction and cDNA synthesis were performed according to the methods described in our previous studies [26, 27].

According to the manufacturer's instructions, and TNF-α (Mm00443258_m1), IL-1β (Mm00434228_m1), IL-6 (Mm00446190_m1), Bcl (Mm00477631_m1), Bax

(Mm00432051_m1), Caspase-3 (Mm01195085_m1), Caspase-9 (Mm00516563_m1), and β-actin (Mm02619580_g1) mRNA expression as a housekeeping gene were analyzed. Compared with the control group, all data were expressed as fold change in expression compared with the cell groups using the 2-ΔΔCt method. Differences between the groups in terms of oxidative stress, inflammation, and apoptosis were evaluated.

Immunohistochemistry Procedure

Serial sections 5-µm in thickness from the prepared paraffin blocks were placed onto adhesive slides. The streptavidin-biotin complex (sABC) staining method was then applied to determine PARP1 and PARP2 reactivity in the sections. Antigen retrieval was performed once the sections had been deparaffinized in xylol and dehydrated by passage through alcohol series. For that purpose, the slides were placed into a 10-fold diluted citrate buffer (citrate buffer heat-induced epitope retrieval pH: 6) solution and heated in a microwave oven at 800 Watts for 20 min. Following this process, the sections were left to cool for 30 min. After washing in phosphate-buffered saline (PBS) solution for 3×5 min, they were next incubated in 3% hydrogen peroxide (hydrogen peroxide 30% Merck: 108597) solution in the dark for 20 min for the purpose of blocking endogenous peroxidase activity [28]. At the end of the incubation procedure, the sections were rewashed with PBS solution for 3×5 min. In order to prevent nonspecific antibody binding, Ultra V Block (TP-125-HL, Thermo Fisher Scientific) solution was dropped onto the tissues, left for 10 min. Then, they were incubated at +4 °C overnight at appropriate concentrations of primary antibodies [anti-PARP-1 (1:200; catalogue no. 13371–1-AP, Proteintech) and anti-PARP-2 (1:200; catalogue no. 20555-1-AP, Proteintech)] without washing. Following incubation, the sections were washed with PBS for 3×5 min. Secondary antibody kits (TP-125-HL, Thermo Fisher Scientific) were then applied in line with the procedure order and duration, after which AEC chromogen (TA-060-HA, Thermo Fisher Scientific) was applied to show the reaction and render the immunoreactivity visible. A water-compatible covering medium (TA-125-UG, Thermo Fisher Scientific) was applied to the sections counterstained with Gill's hematoxylin were and examined under a microscope. In the negative controls, PBS replaced the primary antibody after protein blocking, and the tissues were incubated with that solution overnight [29].

Statistical Analysis

Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by the Tukey test and with GraphPad Prism 9 software (GraphPad Software, Inc.). P values <0.05 were considered statistically significant [30].

RESULTS

Compared to the control group, SOD activity

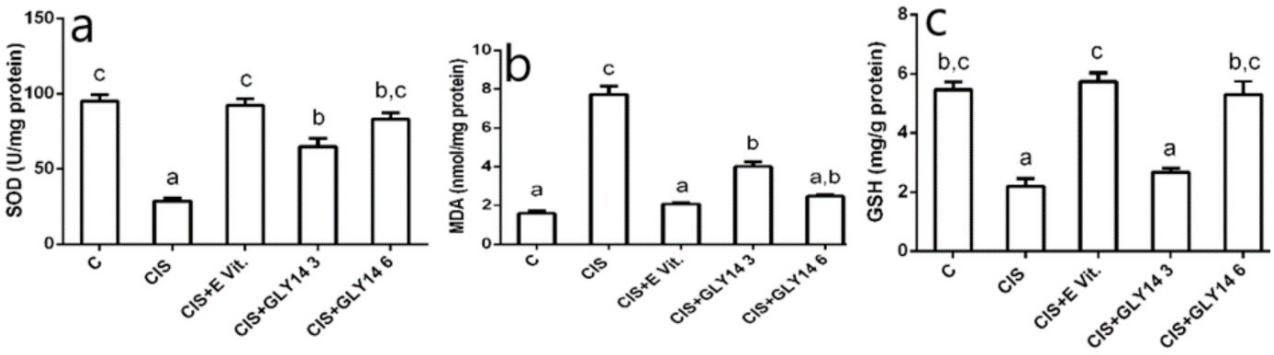

FIGURE 1. Figure 1. Effect of [Gly14]-humanin on antioxidant marker levels in CIS-induced ototoxicity. C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E.
Different letters (a, b, c, d, e) in the same column represent a statistically significant difference (P<0.05).

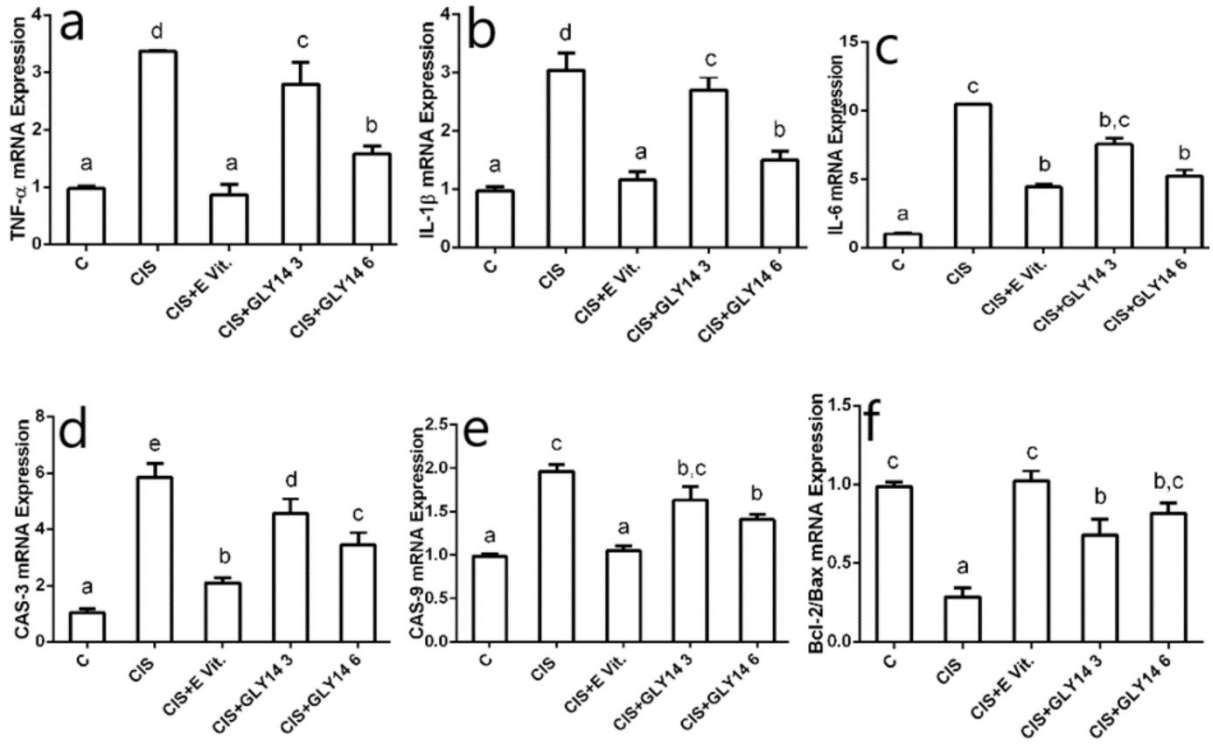

FIGURE 2. The effect of [Gly14]-humanin on inflammation and apoptotic markers mRNA level in CIS-induced ototoxicity. C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E. Different letters (a, b, c, d) in the same column represent a statistically significant difference (P<0.05).

(28.2±9.4) and GSH (28.2±9.4) levels were decreased (P<0.001), while MDA (7.64±1.1) levels were increased (P<0.001) with CIS application in the cochlear tissue. With the application of [Gly14]-humanin, these parameters approached their normal levels (Figure 1). Similar findings were found in brain tissue analyses of mice. Decreased SOD activity and GSH levels, and increased MDA levels with CIS application were regulated by [Gly14]-humanin application.

In comparison to the control group, we found significant increases (P<0.001) in pro-apoptotic markers, CAS-3 (5.81±0.7) (P<0.001), inflammatory markers TNF-α (3.3±0.2) (P<0.05), IL-1β (3.2±0.3) (P=0.035), and IL-6 (10.3±0.8) (P<0.001) after cisplatin challenge. In addition, significant reductions (P<0.05) in pro-apoptotic markers, CAS-9 (1.9±0.1) (P=0.035) gene expression and Bcl-2/Bax ratio (0.2±0.1) (P<0.001) after cisplatin application were observed. Moreover, our results revealed that [Gly14]-humanin expecially high dose treatment significantly decreased CAS-3 (3.4±0.7) (P<0.002) gene expression, CAS-9 (1.3±0.5) (P<0.04), TNF-α (1.5±0.3) (P<0.001), IL-1β (1.5±0.3) (P<0.001), and IL-6 (5.2±0.4) (P<0.001), and increased Bcl-2/Bax ratio (0.8±0.1) (P<0.04) gene expression (Figure 2).

TNF-α, IL-1β, IL-6, CAS-3, and CAS-9 values increased significantly with CIS application to mice, while Bcl-2/BAX values were significantly decreased compared to control. These values were normalized depending on the dose (especially [Gly14]-humanin 6 mg/kg) with the administration of [Gly14]-humanin (Figure 2).

In cis groups, both PARP1 and PARP2 exhibited intense immunoreactivity. It was determined that PARP1 immunoreactivity decreased at a similar rate in the [Gly14]-humanin 6 mg/kg and Vitamin E groups. It was determined that PARP2 immunoreactivity decreased similarly in the [Gly14]-humanin 6 mg/kg and Vitamin E. (Figure 3).

Both PARP1 and PARP2 exhibited intense immunoreactivity in the cisplatin group compared to the control groups. Immunohistochemical analysis showed that increased expression of PARP1 in tissue in the cisplatin group tended to decrease with the application of [Gly14]-humanin and Vitamin E (Figure

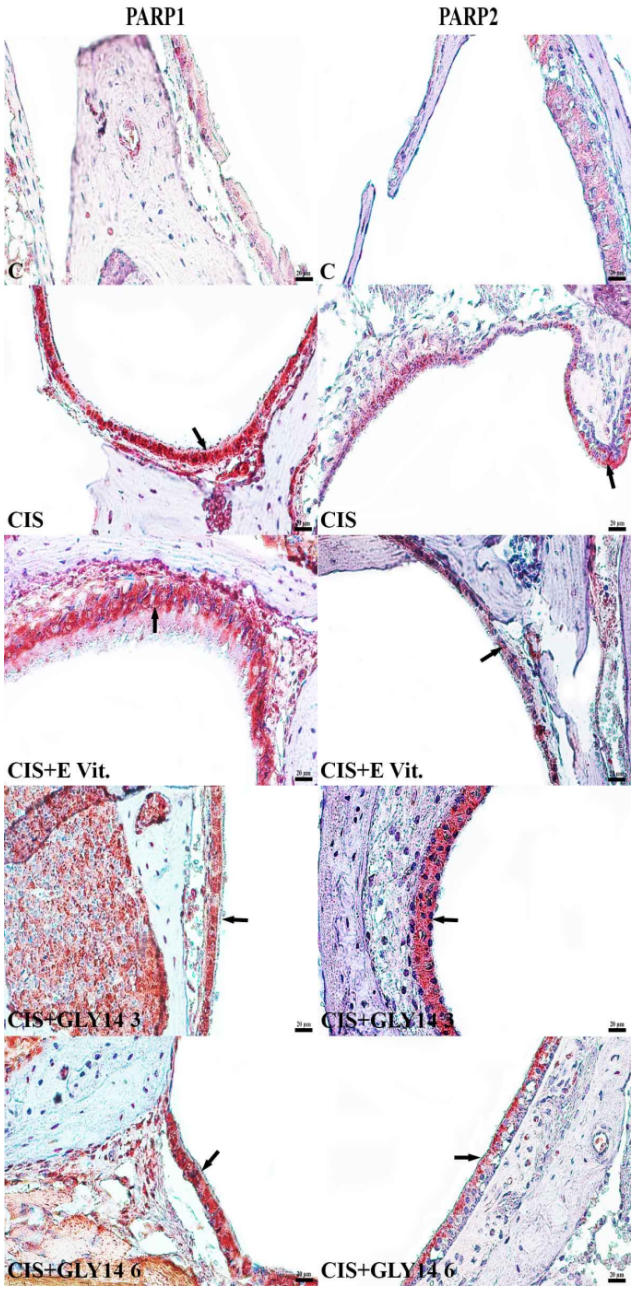

FIGURE 3. Immunohistochemical staining of PARP1 and PARP2 in all groups. Positive-stained cells are shown by arrows. Strept-ABC-IHC, AEC, paraffin section. Scale bar: 20 µm for all panels (C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E; PARP, Poly (ADP-ribose) polymerase).

4A). Additionally, the [Gly14]-humanin 6 mg/kg group and Vitamin E group were similar in terms of healing, and less immunoreactivity was observed compared to [Gly14]-humanin 3 mg/kg. Immunohistochemically, the increased expression of PARP1 in the cisplatin group tended to decrease with the application of [Gly14]-humanin and vitamin E (Figure 4B). The increased PARP2 expression with cisplatin administration also decreased with the application of [Gly14]-humanin and vitamin E. In addition, while [Gly14]-humanin 6 mg/kg and vitamin E applications were statistically similar, the vitamin E group was similar to the control group.

DISCUSSION

Cisplatin can produce an ototoxic effect through complex pathophysiological mechanisms. This ototoxic effect largely involves cellular processes such as excessive ROS production in cochlear tissue, oxidative stress, inflammatory responses, and apoptosis [1, 2, 8, 9]. The purpose of this study was to investigate the potential protective effects of [Gly14]-humanin against CIO. The findings showed that [Gly14]-humanin significantly reduces oxidative stress, inflammation, and apoptosis induced by cisplatin, and thus exhibits a protective effect in cochlear tissue. These results suggest that [Gly14]-humanin may exhibit a potential therapeutic effect against CIO.

Cisplatin induces mitochondrial dysfunction in cochlear hair cells and supporting cells, and leads to ROS accumulation by inhibiting antioxidant enzymatic systems. This causes a severe oxidative stress response characterized by a decrease in SOD activity, depletion of GSH levels, and an increase in lipid peroxidation products such as MDA [10-12]. In agreement with the current literature, cisplatin administration in the present study increased MDA levels while decreasing those of SOD and GSH. This confirms that cisplatin triggers a potent oxidative stress response [2, 5, 9]. In this study, we observed that these parameters (SOD, GSH, and MDA) approached their normal levels with [Gly14]-humanin application. The fact that [Gly14]-humanin application restored these oxidative stress markers to close to normal levels shows that this peptide possesses powerful antioxidant properties. This is consistent with previous studies showing that humanin and derivatives thereof exhibit the ability to lower oxidative stress [23, 31, 32]. In particular, a recent study by Liu *et al*. showed that [Gly14]-humanin improved oligoasthenozospermia by

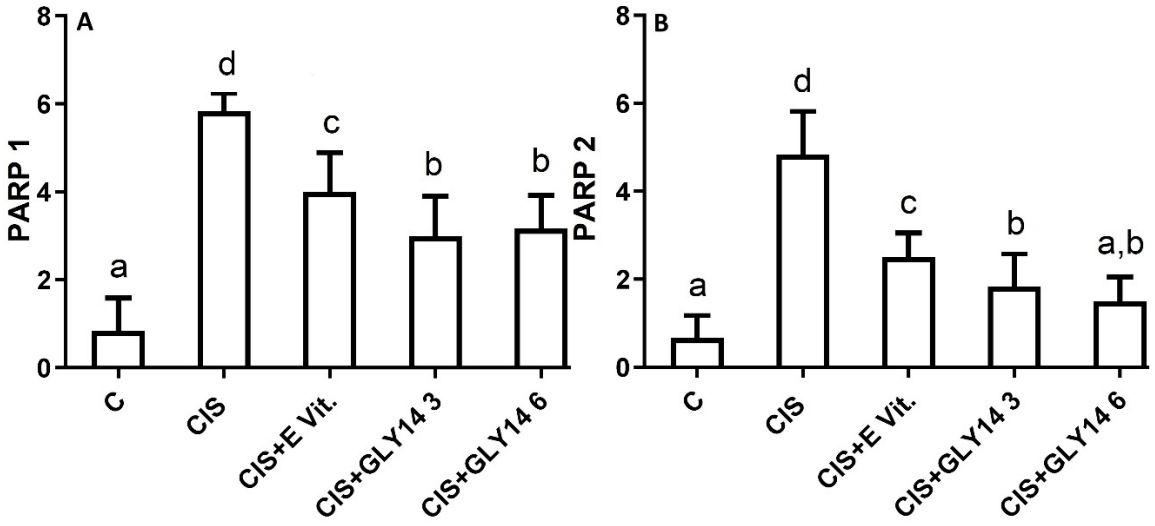

FIGURE 4. Statistical graphical representation of PARP1 (A) and PARP2 (B) IHC scores of inner ear slices. C, Control; CIS, Cisplatin; GLY 3, [Gly14]-humanin 3 mg/kg; GLY 6, [Gly14]-humanin 6 mg/kg; E Vit, Vitamin E; IHC, Immunohistochemistry. Different letters (a, b, c, d, e) in the same column represent a statistically significant difference (P<0.05).

reducing oxidative stress [23]. This suggests that [Gly14]-humanin possesses antioxidant properties at differing doses and in different toxicity models.

Oxidative stress triggers the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and initiates an inflammatory cascade by activating transcription factors, including nuclear factor kappa B. In addition, cisplatin-induced oxidative stress and inflammation activate caspase-dependent and –independent apoptotic pathways, thus leading to the programmed death of cochlear hair cells [2, 8].

In our study, significant increases were found in pro-apoptotic markers CAS-3 (P<0.05), inflammatory markers TNF-α (P<0.05), IL-1β (P<0.05) and IL-6 (P<0.05) after cisplatin administration compared to the control group. In addition, significant decreases (P<0.05) were observed following cisplatin administration in anti-apoptotic markers, in CAS-9 (P<0.05) gene expression, and in the Bcl-2/Bax ratio (P<0.05). These values normalized with the application of [Gly14]-humanin, particularly [Gly14]-humanin 6 mg/kg, in a dose-dependent manner. These findings clearly show that cisplatin triggers programmed cell death and an inflammatory response in cochlear cells. The fact that [Gly14]-humanin treatment significantly lowered these inflammatory and apoptotic markers and increased the Bcl-2/Bax ratio shows that it exhibits anti-inflammatory and anti-apoptotic effects. Previous studies have also reported that humanin and its analogs possess anti-apoptotic and anti-inflammatory characteristics [19, 21]. In particular. Jin *et al*. reported that [Gly14]-humanin prevented cell death by obstructing mitochondrial dysfunction [20]. This suggests that GLY14 may exhibit a protective effect by directly targeting cellular damage derived from cisplatin treatment. Apart from its anti-inflammatory and antioxidant properties, [Gly14]-humanin may also have cytoprotective effects by regulating mitochondrial homeostasis and triggering pro-survival pathways like PI3K/Akt signaling and STAT3, which are recognized downstream targets of humanin analogs. The observed decrease in oxidative stress and cochlear apoptosis in our study could be attributed to these pathways.

PARP is an important enzyme in the DNA damage response and cell death. Its activation is known to play an important role in cisplatin-induced cell damage [24]. Histopathological investigation in the present study revealed intense PARP1 and PARP2 immunoreactivity in the cisplatin group, while the application of GLY-14 and vitamin E reduced PARP1 and PARP2 expression. This suggests that [Gly14]-humanin and vitamin E may have the potential to reduce DNA damage and resulting cell death. In particular, the fact that the GLY-14 6 group exhibited

similar healing outcomes to the group in which vitamin E, a known antioxidant and otoprotective agent, was applied suggests that [Gly14]-humanin may be as effective as vitamin E [25].

To the best of our knowledge, this is the first experimental animal study investigating the effect of GLY14 on ototoxicity. Further clinical research evaluating hearing functions and involving long-term follow-up is now needed to confirm these effects in humans.

Strengths and Limitations

One strength of this study is that it compared the effects of two distinct [Gly14]-humanin dosages, 3 mg/kg and 6 mg/kg. The 6 mg/kg dose normalized oxidative stress and inflammatory parameters more effectively than the 3 mg/kg dose. This dose-dependent effect supports the idea of a potential therapeutic role of [Gly14]-humanin. Another strength of this study is the inclusion of vitamin E, with known antioxidant and otoprotective effects, as a positive control. This allowed us to compare the effectiveness of [Gly14]-humanin and vitamin E. That comparison may yield important data regarding the efficacy of [Gly14]-humanin. Further studies including molecular level analyses and different routes of administration (such as intratympanic injection) are now needed to fully elucidate the effect mechanism of [Gly14]-humanin.

However, there are also a number of limitations to this study. The study used waste materials from the project investigating the neuroprotective effect of [Gly14]-humanin on cisplatin-induced neurotoxicity in mice. Therefore, auditory function testing (ABR/DPOAE) could not be performed, and thus, the hearing functions of the animals before and after the experiment could not be compared. In addition, due to the brief follow-up period, this study does not provide information concerning the long-term effects of CIO or the protective potential of [Gly14]-humanin against those long-term effects.

CONCLUSION

The findings of this study show that [Gly14]-humanin exhibits important protective effects against CIO. It may protect cochlear tissue against damage by reducing oxidative stress, inflammation, and apoptosis induced by cisplatin. [Gly14]-humanin may exhibit more pronounced healing at 6 mg/kg. Our findings show that [Gly14]-humanin exhibits similar effects to vitamin E, a known antioxidant. Further studies evaluating the clinical efficacy and reliability of [Gly14]-humanin are now needed.

Ethics Approval and Consent to Participate

This study was approved by the the Kastamonu University Animal Experiments Local Ethics Committee (Decision No: 2024/11-39; date: 25.10.2024). All experimental procedures involving animals were conducted in accordance with the ethical standards of the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: MY, SG, İÇ, MT; Study Design: MY, YA; Supervision: MY, FA, SG; Funding: MY; Materials: MY, SG, İÇ, MT; Data Collection and/or Processing: SG, İÇ, MT; Statistical Analysis and/or Data Interpretation: MY, SG, İÇ; Literature Review: MY, SG, İÇ, FA, YA, MT; Manuscript Preparation: MY, FA, YA; and Critical Review: MY, FA, YA.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author(s) declare that no artificial intelligence-based tools or applications were used during the preparation process of this manuscript. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. Tang Q, Wang X, Jin H, et al. Cisplatin-induced ototoxicity: Updates on molecular mechanisms and otoprotective strategies. Eur J Pharm Biopharm. 2021;163:60-71. doi: 10.1016/j.ejpb.2021.03.008.
2. Rybak LP, Whitworth CA, Mukherjea D, Ramkumar V. Mechanisms of cisplatin-induced ototoxicity and prevention. Hear Res. 2007;226(1-2):157-67. doi: 10.1016/j.heares.2006.09.015.
3. Nagy JL, Adelstein DJ, Newman CW, Rybicki LA, Rice TW, Lavertu P. Cisplatin ototoxicity: the importance of baseline audiometry. Am J Clin Oncol. 1999;22(3):305-308. doi: 10.1097/00000421-199906000-00020.
4. Dai D, Chen C, Lu C, Guo Y, Li Q, Sun C. Apoptosis, autophagy, ferroptosis, and pyroptosis in cisplatin-induced ototoxicity and protective agents. Front Pharmacol. 2024;15:1430469. doi: 10.3389/fphar.2024.1430469.
5. Sheth S, Mukherjea D, Rybak LP, Ramkumar V. Mechanisms of Cisplatin-Induced Ototoxicity and Otoprotection. Front Cell Neurosci. 2017;11:338. doi: 10.3389/fncel.2017.00338.
6. Liu Z, Zhang H, Hong G, et al. Inhibition of Gpx4-mediated ferroptosis alleviates cisplatin-induced hearing loss in C57BL/6 mice. Mol Ther. 2024;32(5):1387-1406. doi: 10.1016/j.ymthe.2024.02.029.
7. Xu B, Li J, Chen X, Kou M. Puerarin attenuates cisplatin-induced apoptosis of hair cells through the mitochondrial apoptotic pathway. Biochim Biophys Acta Mol Cell Res. 2022;1869(4):119208. doi: 10.1016/j.bbamcr.2021.119208.
8. Lu X, Deng T, Dong H, et al. Novel Application of Eupatilin for Effectively Attenuating Cisplatin-Induced Auditory Hair Cell Death via Mitochondrial Apoptosis Pathway. Oxid Med Cell Longev. 2022;2022:1090034. doi: 10.1155/2022/1090034.
9. Ramkumar V, Mukherjea D, Dhukhwa A, Rybak LP. Oxidative Stress and Inflammation Caused by Cisplatin Ototoxicity. Antioxidants (Basel). 2021;10(12):1919. doi: 10.3390/antiox10121919.
10. Finkel T. Signal transduction by reactive oxygen species. J Cell Biol. 2011;194(1):7-15. doi: 10.1083/jcb.201102095.
11. Sies H, Belousov VV, Chandel NS, et al. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. Nat Rev Mol Cell Biol. 2022;23(7):499-515. doi: 10.1038/s41580-022-00456-z.
12. Gentilin E, Simoni E, Candito M, Cazzador D, Astolfi L. Cisplatin-Induced Ototoxicity: Updates on Molecular Targets. Trends Mol Med. 2019;25(12):1123-1132. doi: 10.1016/j.molmed.2019.08.002.
13. Wang X, Zhou Y, Wang D, et al. Cisplatin-induced ototoxicity: From signaling network to therapeutic targets. Biomed Pharmacother. 2023;157:114045. doi: 10.1016/j.biopha.2022.114045.
14. Kim KH. Intranasal delivery of mitochondrial protein humanin rescues cell death and promotes mitochondrial function in Parkinson's disease. Theranostics. 2023;13(10):3330-3345. doi: 10.7150/thno.84165.
15. Alqahtani SM, Al-Kuraishy HM, Al-Gareeb AI, et al. The neuroprotective role of Humanin in Alzheimer's disease: The molecular effects. Eur J Pharmacol. 2025;998:177510. doi: 10.1016/j.ejphar.2025.177510.
16. Thiankhaw K, Chattipakorn K, Chattipakorn SC, Chattipakorn N. Roles of humanin and derivatives on the pathology of neurodegenerative diseases and cognition. Biochim Biophys Acta Gen Subj. 2022;1866(4):130097. doi: 10.1016/j.bbagen.2022.130097.
17. Karachaliou CE, Livaniou E. Neuroprotective Action of Humanin and Humanin Analogues: Research Findings and Perspectives. Biology (Basel). 2023;12(12):1534. doi: 10.3390/biology12121534.
18. Zhu S, Hu X, Bennett S, Xu J, Mai Y. The Molecular Structure and Role of Humanin in Neural and Skeletal Diseases, and in Tissue Regeneration. Front Cell Dev Biol. 2022;10:823354. doi: 10.3389/fcell.2022.823354.
19. Zapała B, Kaczyński Ł, Kieć-Wilk B, et al. Humanins, the neuroprotective and cytoprotective peptides with antiapoptotic and anti-inflammatory properties. Pharmacol Rep. 2010;62(5):767-777. doi: 10.1016/s1734-1140(10)70337-6.
20. Jin H, Liu T, Wang WX, et al. Protective effects of [Gly14]-Humanin on beta-amyloid-induced PC12 cell death by preventing mitochondrial dysfunction. Neurochem Int. 2010;56(3):417-423. doi: 10.1016/j.neuint.2009.11.015.
21. Hazafa A, Batool A, Ahmad S, et al. Humanin: A mitochondrial-derived peptide in the treatment of apoptosis-related diseases. Life Sci. 2021;264:118679. doi: 10.1016/j.lfs.2020.118679.
22. Li M, Liu Z, Cao X, et al. [Gly14]-Humanin ameliorates high glucose-induced endothelial senescence via SIRT6. Sci Rep. 2024;14(1):30924. doi: 10.1038/s41598-024-81878-x.
23. Liu Y, Feng Q, Zou L, Zhu C, Xia W. Oligoasthenozoospermia is alleviated in a mouse model by [Gly14]-humanin-mediated attenuation of oxidative stress and ferroptosis. Andrology. 2025;13(6):1541-1555. doi:

10.1111/andr.13786.
24. Wang XL, Lin FL, Xu W, Wang C, Wang QQ, Jiang RW. Silybin B exerts protective effect on cisplatin-induced neurotoxicity by alleviating DNA damage and apoptosis. J Ethnopharmacol. 2022;288:114938. doi: 10.1016/j.jep.2021.114938.
25. Aissous I, Benrebai M, Ameddah S, et al. The preventive effects of Centaurea maroccanaBall. extract against oxidative stress induced by cisplatin in mice brains: in vitro and in vivostudies. Drug Chem Toxicol. 2023;46(6):1162-1175. doi: 10.1080/01480545.2022.2139841.
26. Gelen V, Sengul E, Yildirim S, Cinar İ. The role of GRP78/ATF6/IRE1 and caspase-3/Bax/Bcl2 signaling pathways in the protective effects of gallic acid against cadmium-induced liver damage in rats. Iran J Basic Med Sci. 2023;26(11):1326-1333. doi: 10.22038/IJBMS.2023.71343.15525.
27. Çınar İ, Gıdık B, Dirican E. Determination of anti-cancer effects of Nigella sativa seed oil on MCF7 breast and AGS gastric cancer cells. Mol Biol Rep. 2024;51(1):491. doi: 10.1007/s11033-024-09453-1.
28. Tatar M, Tüfekci KK. An investigation of the distributions of ferroptosis and necroptosis mediators in the maternal-fetal interface at different days of rat pregnancy. Anat Histol Embryol. 2024;53(1):e12991. doi: 10.1111/ahe.12991.
29. Tatar M, Tüfekci KK, Uslu S. A determination of the main regulators of necroptosis in testicular tissue under different heat stresses. J Mol Histol. 2025;56(1):74. doi: 10.1007/s10735-024-10350-x.
30. Cinar I, Sirin B, Aydin P, Toktay E, Cadirci E, Halici I, Halici Z. Ameliorative effect of gossypin against acute lung injury in experimental sepsis model of rats. Life Sci. 2019;221:327-334. doi: 10.1016/j.lfs.2019.02.039.
31. Cai H, Liu Y, Men H, Zheng Y. Protective Mechanism of Humanin Against Oxidative Stress in Aging-Related Cardiovascular Diseases. Front Endocrinol (Lausanne). 2021;12:683151. doi: 10.3389/fendo.2021.683151.
32. Klein LE, Cui L, Gong Z, Su K, Muzumdar R. A humanin analog decreases oxidative stress and preserves mitochondrial integrity in cardiac myoblasts. Biochem Biophys Res Commun. 2013;440(2):197-203. doi: 10.1016/j.bbrc.2013.08.055.

The European Research Journal 2026;12(1):129-138 Original Article

DOI: https://doi.org/10.18621/eurj.1797106 *Infectious Diseases and Clinical Microbiology*

Isolated Peripheral Tuberculous Lymphadenitis: Insights into Diagnostic Delays and Clinical Challenges

Emel Gürcüoğlu[1]

[1]*Department of Infectious Diseases and Clinical Microbiology, Bursa City Hospital, Bursa, Türkiye*

ABSTRACT

Objectives: Peripheral tuberculous lymphadenitis (TB-LAP) is the most common form of extrapulmonary tuberculosis (EPTB) and remains diagnostically challenging due to its low bacillary burden. This study aimed to evaluate diagnostic pathways, microbiological confirmation rates, and treatment outcomes in adult TB-LAP cases.

Methods: We retrospectively analyzed 30 adult patients diagnosed with TB- LAP at a tertiary care center between 2021 and 2025. Inclusion criteria were isolated peripheral lymphadenopathy with compatible histopathology, supported by culture, PCR, or clinical-radiological findings. Data regarding diagnostic delay, referral pathways, microbiological yield, and treatment outcomes were collected.

Results: Among 158 peripheral lymphadenopathy cases, 52 were diagnosed as TB-LAP, and 30 with complete follow-up data were included in the final analysis. The mean age was 39.2 years, and 83.3% were female. Culture positivity was 28.6%, Polymerase Chain Reaction (PCR) 42.9%, and acid-fast bacilli were detected in only one patient. Overall microbiological confirmation rate was 23.3%. Treatment was successfully completed in most cases, with treatment-related complication rate of 6.7%. Diagnostic delay ≥2 months was observed in 56.7% of patients. Early referral to the infectious diseases clinic significantly reduced diagnostic delay (<2 months in 72.7% vs. 15.4% in internal medicine; P=0.017). Histopathology most frequently showed granulomatous inflammation, while caseating necrotizing inflammation (23.3%) was the strongest supportive finding.

Conclusions: In this 30-case series of isolated peripheral TB-LAP, histopathology - supported by PCR when available - provided the most reliable diagnostic contribution, while culture remained limited in keeping with the paucibacillary nature of the disease. The findings highlight the need for more sensitive molecular tools, yet indicate that, for now, timely tissue sampling and careful clinical–pathological interpretation remain the most dependable approach in routine practice.

Keywords: Tuberculosis, Lymphadenitis, Extrapulmonary, Tuberculosis, Diagnosis, Histopathology, Diagnostic Delay

Peripheral tuberculous lymphadenitis (TB-LAP) is the most common form of extrapulmonary tuberculosis (EPTB) and typically presents with persistent, non-specific cervical lymphadenopathy [1-3]. Its diagnosis is often complicated by the paucibacillary nature of the disease and the slow turnaround of mycobacterial culture, which together contribute to substantial delays in clinical decision-making. As a result, histopathology frequently becomes the initial basis for diagnosis, although

Received: October 5, 2025 **Accepted:** December 2, 2025 **Available Online:** December 15, 2025 **Published:** January 4, 2025

How to cite this article: Gürcüoğlu E. Isolated Peripheral Tuberculous Lymphadenitis: Insights into Diagnostic Delays and Clinical Challenges. Eur Res J. 2026(1):129-138. doi: 10.18621/eurj.1797106

Corresponding author: Emel Gürcüoğlu, MD., Phone: +90 224 975 00 00, E-mail: emelshen91@gmail.com

This is an **open-access** article distributed under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it.

Available Online at https://dergipark.org.tr/en/pub/eurj

caseating granulomatous inflammation is not specific to tuberculosis and may overlap with sarcoidosis, fungal infections, nontuberculous mycobacteria, or malignancy [4, 5]. Recent multicentre data reporting microbiological confirmation rates of only 20-40% further highlight the practical limitations of current diagnostic tools and the continued reliance on histopathology in routine practice [6].

A further determinant of diagnostic delay is the route by which patients enter the healthcare system. Whether individuals first present to infectious diseases services, internal medicine or surgical clinics, or are referred through the national tuberculosis surveillance pathway may significantly influence biopsy timing, the speed of diagnostic clarification, and subsequent management. In a condition already marked by diagnostic uncertainty, such variations in referral pathways represent an additional source of variability.

This study examines diagnostic pathways in adult TB-LAP, focusing on referral patterns associated with diagnostic delay, and evaluates histopathological and microbiological confirmation rates. It also assesses the diagnostic contribution of culture, Polymerase Chain Reaction (PCR), Interferon-Gamma Release Assay (IGRA), and histopathology in relation to treatment outcomes. The aim is to provide a clearer framework for improving diagnostic confidence and accelerating clinical decision-making in endemic settings.

METHODS

Between 2021 and 2025, we retrospectively evaluated 158 patients who presented to the Health Sciences University Bursa City Hospital with single or multiple isolated peripheral lymph node involvement after the exclusion of acute lymphadenitis. While not a dedicated tuberculosis centre, our institution serves as a tertiary-care hospital with extensive diagnostic capabilities and advanced imaging infrastructure.

Cases diagnosed histopathologically as chronic granulomatous lymphadenitis were initially identified. However, since the presence of granulomas is not specific for tuberculosis, only those supported by culture, PCR, or compatible clinical-radiological findings were classified as tuberculous lymphadenitis. Of the 52 identified cases, 22 were excluded due to incomplete treatment or follow-up data; thus, the final analysis was conducted on 30 patients. Diagnostic delay was defined as the interval between the first hospital admission and the definitive diagnosis and was categorized as <2 months or ≥2 months. Patients were further stratified according to referral pathways. Only adult patients aged 18 years and older were included.

Inclusion criteria were ≥18 years of age, isolated peripheral lymph node involvement, histopathological findings consistent with tuberculous lymphadenitis, and availability of complete follow-up data.

Exclusion criteria were history of malignancy, prior tuberculosis, concomitant pulmonary tuberculosis, and intra-abdominal or intrathoracic lymphadenopathy. Intra-abdominal and intrathoracic lymphadenopathies were excluded due to the possibility of secondary spread from adjacent parenchymal structures and their limited diagnostic value.

Referral pathways were evaluated in three groups. The direct referral group included patients who presented with systemic symptoms and were found to have lymphadenopathy on examination, or those referred directly to the infectious diseases outpatient clinic without further preliminary work-up. Patients initially evaluated in internal medicine or surgical clinics with a presumptive diagnosis of malignancy, later referred to the infectious diseases clinic during the diagnostic process, together with those reported through the Türkiye's National Tuberculosis Surveillance System (NTSS-TR) after pathology demonstrated chronic granulomatous inflammation, were analyzed separately in sub-analyses due to differences in timing and referral processes. Patients first assessed in internal medicine or surgical clinics for suspected malignancy and subsequently referred to the infectious diseases clinic, as well as those identified through NTSS-TR following histopathological reports of chronic granulomatous inflammation, were ultimately classified within the same group. This categorization was made to reflect similarities in diagnostic evaluation despite different referral routes, while also highlighting the impact of referral timing on the diagnostic process. All clinical, microbiological, radiological, and histopathological data were retrospectively obtained from patient files and laboratory records. The following were recorded: Purified Protein Derivative (PPD), IGRA, complete blood count, C-Reactive Protein (CRP), and Erythrocyte Sedimentation Rate (ESR) results. IGRA

was performed using the QuantiFERON-TB Gold Plus platform (Qiagen, Hilden, Germany). Molecular detection of the Mycobacterium tuberculosis complex was undertaken on lymph node tissue samples using the Xpert MTB/RIF Ultra system (Cepheid, Sunnyvale, CA, USA).

The diagnosis of tuberculous lymphadenitis was primarily based on histopathology and, when available, supported by culture or PCR results.

Not all patients underwent all tests; therefore, diagnostic evaluation was performed according to available data, incorporating clinical and radiological compatibility. Lymph node biopsy was performed in all confirmed TB-LAP cases (n=30); mycobacterial culture was requested for 14 samples and PCR was performed in seven samples.

The study was limited to tuberculous lymphadenitis cases. Since histopathology, culture, or PCR results were not available for all patients, alternative causes of lymphadenitis with similar clinical or pathologicaxal presentation were excluded by reviewing patient files.

Specifically, Bartonella henselae, Toxoplasma gondii, Francisella tularensis, Epstein–Barr virus (EBV), Coxiella burnetii, Treponema pallidum, as well as non-infectious causes such as sarcoidosis, reactive lymphadenitis, and malignant lymphadenopathies were excluded based on clinical, serological, and histopathological findings.

Radiological evaluations were reviewed from patient records. Ultrasonography was performed in all cases to confirm peripheral lymph node involvement. When clinically indicated, further evaluation included computed tomography (CT) and magnetic resonance imaging (MRI), while PET-CT was performed in selected cases with suspected malignancy or disseminated disease. Imaging was also used to exclude intra-abdominal and intrathoracic involvement.

Histopathological diagnosis was based on the presence of granulomas (with or without necrosis or caseation). Findings were categorized as granulomatous, necrotizing, caseating necrotizing, necrotizing granulomatous, or atypical/nonspecific. Additionally, records of fluoroquinolone use within 30 days prior to diagnosis were reviewed, as this factor may contribute to diagnostic delay [7]

This study was approved by the Bursa City Hospital Scientific Research Ethics Committee (11.06.2025/2025-12/09). All procedures complied with the principles of the Declaration of Helsinki.

Statistical Analysis

All statistical analyses were performed using SPSS version 25.0 (IBM SPSS, Chicago, IL, USA). Descriptive data were presented as means and standard deviations, while distributions of nominal or ordinal variables were expressed as counts and percentages. Comparisons between groups for

TABLE 1. Diagnostic Distribution of Prolonged Peripheral Lymphadenopathy Cases (n=158)

Diagnostic group	n	%
Hematological malignancies	34	21.5
Reactive lymphadenitis	32	20.3
Tuberculous lymphadenitis	52	32.9
Bartonella henselae (cat scratch disease)	11	7.0
Solid-organ malignancies	11	7.0
Toxoplasma gondii lymphadenitis	9	5.7
Tularemia (*Francisella tularensis*)	2	1.3
Persistent Epstein–Barr virus (EBV) infection	2	1.3
Lymphadenitis secondary to rheumatologic disease	2	1.3
Sarcoidosis	1	0.6
Coxiella burnetii (Q fever)	1	0.6
Syphilis	1	0.6
Total	**158**	**100.0**

categorical variables were conducted using the Chi-square test or Fisher's Exact Test, as appropriate. The normality of distribution for continuous variables was assessed using the Kolmogorov-Smirnov test. For continuous variables not normally distributed, intergroup comparisons were performed using the Mann–Whitney U test. Correlations between ordinal variables were assessed using Spearman's rank correlation coefficient. All results were interpreted with a 95% confidence interval, and a P-value of less than 0.05 was considered statistically significant. The statistical significance of correlations was determined by the p-value, while the strength of the correlation was assessed using the Spearman correlation coefficient (rho). Correlation strength was interpreted using Spearman's rho values as follows [8]: rho = 0 → no correlation 0 < rho < 0.3 → weak correlation 0.3 ≤ rho < 0.7 → moderate correlation 0.7 ≤ rho < 1 → strong correlation rho = 1 → perfect correlation."

RESULTS

A total of 158 adult patients with peripheral lymphadenopathy who met the inclusion criteria were evaluated. The most common etiology was tuberculous lymphadenitis (32.9%, n=52), followed by hematological malignancies (21.5%, n=34) and reactive lymphadenitis (20.3%, n=32). Less frequent causes included Bartonella henselae infection (7.0%, n=11), metastatic lymphadenopathy secondary to solid organ malignancies (7.0%, n=11), and Toxoplasma gondii lymphadenitis (5.7%, n=9). Rare etiologies were tularemia (1.3%), persistent Epstein–Barr virus (EBV) infection (1.3%, n=2), rheumatologic disorders (1.3%, n=2), sarcoidosis (0.6%, n=1), Q fever (0.6%, n=1), and syphilis (0.6%, n=1) (Table 1). Of the 52 patients diagnosed with tuberculous lymphadenitis (TB-LAP), 22 (42.31%) were excluded due to incomplete follow-up data or missing records, leaving 30 patients for the final analysis. Among these, 25 (83.3%) were female and 5 (16.7%) male, with a mean age of 39.2±13.4 years. Nineteen (63.3%) patients were under 50 years of age, while 11 (36.7%) were ≥50 years. Seven (23.3%) patients were of foreign origin, and five (16.7%) had a history of immunosuppression. The most frequent site of lymph node involvement was cervical (53.3%, n=16), followed by supraclavicular (26.7%, n=8), axillary (16.7%, n=5), and epitrochlear (3.3%, n=1). All patients underwent thoracic and abdominal imaging to exclude additional involvement. The tuberculin skin test (PPD) was performed in 24 (80%) patients, with 17 (70.8%) yielding positive results. IGRA was conducted in 18 (60%) patients and was positive in 8 (44.4%). Laboratory evaluation showed ESR in 17 (60.7%) patients and elevated CRP in 94.7% (18/19) tested patients. CRP levels were significantly higher in male patients compared to females (P=0.014). Leukocytosis was observed in four (14.3%) patients. Microbiological analysis demonstrated culture positivity in 4 (28.6%) of 14 biopsies. Ziehl-Neelsen staining revealed acid-fast bacilli in only one case. PCR testing was performed in seven biopsies and detected Mycobacterium tuberculosis DNA in 3 (42.9%). Histopathological examination was positive in 26 (86.7%) of the 30 confirmed TB-LAP cases, making it the most sensitive diagnostic modality in

TABLE 2. Diagnostic Delay in Peripheral Tuberculous Lymphadenitis by Referral Pathway

Referral source	<2 Months n (%)	≥2 Months n (%)	Total (n)
Direct infectious diseases (pathology report+TB Surveillance Registry* or symptomatic referral)	8 (72.7%)	3 (27.3%)	11
Internal medicine clinics	2 (15.4%)	11 (84.6%)	13
Surgical clinics	3 (50.0%)	3 (50.0%)	6
Chi-square (χ^2) value	8.11		
P value	**0.017**		

*Türkiye's National Tuberculosis Surveillance System (NTSS-TR). Statistically significant P-value is shown in bold.

TABLE 3. Demographic, Clinical, and Laboratory Characteristics of Patients (n=30)

Variable	n	%
Gender		
Male	5	16.7
Female	25	83.3
Age group (years)		
<50	19	63.3
≥50	11	36.7
Immunosuppression		
Absent	25	83.3
Present	5	16.7
Nationality		
Turkish citizen	23	76.7
Foreign national	7	23.3
PPD result		
Normal	7	29.2
Elevated	17	70.8
Interferon-gamma release assay		
Negative	10	55.6
Positive	8	44.4
Biopsied lymph node site		
Axillary	5	16.7
Epitrochlear	1	3.3
Cervical	16	53.3
Supraclavicular	8	26.7
Imaging modality		
CT	3	10.0
MRI	1	3.3
Ultrasound	26	86.7
PET imaging		
Not performed	15	71.4
Performed	6	28.6
Fluoroquinolone use		
No	21	70.0
Yes	9	30.0
Culture result		
Negative	10	71.4
Positive	4	28.6
Acid-fast bacilli stain		
Negative	13	92.9
Positive	1	7.1

TABLE 3 Continued. Demographic, Clinical, and Laboratory Characteristics of Patients (n=30)

Variable	n	%
PCR result		
Negative	4	57.1
Positive	3	42.9
White blood cell count		
Normal	24	85.7
Elevated	4	14.3
C-reactive protein		
Normal	1	5.3
Elevated	18	94.7
Erythrocyte sedimentation rate		
Normal	11	39.3
Elevated	17	60.7
Histopathological findings		
Caseating inflammation	1	3.3
Necrotizing inflammation	3	10.0
Granulomatous inflammation	9	30.0
Caseating granulomatous inflammation	4	13.3
Caseating necrotizing inflammation	7	23.3
Necrotizing granulomatous inflammation	5	16.7
Atypical / nonspecific	1	3.3
Treatment duration		
6–9 months	15	50.0
>9 months	11	36.7
Ongoing (3–4 months)	4	13.3
Complications		
Absent	28	93.3
Present	2	6.7
Total	30	100.0

CT, computed tomography; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PET, positron emission tomography.

this cohort. While mycobacterial culture yielded positivity in only 4 (28.6%) of 14 samples, PCR detected Mycobacterium tuberculosis complex DNA in 3 (42.9%) of 7 samples, illustrating the limited microbiological yield typically associated with the low bacillary burden of TB-LAP.

When interpreted together, histopathology and PCR provided the strongest diagnostic support, with PCR offering molecular confirmation that complemented granulomatous inflammation observed in tissue samples. In contrast, culture contributed minimally to combined diagnostic assessment due to its low positivity rate.

Because culture and PCR were performed in a restricted subset of cases (n=14 and n=7, respectively), a formal comparison of sensitivity or specificity between diagnostic combinations was not feasible. Nevertheless, the available data indicate that the

histopathology + PCR combination provides the highest diagnostic value among the approaches used in this study.

Ultrasonography was the most frequently used imaging method (86.7%), followed by computed tomography (10.0%) and magnetic resonance imaging (3.3%). Positron emission tomography– computed tomography (PET-CT) was employed in selected cases (28.6%) (Table 3). These findings highlight the role of ultrasonography as the primary diagnostic tool, while advanced imaging was reserved for selected indications. Histopathological examination was available in all patients and showed heterogeneous patterns. The most common findings were granulomatous inflammation (30.0%), caseating necrotizing inflammation (23.3%), and necrotizing granulomatous inflammation (16.7%). Less frequent features included caseating granulomatous inflammation (13.3%), necrotizing inflammation (10.0%), caseating inflammation (3.3%), and atypical/nonspecific changes (3.3%). Regarding treatment duration, 15 (50.0%) patients completed therapy within 6-9 months, 11 (36.7%) required >9 months of treatment, and 4 (13.3%) were still under therapy at the time of data cutoff. The complication rate was 6.7%, most commonly presenting as fistula formation or fluctuation. Diagnostic delay ≥2 months was observed in 17 (56.7%) patients. Among those presenting directly to the infectious diseases clinic, 72.7% were diagnosed within 2 months, compared with 15.4% in internal medicine clinics and 50.0% in surgical clinics (χ^2=8.11, P=0.017) (Table 2).

Spearman correlation analysis revealed a moderate, statistically significant association between elevated ESR and IGRA positivity (rho = 0.624; P=0.010) (Table 4).

DISCUSSION

EPTB in adults is typically defined by a low bacillary load and prolonged turnaround times for microbiological confirmation, often compelling clinicians to rely on histopathological assessment. Yet, the diagnostic accuracy of histopathology alone remains debated, and in endemic regions it is regarded more as a pragmatic necessity than a definitive standard [1, 9]. In our cohort, despite the limited rate of microbiological confirmation, antituberculous therapy initiated on the basis of histopathological findings led to high clinical response rates and minimal complications. Although this does not render histopathology infallible, the strong concordance between clinical presentation and tissue pathology - reinforced by favorable treatment outcomes -supports its continued diagnostic value in endemic settings.

A closer examination of test performance underscores the inherent limitations of microbiological methods in TB-LAP. Culture positivity was low (28.6%), consistent with previous reports highlighting the difficulty of recovering viable bacilli from necrotic, paucibacillary lymph node tissue [10, 11]. PCR positivity was higher (42.9%), reflecting the superior sensitivity of molecular assays in detecting low-level bacillary DNA and their complementary role alongside histopathology. The combination of histopathology and PCR provided the greatest diagnostic confidence, whereas culture contributed minimally due to its limited yield. Although the subset of specimens subjected to culture and PCR was small - precluding formal sensitivity and specificity analysis - the overall pattern strongly suggests that the histopathology - PCR combination offers the highest diagnostic value in routine clinical practice. These

TABLE 4. Correlation Analyses

	PPD	IGRA
WBC (rho/P)	0.290/0.191	0.181/0.488
CRP (rho/P)	-0.130/0.657	0.319/0.312
ESR (rho/P)	0.080/0.722	0.624**/0.010
PPD (rho/P)	1.000/-	0.488/0.108

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IGRA, interferon-gamma release assay; PPD, purified protein derivative. Statistical test: Spearman's correlation analysis.

**A moderate positive and statistically significant correlation was found between ESR and IGRA levels (rho = 0.624; P=0.010).

findings reinforce the principle that, particularly in endemic regions, diagnosis must be grounded in an integrated assessment of clinical, epidemiological, and histopathological data, rather than relying exclusively on microbiological confirmation.

Histopathological evaluation was available in all cases, with caseating necrotizing granulomatous inflammation identified in 23.3%, representing the most characteristic morphological hallmark of tuberculosis. Microbiological confirmation was achieved in 23.3% of patients, consistent with the 20–40% range reported in prior TB-LAP cohorts [10, 11]. All patients received standard antituberculous therapy, and the majority completed treatment successfully, further reinforcing the reliability of clinical–pathological concordance as a basis for initiating therapy in endemic settings [9, 12].

Regarding immunological and inflammatory markers, tuberculin skin test (PPD) positivity (70.8%) was comparable to previously reported rates among immunocompetent adults [13], though universal BCG vaccination and latent infection continue to limit its specificity [4]. IGRA positivity (44.4%) aligned with the expected 30–60% positivity range in EPTB [14, 15]. Elevated ESR was observed in 60.7% of patients and correlated strongly with IGRA positivity (rho = 0.624; P=0.010), suggesting that IGRA may reflect both immune sensitization and underlying inflammatory activity [6]. CRP was elevated in 94.7% of tested patients and was significantly higher in men, supporting its utility as an adjunctive marker that strengthens clinical suspicion and aids in monitoring disease activity despite its limited specificity [16].

Diagnostic delay was markedly influenced by referral pathways. Patients presenting directly to the infectious diseases clinic were more frequently diagnosed within two months (72.7%) compared with those evaluated in internal medicine (15.4%) or surgical clinics (50.0%) (χ^2 = 8.11; P=0.017). These discrepancies likely reflect differences in how prominently tuberculosis features in the differential diagnosis across specialties - even in endemic areas. Cases reported through Türkiye's National Tuberculosis Surveillance System (NTSS-TR) commonly underwent early biopsy due to suspected malignancy and were subsequently referred to infectious diseases specialists once granulomatous inflammation was identified histologically. Although indirect, this pathway likewise facilitated relatively early specialist evaluation. However, combining NTSS-TR–reported cases with other referral routes introduced heterogeneity that should be acknowledged as a limitation.

Comparison with recent multicenter studies from Türkiye further supports our findings. Celik *et al*. [9] described similar diagnostic challenges across EPTB presentations, while Yenilmez *et al*. [13] emphasized the persistently low microbiological confirmation rates in peripheral TB lymphadenitis. Our single-center results align closely with these observations, underscoring that diagnostic delay and reliance on histopathology remain ongoing challenges in endemic settings.

Strengths and Limitations

This study reflects real-world clinical practice in a tertiary care setting and focuses on adult isolated peripheral tuberculous lymphadenitis, a frequently under-recognised and diagnostically challenging form of extrapulmonary tuberculosis. A major strength of the study is the detailed assessment of the diagnostic process, including clinical presentation, timing of biopsy, histopathological evaluation, and the limited yield of microbiological methods. By analysing routine clinical data, the study highlights practical diagnostic challenges and underscores the importance of clinicopathological correlation in daily infectious diseases practice.

The study is limited by its retrospective, single-centre design and relatively small sample size. Molecular tests were not performed uniformly across all specimens, and culture positivity was low, restricting detailed microbiological analyses. Nevertheless, the findings provide clinically relevant insights into real-world diagnostic decision-making in peripheral TB lymphadenitis.

CONCLUSION

This series demonstrates clearly how peripheral tuberculous lymphadenitis behaves in day-to-day clinical practice. The low rate of microbiological confirmation is entirely expected; given the tissue characteristics and paucibacillary nature of the disease,

it would be unreasonable to anticipate more. What ultimately drives the diagnosis is obtaining tissue at the right time and ensuring that the case reaches a clinician who can recognise the pattern early.

Histopathology, when interpreted in an appropriate clinical context, provided adequate clarity to initiate treatment without delay in most patients. PCR supported the histological impression in the small number of samples in which it was performed, while culture contributed little, as anticipated. Current diagnostic tools remain limited; there is a clear need for more sensitive molecular assays and for studies involving larger, well-characterised cohorts. Even so, this work reinforces a familiar lesson: in TB-LAP, timely biopsy combined with thoughtful clinical–pathological correlation remains the most dependable route to a confident diagnosis.

Ethics Approval and Consent to Participate

This study was approved by the Bursa City Hospital Scientific Research Ethics Committee (Decision No: 2025-12/09; date: 11.06.2025). All procedures were conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

Data Availability

All data generated or analyzed during this study are included in this published article. The data that support the findings of this study are available on request from the corresponding author, upon reasonable request.

Authors' Contribution

Study Conception: EG; Study Design: EG; Supervision: EG; Funding: EG; Materials: EG; Data Collection and/or Processing: EG; Statistical Analysis and/or Data Interpretation: EG; Literature Review: EG; Manuscript Preparation: EG; and Critical Review: EG.

Conflict of Interest

The author(s) disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The author(s) disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgments

The authors have no acknowledgments to declare.

Generative Artificial Intelligence Statement

The author used artificial intelligence tools (Chat GPT, OpenAI) only for language editing and reference formatting. The all content of the study was produced by the author(s) in accordance with scientific research methods and academic ethical principles.

Editor's Note

All statements made in this article are solely those of the authors and do not represent the views of their affiliates or the publisher, editors, or reviewers. Any claims made by any product or manufacturer that may be evaluated in this article are not guaranteed or endorsed by the publisher.

REFERENCES

1. World Health Organization. Global Tuberculosis Report 2023. Geneva: WHO; 2023. Available at: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2023.(Accessed:August 31, 2025)
2. Polesky A, Grove W, Bhatia G. Peripheral tuberculous lymphadenitis: epidemiology, diagnosis, treatment, and outcome. Medicine (Baltimore). 2005v;84(6):350-362. doi: 10.1097/0.md.0000189090.52626.7a.
3. Mathiasen VD, Eiset AH, Andersen PH, Wejse C, Lillebaek T. Epidemiology of tuberculous lymphadenitis in Denmark: A nationwide register-based study. PLoS One. 2019;14(8):e0221232. doi: 10.1371/journal.pone.0221232.
4. Sivaratnam L, Nawi AM, Abdul Manaf MR. An evidence-based clinical pathway for the diagnosis of tuberculous lymphadenitis: A systematic review. Int J Mycobacteriol. 2020;9(2):107-115. doi: 10.4103/ijmy.ijmy_207_19.
5. Purohit M, Mustafa T. Laboratory Diagnosis of Extra-pulmonary Tuberculosis (EPTB) in Resource-constrained Setting: State of the Art, Challenges and the Need. J Clin Diagn Res. 2015;9(4):EE01-6. doi: 10.7860/JCDR/2015/12422.5792.
6. Kumar N, Khan A, Boora S, et al. Diagnosis of tuberculous lymphadenitis by molecular and immunological tools. Med Microecol. 2024;22:100116. doi: 10.1016/j.medmic.2024.100116.
7. Republic of Türkiye, Ministry of Health. Tuberculosis Control in Türkiye 2021 Report. Ankara: Republic of Türkiye Ministry of Health; 2021. Available at: https://hsgm.saglik.gov.tr. (Accessed: August 31, 2025).

8. Akoglu H. User's guide to correlation coefficients. Turk J Emerg Med. 2018 ;18(3):91-93. doi: 10.1016/j.tjem.2018.08.001.
9. Celik M, Gurbuz E, Cicek Y, et al. Demographic, clinical and laboratory characteristics of extrapulmonary tuberculosis: Eight-year results of a multicenter retrospective study in Turkey. J Investig Med. 2025;73(2):206-217. doi: 10.1177/10815589241299367.
10. Gupta V, Bhake A. Diagnosis of clinically suspected and unsuspected tubercular lymphadenopathy by cytology, culture, and smear microscopy. Indian J Tuberc. 2017;64(4):314-317. doi: 10.1016/j.ijtb.2016.11.014.
11. Méchaï F, Macaux L, Jeny F, et al. Granulomatous lymphadenopathy and tuberculosis: accuracy of histology. Int J Infect Dis. 2025;156:107920. doi: 10.1016/j.ijid.2025.107920.
12. Walker CW, Hulsey B, Pompa M. Evaluating lymphadenopathy. JAAPA. 2025;38(6):20-27. doi: 10.1097/01.JAA.0000000000000212.
13. Yenilmez E, Özakınsel D, Köse A, et al. Diagnostic approach of tuberculous lymphadenitis in a multicenter study. J Infect Dev Ctries 2024;18(5):742-750. doi: 10.3855/jidc.19502.
14. Fukushima K, Akagi K, Kondo A, Kubo T, Sakamoto N, Mukae H. First clinical evaluation of the QIAreachTM QuantiFERON-TB for tuberculosis infection and active pulmonary disease. Pulmonology. 2022;28(1):6-12. doi: 10.1016/j.pulmoe.2021.07.003.
15. Mohseni S, Shojaiefard A, Khorgami Z, Alinejad S, Ghorbani A, Ghafouri A. Peripheral lymphadenopathy: approach and diagnostic tools. Iran J Med Sci. 2014;39(Suppl):158-170.
16. Sunnetcioglu A, Sunnetcioglu M, Binici I, Baran AI, Karahocagil MK, Saydan MR. Comparative analysis of pulmonary and extrapulmonary tuberculosis of 411 cases. Ann Clin Microbiol Antimicrob. 2015;14:34. doi: 10.1186/s12941-015-0092-2.
17. Öztomurcuk D, Terzi Ö, Demirci C, Kılıçaslan Z. Investigation of Granulomatous Inflammations in Terms of Tuberculosis Diagnosis: A 5-Year Multi-center Laboratory Study. Turk Thorac J. 2022;23(1):11-16. doi: 10.5152/TurkThoracJ.2022.20314.