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Evaluation of Pediatricians' Awareness, Knowledge, and Attitudes Toward Avoidant/Restrictive Food Intake Disorder

Pediatristlerin Kaçıngan Kısıtlayıcı Gıda Alım Bozukluğu Hakkındaki Farkındalık, Bilgi ve Tutumlarının Değerlendirilmesi

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ABSTRACT

Objective: Despite the inclusion of Avoidant/Restrictive Food Intake Disorder (ARFID) in the DSM-5 in 2013, there is limited research assessing physicians' awareness and knowledge of the disorder. This study aimed to evaluate pediatricians' knowledge, awareness, clinical experience, and referral practices related to ARFID.

Materials and Methods: This cross-sectional study was conducted with 216 pediatricians (specialists and residents) working in three major hospitals in Ankara, Türkiye. Data were collected using an original, literature-based questionnaire developed by the researchers.

Results: Among participants, 28.5% correctly identified the expanded form of ARFID, and 38% recognized it as a DSM-5-defined eating disorder. A total of 52.3% misclassified body image disturbance as a subtype of ARFID, suggesting a conceptual overlap with anorexia nervosa. While 34.9% reported encountering ARFID cases in their clinical practice, 29.8% had referred such patients to other healthcare professionals. The most commonly reported referral disciplines were child and adolescent psychiatry, pediatric gastroenterology, and dietetics. Only 4.3% of respondents reported feeling competent in managing ARFID, while 90.7% expressed a need for further education and training.

Conclusion: The findings highlight significant gaps in pediatricians' knowledge and clinical management of ARFID. The development of structured guidelines, standardized diagnostic tools, and targeted training programs is warranted to improve early identification and multidisciplinary management of ARFID in pediatric settings.

Keywords: Avoidant/Restrictive food intake disorder, awareness, pediatricians

ÖZET

Amaç: Kaçıngan/Kısıtlayıcı Gıda Alım Bozukluğu (ARFID), DSM-5'te 2013'de tanımlanmasına rağmen hekimlerin bu konu hakkındaki farkındalık ve bilgi seviyesi literatürde yeteri kadar çalışılmamıştır. Bu çalışma, pediatri hekimlerinin ARFID'e ilişkin bilgi düzeyini, farkındalığını, klinik deneyimlerini ve yönlendirme davranışlarını değerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Ankara'daki üç büyük hastanede görev yapan 216 pediatri uzmanı ve asistanıyla yürütülen bu kesitsel araştırmada, veri toplama aracı olarak literatüre dayalı özgün bir anket formu kullanılmıştır.

Bulgular: Çalışmaya katılan pediatristlerin %28,5'i ARFID'nin açılımını bildiğini, %38'i ise bu bozukluğun DSM-5'te tanımlı olduğunu ifade etmiştir. Ayrıca, %52,3'lük bir kısmı beden algısı bozukluğunu ARFID alt tipi olarak belirtmiş, bu da anoreksiya nervoza ile kavramsal karışıklık yaşandığını göstermektedir. Katılımcıların %34,9'u mesleki yaşamlarında ARFID olgusu ile karşılaştığını, %29,8'i ise bu grup hastayı başka bir uzmana yönlendirdiğini ifade etmiştir. En sık yönlendirilen disiplinler çocuk ve ergen psikiyatrisi, pediatrik gastroenteroloji ve diyetisyenlik olmuştur. Hekimlerin yalnızca %4,3'ü ARFID yönetiminde kendini yetkin hissettiğini ifade etmiş, %90,7'si ise bu konuda ek eğitim ihtiyacı olduğunu belirtmiştir.

Sonuç: Bu çalışmada pediatristlerin ARFID'e yönelik bilgi düzeyi, tanı süreci ve yönlendirme uygulamalarında yetersizlikler olduğu bulunmuştur. Bu bağlamda yapılandırılmış rehberlerin, tanısal araçların ve hedefe yönelik eğitim programlarının geliştirilmesinin pediatri hasta bakım uygulamalarına katkı sağlayabileceğini düşünmekteyiz.

Anahtar kelimeler: Kaçıngan/kısıtlayıcı gıda alım bozukluğu, farkındalık, pediatristler

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Introduction

Avoidant/Restrictive Food Intake Disorder (ARFID) is an eating disorder first introduced in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) in 2013 (1). According to the DSM-5 criteria, ARFID is characterized by a persistent restriction of food intake due to low appetite, sensory sensitivities, or fear-based aversions stemming from negative eating experiences. This restrictive intake can result in significant weight loss, faltering growth, nutritional deficiencies, dependence on enteral or oral nutritional supplementation, and marked impairment in psychosocial functioning (1,2,3). Unlike anorexia nervosa (AN) or bulimia nervosa (BN), ARFID is not associated with body image disturbances, which may lead to underrecognition or misdiagnosis in clinical settings (4).

Individuals with ARFID often present with comorbid psychiatric conditions such as anxiety disorders, mood disorders, or substance use, alongside nutritional deficiencies severe enough to impact growth and development (5). Although data have been collected from both community and clinical samples, the prevalence and clinical characteristics of ARFID remain insufficiently explored in the literature, partly due to the recency of its inclusion in the DSM-5 (6).

Limited awareness of ARFID among pediatricians may contribute to delays in diagnosis and intervention (3,7). Given the relatively high prevalence of feeding difficulties and psychiatric comorbidities in both community and pediatric healthcare settings, it is likely that pediatric healthcare professionals encounter individuals with ARFID in routine clinical practice. Notably, an international expert consensus on eating disorders reported that individuals with ARFID frequently present to non-psychiatric medical services, underscoring the importance of cross-disciplinary awareness among healthcare providers who may encounter this population (3,7,9).

Since the inclusion of Avoidant/Restrictive Food Intake Disorder (ARFID) in the DSM-5, awareness among healthcare professionals has increased; however, significant gaps in knowledge and clinical experience regarding diagnosis and management persist (4,10). In a study conducted in Canada by Coelho et al.(10), only 37% of pediatric healthcare professionals were able to fully identify the DSM-5 diagnostic criteria for ARFID. Moreover, marked inconsistencies in diagnostic practices were observed when participants were presented with clinical vignettes. Similarly, Harrison (2) reported that primary care providers in the United Kingdom experienced difficulty recognizing ARFID in children, and even when a diagnosis was made, there was often insufficient knowledge regarding appropriate referral pathways. Healthcare professionals in that study identified the absence of a specific treatment protocol and referral framework for ARFID as a systemic barrier (2,11).

The lack of standardized clinical guidelines for the management of ARFID remains a critical issue (5,12) A physician survey conducted in the United States revealed considerable variation in inpatient treatment protocols for ARFID cases, with many clinicians applying anorexia nervosa–based protocols. Due to the absence of ARFID-specific treatment pathways, providers reported challenges in developing appropriate management plans (5). These findings underscore the need for enhanced training for healthcare professionals to differentiate ARFID from other eating disorders and to implement tailored management strategies (13).

The diagnosis of Avoidant/Restrictive Food Intake Disorder (ARFID) in childhood and adolescence is closely linked to the knowledge and awareness levels of pediatricians (6,10,14). In Türkiye, there is currently no awareness study specifically targeting pediatricians in relation to ARFID. This study aims to assess the knowledge, attitudes, and awareness levels of pediatric healthcare professionals regarding ARFID and to contribute to the existing body of literature in this field (15).

Methods

Participants and Sampling

This cross-sectional study was conducted among pediatricians and pediatric residents working in the pediatric clinics of three major hospitals in Ankara, each serving diverse patient populations. A conve-

nience sampling method was used. Inclusion criteria were: being an actively practicing pediatrician or pediatric resident providing outpatient or inpatient care in any pediatric unit, and willingness to participate voluntarily. Since there was no existing validated scale for ARFID awareness targeting pediatricians and the questionnaire was developed specifically for this study, the sample size calculation assumed a prevalence of 50%. Using the G*Power 3.1 software, with 50% prevalence, 95% power, and a Type I error rate (α) of 0.05, a minimum of 48 participants per hospital was required to detect a medium effect size (0.5). The study aimed to reach the maximum number of pediatricians and residents across different hospitals to ensure diversity in professional background and institutional settings, thereby increasing the representativeness of the sample. Data were collected using an online and paper-based questionnaire developed by the researchers, informed by prior studies in the literature. A total of 216 pediatricians who completed the questionnaire in full were included in the analysis.

The survey form consisted of four main components: (1) demographic questions; (2) items assessing participants' knowledge of the definition and subtypes of ARFID; (3) questions evaluating clinical diagnostic and referral behaviors; and (4) open-ended qualitative questions exploring participants' educational needs, diagnostic challenges, and referral practices. The content and clarity of the questionnaire were evaluated through a pilot test conducted with 10 pediatricians working in clinical settings, and necessary modifications were made based on their feedback.

Participants were informed about the study's purpose, the voluntary nature of participation, and confidentiality principles. Written informed consent was obtained prior to participation. Each participant was permitted to complete the survey only once, and all responses were collected anonymously. The survey link and study information were distributed through professional communication platforms (e.g., WhatsApp, email, social media) and via pediatricians who were co-authors of the study. Participation in the survey was entirely voluntary. The online format was preferred to enhance participant accessibility, reduce response time, and improve data collection efficiency.

Data Analysis and Statistical Evaluation

Data were analyzed using IBM SPSS Statistics for Windows, Version 30.0. Descriptive statistics, including mean, standard deviation, median, minimum, maximum, frequency, and percentage, were calculated. Chi-square tests were used to compare categorical variables according to independent factors. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 216 pediatricians participated in this study, of whom 170 (78.7%) were female and 46 (21.3%) were male. The participants included 133 (61.6%) pediatric residents (specialty trainees), 33 (15.3%) general pediatric specialists, 31 (14.4%) pediatric subspecialty residents, and 19 (8.7%) pediatric subspecialists. The mean age of participants was 32.6 ± 6.4 years (range: 23–55), and the mean duration of pediatric clinical experience was 5.8 ± 5.9 years (range: 0.4–26). On average, participants reported seeing 495.4 ± 398.9 patients per month (range: 17–2500). A total of 154 participants (71.3%) were working in institutions with a dedicated adolescent health unit.

Awareness, Knowledge, and Attitudes of Pediatricians Regarding ARFID

The awareness and knowledge levels of pediatricians regarding ARFID are summarized in Table 1. Prior to reading the study title, only 61 participants (28.5%) reported that they knew the full term for ARFID. While 82 participants (38.0%) recognized ARFID as a DSM-5-defined eating disorder, 127 (58.8%) stated they were uncertain. Regarding subtypes of ARFID, 157 (72.7%) identified "food-related fears," 135 (62.5%) identified "lack of interest in eating," and 113 (52.3%) incorrectly identified "body image disturbance" as a subtype, indicating a potential conceptual confusion with anorexia nervosa.

Table 1. Evaluation of pediatricians' awareness, knowledge, attitudes, and practices regarding ARFID

	n	%
Were you familiar with the term "ARFID" before reading the title of this study?		
Yes	61	28.5
No	155	71.5
Is ARFID a diagnosis included in the DSM-5?		
Yes	82	38.0
No	7	3.2
Not Sure	127	58.8
Which of the following are diagnostic presentations of ARFID according to DSM-5? *[Multiple responses allowed]*		
Avoidance based on the sensory characteristics of food	109	50.5
Apparent lack of interest in eating or food	135	62.5
Body image disturbance	113	52.3
Concern about aversive consequences of eating	157	72.7
Are you aware of common psychiatric and developmental comorbidities of ARFID?		
Yes (anxiety disorders, autism spectrum disorder, ADHD)	153	70.8
No	61	28.2
ARFID may present with diminished appetite or disinterest in eating.		
Yes	185	85.6
No	1	0.5
I am not familiar	30	13.9
ARFID may involve avoidance of food due to sensory sensitivities.		
Yes	178	82.4
No	7	3.2
I am not familiar	31	14.4
ARFID may develop following fear of negative consequences related to eating.		
Yes	167	77.3
No	8	3.7
I am not familiar	41	19.0
ARFID may result in significant weight loss or failure to achieve expected weight gain.		
Yes	179	82.9
No	4	1.9
I am not familiar	33	15.3
ARFID may lead to nutritional deficiencies requiring oral supplements or enteral feeding.		
Yes	160	74.1
No	9	4.2
I am not familiar	47	21.8
ARFID can cause marked psychosocial impairment.		
Yes	178	82.4
No	4	1.9

I am not familiar	34	15.7
Restrictive eating in ARFID cannot be better explained by a medical condition, cultural practice, or food unavailability.		
Yes	120	56.1
No	38	17.8
I am not familiar	56	26.1
Individuals with ARFID fear gaining weight.		
Yes	98	45.6
No	57	26.5
I am not familiar	60	27.9
Have you ever diagnosed or suspected ARFID in your clinical practice?		
Yes	75	34.9
No	140	65.1
Have you ever referred a patient suspected of ARFID to another specialty?		
Yes	64	29.8
No	151	70.2
Which specialties do you referr to? *[Multiple responses allowed]*		
Child and Adolescent Psychiatry	117	54.2
Pediatric Gastroenterology	99	45.8
Nutrition and Dietetics	98	45.4
I don't reffer	78	36.1
Other (e.g., Adolescent Health, Occupational Therapy, Developmental Pediatrics)	9	4.2
How competent do you feel in diagnosing and managing ARFID?		
Not competent at all	86	41.0
Slightly competent	85	40.5
Moderately competent	30	14.3
Quite/Very competent	9	4.3
Have you ever received any formal education or training on ARFID?		
Yes	14	6.5
No	202	93.5
Do you think pediatricians need additional training regarding ARFID?		
Yes	195	90.7
No	20	9.3
Would you participate in ARFID-related training if offered?		
Yes	186	87.3
No	27	12.7

n = frequency; % = percentage of respondents.

Awareness of Clinical Features and Referral Practices Related to ARFID

When asked about their awareness of the clinical characteristics of Avoidant/Restrictive Food Intake Disorder (ARFID), 178 participants (82.9%) indicated that ARFID could lead to weight loss or faltering growth in children. Additionally, 160 participants (74.1%) acknowledged that ARFID may result in significant nutritional deficiencies requiring oral supplements or tube feeding. A total of 185 respondents (85.6%) reported that loss of appetite could be a presenting feature of ARFID, 178 (82.4%) mentioned avoidance due to sensory sensitivities, and 167 (77.3%) cited fear of adverse eating consequences (e.g., choking or vomiting) as possible manifestations.

In contrast, 98 participants (45.6%) incorrectly stated that individuals with ARFID experience a fear of gaining weight. Furthermore, 120 participants (56.1%) correctly noted that the food restriction seen in ARFID cannot be attributed to medical conditions, cultural practices, or limited access to food.

Pediatricians' clinical experience with ARFID and their referral behaviors are presented in Table 1. Of the respondents, 75 (34.9%) reported having diagnosed or suspected a case of ARFID during their professional practice. Additionally, 64 participants (29.8%) had referred such patients to other specialists. The most frequently reported referral destinations were child and adolescent psychiatry (117; 54.2%), pediatric gastroenterology (99; 45.8%), and dietetics (98; 45.4%). Nine respondents (4.2%) selected "other" as the referral category, specifying adolescent health, occupational therapy, and developmental pediatrics as relevant fields.

Pediatricians' perceived competence in ARFID management and their educational background are also summarized in Table 1. Only 9 participants (4.3%) reported feeling "very competent" or "quite competent" in diagnosing and managing ARFID, whereas 86 (41.0%) stated they did not feel competent at all, and 85 (40.5%) reported feeling minimally competent. A total of 14 respondents (6.5%) had previously received any formal training on ARFID. However, 195 participants (90.7%) believed that pediatricians need additional training on the topic, and 186 (87.3%) expressed willingness to attend such training if offered.

The comparison of pediatricians' knowledge levels regarding ARFID based on their professional titles is presented in Table 2. Pediatric subspecialists demonstrated significantly higher rates of correct responses regarding core clinical criteria of ARFID, such as weight loss and nutritional deficiencies, compared to other groups ($p < 0.05$). They also reported the highest perceived competence in diagnosing and managing ARFID ($p = 0.020$). Pediatric residents were more likely to incorrectly endorse "fear of gaining weight"—a feature associated with anorexia nervosa—as a characteristic of ARFID ($p = 0.020$).

When pediatricians were stratified by the presence of an adolescent health unit in their institution, those working in institutions with such a unit were significantly more knowledgeable. Among them, 51 participants (33.1%) reported knowing the expanded form of ARFID prior to reading the study title, compared to 10 (16.1%) in institutions without an adolescent health unit ($p = 0.012$). Awareness that ARFID is a DSM-5-defined disorder was higher among the former group (67; 43.5%) than the latter (15; 24.2%) ($p = 0.014$). Additionally, recognition that ARFID can lead to significant weight loss or growth faltering was reported by 135 participants (87.7%) in institutions with adolescent health units versus 44 (71.0%) in those without ($p = 0.013$). Awareness that ARFID can cause psychosocial impairment was similarly higher among pediatricians in adolescent health-equipped institutions (87.1% vs. 71.0%, $p = 0.011$). Furthermore, more pediatricians from these institutions had encountered ARFID cases (39.9% vs. 22.6%, $p = 0.016$) and had referred such cases to other specialists (35.3% vs. 16.1%, $p = 0.005$).

Regarding clinical experience, knowledge levels improved with increasing years in practice. The proportion of pediatricians who reported knowing the expanded form of ARFID prior to reading the study title was 16.1% among those with ≤2 years of experience, 30.3% in those with 2–4 years, and 36.8% among those with more than 4 years ($p = 0.025$). Similarly, the percentage of those who had referred an ARFID

case to another specialist increased with experience (17.7% for ≤2 years, 36.8% for 2–4 years, and 32.0% for >4 years; p = 0.043).

No statistically significant differences were observed in ARFID awareness or attitudes based on the number of patients seen monthly.

Responses to open-ended questions were thematically analyzed. Barriers to diagnosis and management included lack of familiarity with the disorder, difficulty in differential diagnosis, insufficient time during outpatient visits to assess for eating disorders, difficulty accessing dietitians and child/adolescent psychiatry teams, limited physician experience in managing eating disorders, and communication challenges with pediatric and adolescent patients. Recommendations for improving ARFID management included: structured educational programs covering diagnosis, differential diagnosis, and treatment tailored to pediatricians; increased awareness and early detection training for family physicians; extended consultation time for outpatient visits; enhanced awareness among schoolteachers and guidance counselors; and incorporating mandatory rotations in adolescent health units during pediatric residency training.

Discussion

This study revealed that pediatricians have limited awareness and knowledge regarding ARFID, experience difficulties in its clinical management, and express a need for further training. The findings indicate that the proportion of pediatricians who recognized ARFID as a DSM-5-defined eating disorder was relatively low. While participants most frequently and correctly identified “food-related fears” and “lack of interest in eating” as ARFID subtypes, a considerable proportion also incorrectly identified “body image disturbance”—a feature not associated with ARFID—suggesting conceptual confusion with anorexia nervosa. Pediatric subspecialists demonstrated higher accuracy in identifying clinical features such as weight loss and nutritional deficiencies associated with ARFID compared to other professional groups.

The results are consistent with those of Harrison (2), who conducted a study in the United Kingdom assessing healthcare professionals’ knowledge and referral practices related to ARFID. Among 45 participants, 40% were working in primary care (e.g., general practitioners, school nurses), and 60% in secondary care (e.g., pediatricians, speech therapists, psychologists, dietitians). In the primary care group, 67% reported difficulty diagnosing ARFID and determining appropriate referral pathways. Although 74% of secondary care providers were familiar with the diagnosis, only 33% expressed confidence in making appropriate referrals. The study highlighted key barriers including lack of knowledge, confusion with picky eating, insufficient training, and the absence of multidisciplinary teams. Notably, 88% of participants believed that children with ARFID were falling through systemic gaps without receiving proper care. Similarly, Coelho et al. (4) conducted a study in Canada involving 93 healthcare professionals from various disciplines, including pediatricians, adolescent health specialists, dietitians, psychiatrists, nurses, family physicians, psychologists, occupational therapists, and social workers. Only 37% of participants could accurately identify all DSM-5 diagnostic criteria for ARFID, and most clinicians reported a lack of confidence in their diagnostic abilities.In another qualitative study by Magel et al. (6), more than half of participating professionals—including pediatricians, psychologists, nutritionists, and social workers—had never heard of ARFID, and only 8.6% had received specific training. Participants emphasized the absence of diagnostic and referral protocols and the ambiguity of professional roles in the management of ARFID.Guss et al. (5) surveyed U.S.-based physicians, including pediatricians, child and adolescent psychiatrists, adolescent medicine specialists, and clinicians in nutrition medicine. The study showed that during inpatient refeeding of adolescents with ARFID, protocols designed for anorexia nervosa were commonly used, revealing a lack of standardized approaches. Although multidisciplinary methods were frequently employed, pharmacologic interventions were reported less often. These findings align closely with our own results, which collectively suggest that ARFID remains under-recognized within health systems, and that structural and educational gaps

Table.2 Comparison of pediatricians' knowledge of ARFID according to professional title

	Pediatric Resident	General Pediatrician	Subspecialty Fellow	Subspecialist
	n (%)	n (%)	n (%)	n (%)
ARFID may result in significant weight loss or failure to achieve expected weight gain.				
Yes	112 (84.2)	21 (63.7)	28 (90.3)	18 (94.7)
No	3 (2.3)	1 (3.0)	-	-
Not familiar	18 (13.5)	11 (33.3)	3 (9.7)	1 (5.3)
p :0.051 chi square : 12.513				
ARFID may lead to nutritional deficiencies requiring oral supplements or enteral feeding.				
Yes	98 (73.7)	16 (48.5)	28 (90.3)	18 (94.7)
No	7 (5.3)	2 (5.1)	-	-
Not familiar	28 (21.1)	15 (45.5)	3 (9.7)	1(5.3)
p :0.002 chi square : 20.873				
Individuals with ARFID fear gaining weight.				
Yes	72 (54.1)	10 (31.2)	10 (32.3)	6 (31.6)
No	25 (18.8)	11 (34.4)	12 (38.7)	9 (47.4)
Not familiar	36 (27.1)	11(34.4)	9 (29.0)	4 (21.1)
p:0.020 chi square : 15.038				
ARFID can cause marked psychosocial impairment.				
Yes	111 (83.5)	22 (66.7)	27 (87.1)	18(94.7)
No	3 (2.3)	-	1(3.2)	-
Not familiar	19 (14.3)	11(33.3)	3 (9.7)	1(5.3)
p:0.073 chi square : 11.556				
Restrictive eating in ARFID cannot be better explained by a medical condition, cultural practice, or food unavailability.				
Yes	72(54.1)	13(40.6)	21(70.0)	14(73.7)
No	27(20.3)	5(15.6)	4(13.3)	2(10.5)
Not familiar	34(25.6)	14(43.8)	5(16.7)	3(15.8)
p:0.101 chi square : 10.612				

n = frequency; % = percentage of respondents.

persist in both diagnosis and management. Greater efforts are needed to implement training programs, clinical guidance, and interdisciplinary collaboration.

In our study, pediatricians working in tertiary university and training hospitals were assessed in terms of their awareness, knowledge, clinical experience, and referral behaviors regarding ARFID. The results mirror international findings. Only 28.5% of participants reported knowing the expanded form of ARFID, and 38% identified it as a DSM-5-defined disorder. These figures are comparable to Harrison's (2) findings, where 67% of primary care providers reported difficulty identifying ARFID. Moreover, 52.3% of participants in our study mistakenly identified "body image disturbance" as an ARFID subtype, reflecting the diagnostic confusion noted by Coelho et al. (4), who found variability in clinicians' interpretation of diagnostic criteria. The fact that 45.6% of pediatricians believed individuals with ARFID feared weight gain further illustrates a misunderstanding of a key distinguishing feature of the disorder.

With respect to clinical experience, only 34.9% of participants reported having diagnosed or suspected an ARFID case, and 29.8% had referred a patient to another specialist. These findings support the notion, emphasized in Harrison et al.'s research (2), that children with ARFID often fall through gaps between disciplines due to insufficient referral pathways. Likewise, Magel et al. (6) reported that only 8.6% of clinicians had received ARFID-specific train-

ing, while 57.1% had managed ARFID cases without formal training. Consistent with our data, many participants lacked knowledge about appropriate healthcare services to which ARFID patients should be referred and often continued treatment themselves without involving specialized care.

In both the current and previous studies, the lack of training emerged as a key barrier. Notably, 90.7% of pediatricians in our sample emphasized the need for formal education on ARFID. Compared to the above-mentioned studies, our findings indicate even lower rates of ARFID awareness and referral practices among pediatricians. In Türkiye, the prevalence of ARFID in childhood is unknown. Therefore, the frequency of encountering ARFID cases in outpatient or inpatient settings may differ from countries where previous studies were conducted, suggesting that population-level differences could account for the observed disparities.

The diagnostic complexity of ARFID—particularly when symptom profiles overlap with neurodevelopmental conditions such as autism spectrum disorder—was reported both in our findings and in the study by Magel et al. (6) .Additionally, Guss et al. (5) found that during hospital admissions of ARFID-diagnosed adolescents, inpatient refeeding protocols were often modeled after those for anorexia nervosa, reflecting a lack of disorder-specific treatment models and considerable heterogeneity in clinical practice. These findings align with our data, where 76.3% of pediatricians did not feel competent in managing ARFID.

Coelho et al. (4) showed that experienced clinicians demonstrated higher confidence in diagnosis and greater likelihood of referring ARFID cases. This finding corresponds with our result that pediatricians working in institutions with adolescent health units and those with more than four years of experience exhibited significantly greater awareness and referral behaviors.

In summary, insufficient knowledge, diagnostic confusion, limited referral pathways, and ambiguity surrounding refeeding practices for ARFID appear to be prevalent across healthcare systems. These challenges underscore the urgent need for interdisciplinary collaboration, standardized diagnostic and treatment protocols, and targeted education programs, as consistently highlighted in the international literature.

In addition to variability in the interpretation of ARFID diagnostic criteria, the impact of these differences on patient management practices may partially explain discrepancies in prevalence data. Distinguishing ARFID presentations that involve only psychosocial impairment—but not medical compromise—also remains diagnostically challenging (3). Furthermore, validated assessment tools developed to objectively evaluate ARFID symptoms (e.g., PARDI, NIAS) have only recently been introduced into clinical practice, contributing to diagnostic heterogeneity (8,9). We believe that achieving greater diagnostic consensus and integrating high-validity screening instruments into routine practice are crucial steps toward standardization of ARFID diagnosis.

Strengths

One of the key strengths of this study is that it represents the first investigation in Türkiye to assess ARFID awareness among pediatricians. All participants were actively practicing physicians, including both pediatric residents and specialists, from tertiary care hospitals providing inpatient and outpatient services. The questionnaire used in this study was developed through a comprehensive review of the current literature, and unlike previous studies (2,4), it addressed DSM-5 diagnostic criteria alongside referral practices and educational needs. Furthermore, inclusion of physicians from university and city hospitals that serve diverse patient populations, along with a relatively large sample size compared to similar studies, enhances the generalizability of the findings.

Limitations

This study included only pediatricians and did not assess other professional groups involved in ARFID management, such as child and adolescent psychiatrists, psychologists, and dietitians. Although this may be viewed as a limitation, it is important to note that in Türkiye's healthcare system, pediatricians are often the first point of contact in the diagnostic process. Therefore, we do not consider this exclusion to be a significant limitation.

Conclusion

The findings of this study reflect challenges that align with those reported in the international literature, including limited recognition of DSM-5 diagnostic criteria for ARFID, confusion with anorexia nervosa, gaps in referral systems, and unmet educational needs. Most participants had only limited familiarity with the concept of ARFID and reported uncertainty regarding appropriate referral pathways following diagnosis.

Factors such as length of clinical experience and institutional structure—particularly the presence of adolescent health units—were significantly associated with better awareness and referral behavior. In light of these findings, we conclude that implementation of structured training programs, interdisciplinary referral guidelines, and standardized clinical protocols is necessary to improve early and accurate identification of ARFID in pediatric practice. Moreover, due to the heterogeneous clinical presentations and high rates of psychiatric comorbidities, enhancing both the conceptual and practical clarity of ARFID diagnostic criteria—and promoting the use of validated assessment tools—will be essential to achieving diagnostic and treatment standardization.

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Ethics: The study received approval from the Non-Interventional Clinical Research Ethics Committee of Non-Interventional Clinical Research Ethics Committee of Ankara University (Approval No: İ05-421-25). All participant data were treated with strict confidentiality. Participation was entirely voluntary, and no identifying personal information, such as names or contact details, was collected.

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Research Article / Araştırma Makalesi

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Determinants of ICU Outcomes in Fully Active Patients Before Critical Illness

Kritik Hastalık Öncesi Performans Durumu Tam Aktif Bireylerin Yoğun Bakım Sonuçlarına Etki Eden Faktörlerinin Değerlendirilmesi

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ABSTRACT

Aim: To investigate ICU outcomes and early mortality predictors in fully active patients prior to critical illness, a population often considered resilient, yet underrepresented in outcome studies.

Materials and Methods: This retrospective cohort study included 139 patients admitted to the medical ICUs of a tertiary hospital between November 2022 and February 2025. Only patients with preserved pre-ICU performance status [Eastern Cooperative Oncology Group (ECOG)≤1 or Clinical Frailty Scale (CFS)≤3] were included. Demographics, comorbidities, admission diagnoses, severity scores (Acute Physiology and Chronic Health Evaluation II [APACHE-II], Sequential Organ Failure Assessment [SOFA]), interventions, and laboratory parameters were recorded. Logistic regression was used to identify the independent predictors for ICU mortality.

Results: The ICU mortality rate was 16.5% in fully active critically ill patients. Non-survivors had significantly higher APACHE II and SOFA scores, abnormal laboratory findings, and a greater requirement for mechanical ventilation and renal replacement therapy. Cardiac arrest prior to admission and concominant septic shock were more frequently in non-survivors. ICU admission from inpatient wards (OR 5.69,p=0.023), cardiac arrest before ICU admission (OR 33.45,p=0.002), pulmonary disease as a comorbidity (OR 15.19,p=0.002), concomitant septic shock (OR 5.08,p=0.032), and acute kidney injury (AKI)(OR 10.02,p=0.032) were independent risk factors for ICU mortality, Moreover, SOFA and APACHE II scores showed strong discriminative ability for ICU mortality (AUCs:0.916 and 0.873).

Conclusion: Although fully active before critical illness, these patients are not immune to poor ICU outcomes. Our findings highlight that specific clinical presentations like septic shock, cardiac arrest, and AKI carry substantial prognostic value, even in patients considered low-risk by baseline performance.

Keywords: fully active, performance status, ECOG, clinical fraiilty score, mortality, critical illness

ÖZET

Amaç: Bu çalışma ile kritik hastalık öncesinde tam fonksiyonel durumda olan ve bu sebeple fiziki olarak dayanıklı kabul edebileceğimiz hastalarda yoğun bakım sonuçları ve erken mortalite belirteçlerini incelenmeyi amaçlanmıştır.

Gereç ve Yöntem: Kasım 2022-Şubat 2025 tarihleri arasında üçüncü basamak bir hastanenin dahili yoğun bakım ünitelerine kabul edilen, kritik hastalık öncesi performans durumu Eastern Cooperative Oncology Group Performance Status (ECOG)≤1 veya Clinical Frailty Scale (CFS)≤3 olan 139 erişkin hasta retrospektif olarak değerlendirildi. Demografik özellikler, komorbiditeler, yatış tanıları, Acute Physiology and Chronic Health Evaluation II (APACHE-II) ve S equential Organ Failure Assessment (SOFA) skorları, organ destek tedavileri ve laboratuvar verileri kaydedildi. Birincil sonlanım noktası yoğun bakım mortalitesi olup, bağımsız belirleyiciler lojistik regresyonla (LR) ile değerlendirildi.

Bulgular: Mortalite oranı %16,5 olarak hesaplanan kohortta ölen hastalarda APACHE-II ve SOFA skorları, mekanik ventilasyon ve renal replasman gereksinimleri daha yüksek saptandı. Ölen grupta, servislerden yatış, kabul öncesi kardiyak arrest ve sepsis varlığında şok daha fazla gözlemlendi. LR analizinde servislerden yatış (OR 5.69, p=0.023), kabul öncesi kardiyak arrest (OR 33.45, p=0.002), pulmoner hastalık varlığı (OR 15.19, p=0.002), septik şok varlığı (OR 5.08, p=0.032), ve ABH varlığı (OR 10.02, p=0.032) mortalitenin bağımsız belirleyicileri olarak bulundu. SOFA ve APACHE-II skorları mortaliteyi yüksek doğrulukla öngördü (AUC: 0,916 ve 0,873).

Sonuç: Kritik hastalık öncesinde tam fonksiyonel durumda olmalarına rağmen, bu hastalar kötü prognoz riski taşımaktadır. Bulgularımız, septik şok, kardiyak arrest ve akut böbrek hasarı gibi belirli klinik tabloların, başlangıçtaki iyi performans durumuna rağmen düşük riskli kabul edilen hastalarda bile anlamlı prognostik değere sahip olduğunu ortaya koymaktadır.

Anahtar Kelimeler: tam aktif birey, performans durumu, ECOG, klinik kırılganlık skoru, mortalite, kritik hastalık

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Introduction

Functional status prior to critical illness has been increasingly recognized as a key determinant of intensive care unit (ICU) outcomes, especially in older and comorbid populations (1). Among the available tools, the Eastern Cooperative Oncology Group (ECOG) Performance Status Scale has long been utilized to assess the baseline functional reserve of patients, predominantly in oncology and geriatrics (2-5). It provides a graded evaluation of a patient's capacity to perform daily activities independently, with scores ranging from 0 (fully active) to 5 (deceased) (2).

In recent years, ECOG performance status has also been applied to critically ill patients as a surrogate marker for functional status prior to critical illness. Several studies have demonstrated a strong association between worse ECOG scores, increased ICU and in-hospital mortality, and reduced likelihood of discharge to home (1,3,4). Moreover, these associations appear to be independent of age, comorbidities, or illness severity scores, underscoring the prognostic value of pre-critical illness performance status in intensive care settings (3,6).

In parallel frailty, quantified through tools such as the Clinical Frailty Scale (CFS), has emerged as an important predictor of adverse outcomes among ICU patients, particularly those over 65 years of age (7). Patients with higher frailty scores exhibit prolonged ICU stays, increased risk of complications, and higher short-term mortality (8-10). Despite the growing literature, most studies have focused on individuals with impaired pre-critical illness functionality (e.g., ECOG ≥2 or CFS ≥5), while relatively little is known about patients with preserved baseline functional capacity (11,12).

Patients who are fully active prior to critical illness (ECOG≤1 or CFS≤3) represent a unique and understudied subgroup (11,12). These individuals are theoretically expected to have better outcomes, yet may still experience significant morbidity and mortality during critical illness. Understanding the factors that influence prognosis in this population may help identify modifiable risks and guide early, targeted interventions.

Therefore, in this retrospective cohort study, we aimed to evaluate the ICU outcomes of patients with a fully active functional status prior to critical illness and to identify the clinical and contextual factors associated with poor prognosis in this seemingly low-risk group. By focusing on a high-functioning cohort, this study seeks to inform personalized care strategies and improve clinical decision-making in intensive care medicine.

Materials and Methods

Study Design and Setting

This retrospective, single-center cohort study was conducted in the internal medicine ICUs of Gazi University Hospital, a tertiary academic hospital in Ankara, Turkey. Data were obtained from ICU patients admitted between November 17, 2022, and February 31, 2025.

Ethical Statement

This study was approved by the Gazi University Ethics Committee (Date: April 15, 2025, Number: 2025–627). Informed consent was not obtained because of the retrospective nature of the study. All patient data were anonymized and handled according to institutional and national ethical guidelines.

Study Population and Inclusion Criteria

The study included adult patients (≥18 years) with a documented ECOG performance status ≤1 and/or a CFS score ≤3 prior to ICU admission. Only patients who were followed in the internal medicine ICUs during the specified period were eligible. Patients with incomplete medical records or lacking documentation of baseline performance status were excluded. Only the patient's first admission data was used for the study in multiple admissions.

Data Collection

Demographic information, comorbidities, ICU admission diagnoses, acute illness severity [Acute Physiology and Chronic Health Evaluation II (APACHE II)] and organ disfunction scores [Sequential Organ Failure Assessment (SOFA) and Glasgow Coma Scale (GCS), calculated on the first day of ICU

admission], source of ICU referral, and ICU interventions, including mechanical ventilation, vasopressor use, and renal replacement therapy, were recorded. Additional variables such as sepsis during the ICU stay and laboratory parameters on the day of ICU admission were also recorded. Data were retrospectively collected from electronic medical records and paper-based ICU charts using a standardized case report form.

Outcome Measures

The primary outcome was ICU mortality. Patients were grouped as survivors or non-survivors, and clinical characteristics, interventions, and baseline features were compared between the two groups. Associations between clinical variables and ICU mortality were further analyzed to identify independent risk factors.

Statistical Analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics. Continuous variables were presented as means ± standard deviation (SD) or medians with interquartile ranges (Q1-Q3), depending on the data distribution. Categorical variables were expressed as frequencies and percentages. Continuous variables were compared using Student's t-test or the Mann-Whitney U test. Categorical variables were analyzed using the Chi-square test. Multivariate logistic regression analysis was conducted to identify independent predictors of ICU mortality. Receiver operating characteristic (ROC) curve analysis was performed to assess the ability of severity scores to predict mortality. The area under the curve (AUC) was calculated to quantify the overall discriminative power of each score. ROC curves were also generated to illustrate the trade-off between sensitivity and specificity across a range of cut-off values. A p-value ≤0.05 was considered statistically significant. All analyses were performed by using IBM SPSS Statistics (version 22).

Results

A total of 139 fully active critically ill patients were included in the final analysis. Among them, 116 (83.5%) survived and 23 (16.5%) died during their ICU stay. Baseline characteristics, ICU-related data and laboratory findings according to ICU mortality status are presented in Tables 1 and Supplemental Table.

Non-survivors had significantly higher severity scores upon admission, including APACHE-II (30 [19–39] vs. 15 [10–18], p<0.001), SOFA (10 [7–13] vs. 3 [2–5], p<0.001), and modified Nutrition Risk in the Critically ill (NUTRIC) (5 [4–6] vs. 3 [1–4], p<0.001). The Charlson Comorbidity Index (CCI) and GCS were also worse in non-survivors (CCI: 5 [3–8] vs. 3 [1–5], p=0.001; GCS: 7 [3–15] vs. 15 [15–15], p<0.001). Non-survivors were more frequently admitted from inpatient wards (60.9% vs. 27.6%, p=0.002) and less often from emergency departments (26.1% vs. 62.9%, p=0.001). Length of hospital stay prior to ICU admission was also longer in non-survivors (3 [1–12] vs. 1 [0–1.5] days, p=0.005).

Cardiac decompensation and renal failure as an admission diagnosis were more prevalent in non-survivors (30.4% vs. 6.9%, p<0.001; and 47.8% vs. 26.7%, p=0.044, respectively). Pulmonary disease (52.5% vs. 14.3%, p<0.001), and gastrointestinal disease as comorbidities (30.4% vs. 10.3%, p=0.018) were also significantly more common in the non-survivor group. Cardiac arrest before ICU admission (26.1% vs. 2.6%, p<0.001) and concominant shock with septic pateints were observed more frequently in non-survivors (100% vs. 40% within septic patients (n)=57, p<0.001)

In terms of interventions and organ support, non-survivors more frequently required invasive mechanical ventilation (IMV) (95.7% vs. 16.4%, p<0.001), continuous renal replacement therapy (CRRT; 60.9% vs. 2.6%, p<0.001), albumin replacement (52.2% vs. 9.6%, p<0.001), and blood product transfusion (34.8% vs. 7.8%, p = 0.002) than survivors. Parenteral nutritional support was also more common in non-survivors (43.5% vs. 12.9%, p = 0.002).

Non-survivors exhibited significantly abnormal laboratory parameters, including higher blood urea nitrogen, creatinine, magnesium, liver transaminases, INR, direct bilirubin, and lactate levels, alongside lower albumin and hemoglobin concentrations (all

Table 1. Comparison of baseline characteristics and ICU-related data according to ICU outcome in fully active critically ill patients

	All Patients n=139	Survivors n=116	Non-survivors n=23	**P value**
Baseline Characteristics and ICU Admission Data				
Age*	59 [41-67]	56 [38-67]	61 [51-70]	0.253
Gender, n (%)				0.233
Female	45 (32.4)	40 (34.5)	5 (21.7)	
Male	94 (67.6)	76 (65.5)	18 (78.3)	
BMI (kg/m^2)*	24.6 [22.2-28.3]	24.9 [22.5-28.3]	24.2 [22-29.2]	0.747
APACHE II Score*	15 [11-21]	15 [10-18]	30 [19-39]	**<0.001**
SOFA Score*	4 [2-7]	3 [2-5]	10 [7-13]	**<0.001**
mNUTRIC Score*	3 [1-4]	3 [1-4]	5 [4-6]	**<0.001**
CCI*	3 [1-5]	3 [1-5]	5 [3-8]	**0.001**
Glasgow Coma Scale*	15 [13-15]	15 [15-15]	7 [3-15]	**<0.001**
ECOG Performance Status, n (%)				0.051
0	62 (44.6)	56 (48.3)	6 (26.1)	
1	77 (55.4)	60 (51.7)	17 (73.9)	
Clinical Frailty Scale, n (%)				
1	16 (11.5)	16 (13.8)	0	0.074
2	37 (26.6)	32 (27.6)	5 (21.7)	0.562
3	86 (61.9)	68 (58.6)	18 (78.3)	0.076
Length of ICU stay (day)*	4 [2-7]	4 [2-7]	5 [2-10]	0.619
Length of Hospital stay before ICU (day)*	1 [0-3]	1 [0-1.5]	3 [1-12]	**0.005**
Admission source, n (%)				
Emergency department	79 (56.8)	73 (62.9)	6 (26.1)	**0.001**
Inpatient wards	46 (33.1)	32 (27.6)	14 (60.9)	**0.002**
Transfer from other ICUs	4 (2.9)	3 (2.6)	1 (4.3)	0.519
Transfer from other hospitals	10 (7.2)	8 (6.9)	3 (8.7)	0.671
Admission Diagnosis, n (%)				
Respiratory failure	61 (43.9)	50 (43.1)	11 (47.8)	0.677
Sepsis	57 (41.0)	45 (38.8)	12 (52.2)	0.233
Renal failure	42 (30.2)	31 (26.7)	11 (47.8)	**0.044**
Acute GIS disorders	22 (15.8)	18 (15.5)	4 (17.4)	0.762
Cardiac decompensation	15 (10.8)	8 (6.9)	7 (30.4)	**<0.001**
Intoxication	13 (9.4)	13 (11.2)	0	0.92
Acute neurological disorders	13 (9.4)	10 (8.6)	3 (13.0)	0.451
Metabolic disturbances	9 (6.5)	9 (7.8)	0	0.356
Surgery	4 (2.9)	3 (2.6)	1 (4.3)	0.519
Trauma	4 (2.9)	4 (3.4)	0	1.0
Cardiac arrest before ICU admission, n (%)	9 (6.5)	3 (2.6)	6 (26.1)	**<0.001**

Comorbidities, n (%)				
Hypertension	52 (37.4)	41 (35.3)	11 (47.8)	0.258
Endocrinological	46 (33.1)	40 (34.5)	6 (26.1)	0.434
Cardiac disorders	36 (25.9)	27 (23.3)	9 (39.1)	0.113
Pulmonary disease	29 (20.9)	17 (14.3)	12 (52.5)	**<0.001**
Chronic renal disease	21 (15.1)	19 (16.4)	2 (8.7)	0.527
Malignancy	26 (18.7)	19 (16.4)	7 (30.4)	0.143
Gastroenterologic disorders	19 (13.7)	12 (10.3)	7 (30.4)	**0.018**
Rheumatological	14 (10.1)	12 (10.3)	2 (8.7)	1.0
Neurological	8 (5.8)	5 (4.3)	3 (13.0)	0.127
Source of sepsis (n=57), n (%)				
Respiratory system	28 (49.1)	23 (51.1)	5 (41.7)	0.561
Abdominal	12 (21.1)	8 (17.8)	4 (33.3)	0.25
BSI	8 (15.0)	6 (13.3)	2 (16.7)	0.67
Urinary tract	6 (10.5)	5 (11.1)	1 (8.3)	1.0
Soft tissue	2 (3.5)	2 (4.4)	0	1.0
CNS	1 (1.8)	0	1 (8.3)	0.211
Concominant shock with septic patients (n=57), n (%)	32 (56.1)	18 (40.0)	12 (100)	**<0.001**
Immunosuppression at ICU admission, n (%)	23 (16.5)	16 (13.8)	7 (30.4)	**0.05**
Nutritional support, n (%)				
Parenteral	25 (18.0)	15 (12.9)	10 (43.5)	**0.002**
Enteral	51 (36.7)	42 (36.2)	9 (39.1)	0.790
Requirement of respiratory support, n (%)				
Invasive mechanical ventilation	41 (29.5)	19 (16.4)	22 (95.7)	**<0.001**
Non-invasive mechanical ventilation	25 (18)	21 (18.1)	4 (17.4)	1.0
HFNO	24 (17.3)	21 (18.1)	3 (13.0)	0.765
AKIN stage, n (%)				<0.001
I	36 (25.9)	24 (20.7)	12 (52.2)	
II	10 (7.2)	8 (6.9)	2 (8.7)	
III	20 (14.4)	13 (11.2)	7 (30.4)	
Requirement of RRT, n (%)				
Intermittent hemodialysis	28 (20.1)	22 (19.0)	6 (26.1)	0.409
CRRT	17 (12.2)	3 (2.6)	14 (60.9)	**<0.001**
Blood Product Replacement, n (%)	17 (12.2)	9 (7.8)	8 (34.8)	**0.002**
Albumin Replacement, n (%)	23 (16.7)	11 (9.6)	12 (52.2)	**<0.001**

*median [25th percentile-75th percentile]

ICU: Intensive Care Unit, **BMI:** Body Mass Index, **APACHE:** Acute Physiology and Chronic Health Evaluation, **SOFA:** Sequential Organ Failure Assessment, **mNUTRIC:** modified Nutrition Risk in Critically Ill, **CCI:** Charlson Comorbidity Index, **ECOG:** Eastern Cooperative Oncology Group, **GIS:** Gastrointestinal System, **BSI:** Bloodstream infection, **CNS:** Central Nervous System, **HFNO**: High Flow Nasal Oxygen, **AKIN:** Acute Kidney Injury Network, **RRT:** Renal Replacement Therapy, **CRRT:** Continuous Renal Replacement Therapy

Supplemental Table. Comparison of baseline laboratory findings according to ICU outcome in fully active critically ill patients

	All Patients n=139	Survivors n=116	Non-survivors n=23	P value
Blood Urea Nitrogen(mg/dL)	24 [13-40]	19.5 [11.5-36.5]	97.8 [21.8-186]	**<0.001**
Creatinine (mg/dL)	1.07 [0.74-1.96]	0.94 [0.72-1.8]	1.82 [1.27-2.11]	**<0.001**
Sodium (mEq/L)	128 [133-141]	137 [133-140]	139 [135-142]	0.124
Chloride (mEq/L)	104 [100-108]	103.5 [100-108]	104 [100-107]	0.948
Potassium (mEq/L)	4.2 [3.7-4.7]	4.2 [3.8-4.7]	4.2 [3.6-4.7]	0.860
Calcium (mg/dL)	8.6 [8.3-9.1]	8.6 [8.3-9]	8.7 [8.2-9.7]	0.338
Phosphorus (mg/dL)	3.6 [2.7-]	3.6 [2.7-4.2]	4.7 [3.3-6.7]	**0.002**
Magnesium (mg/dL)	1.8 [1.7-2.1]	1.8 [1.6-2.1]	2.3 [1.8-2.5]	**<0.001**
Alanine transaminase (U/L)	27 [14-56]	24 [14-.44]	87 [30.-152]	**<0.001**
Aspartat transaminase (U/L)	34 [21-74]	30 [20-61]	154 [31-275]	**<0.001**
INR	1.12 [1.03-1.29]	1.08 [1.02-1.25]	1.37 [1.14-1.7]	**<0.001**
Total Bilirubin (mg/dL)	0.6 [0.4-1.0]	0.6 [0.4-0.9]	0.9 [0.4-2.2]	0.065
Direct Bilirubin (mg/dL)	0.23 [0.16-0.43]	0.22 [0.15-0.37]	0.43 [0.18-1.27]	**0.014**
Albumin (g/dL)	3.3 [2.9-3.9]	3.5 [3.0-3.9]	2.8 [2.4-3.3]	**<0.001**
Blood gas sampling				
pH	7.38 [7.32-7.43]	7.38 [7.34-7.43]	7.24 [7.08-7.42]	0.003
PCO_2 (mmHg)	37 [32-45]	37 [32-45]	38 [32-50]	0.658
HCO_3 (mEq/L)	21.9 [18.7-25.2]	22.4 [19.5-25]	18.6 [12.8-23]	**0.002**
Lactate (mmol/L)	1.8 [1.3-3.4]	1.7 [1.3-2.7]	5.5 [1.5-9.5]	**<0.001**
WBC ($x10^3$/µl)	10 [6.8-15.6]	10 [7.36-14.7]	12.8 [3.8-24.3]	0.584
PMNL ($x10^3$/µL)	7.9 [4.8-12.3]	7.75 [5.0-11.7]	10.1 [2.5-17.9]	0.677
Lmyphocytes ($x10^3$/µL)	0.98 [0.5-1.5]	1.0 [0.54-1.5]	0.6 [0.39-1.4]	0.227
Hgb (g/dL)	11.7[9.4-13.8]	11.9 [9.9-13.8]	9.1 [7.8-11.9]	**0.012**
PLT ($x10^3$/µL)	203 [140-295]	210 [159-295]	181 [50-243]	**0.036**
C-reactive protein (mg/L)	85.8 [16.2-179]	85.8 [162-158]	97.8 [21.8-186]	0.653
Procalcitonin (ng/mL)	0.43 [0.1-4.12]	0.35 [0.09-2.89]	34 [26-62]	**0.026**

*median [25th percentile-75th percentile]
ICU: Intensive Care Unit, **INR:** International Normalized Ratio, **PCO_2:** Partial pressure of carbon dioxide, **HCO_3:** Bicarbonate, **WBC:** white blood cell count, **PMNL:** polymorphonuclear leukocytes, **Hgb:** Hemoglobin, **PLT:** Platelets,

p<0.05) (Supplemental Table). Additionally, arterial blood gas analysis revealed significantly lower pH and bicarbonate levels in non-survivors (p=0.003 and p=0.002, respectively), suggesting more pronounced metabolic acid-base disturbances.

Multivariate logistic regression analysis identified that ICU admission from inpatient wards [OR 5.69, 95% CI:1.270-25.518, p=0.023], cardiac arrest before ICU admission [OR 33.45, 95% CI:3.813-293-468, p=0.002], pulmonary disease as a comorbidity [OR 15.19, 95% CI:2.826-81.677, p=0.002], concomitant shock in septic patients [OR 5.08, 95% CI:1.147-22.535, p=0.032], and AKI according to AKIN classification [OR 10.02, 95% CI:1.435-69.909, p=0.032] were independent risk factors for ICU mortality (Table 2).

Table 2. Multivariate analysis for independent risk factors of ICU mortality in fully active critically ill patients

	Adjusted OR (95% CI)	P Value
ICU admission from inpatient wards	5.694 (1.270-25.518)	**0.023**
Cardiac arrest before ICU admission	33.453 (3.813-293-468)	**0.002**
Pulmonary disease as a comorbidity	15.193 (2.826-81.677)	**0.002**
Gastroenterologic disorders as a comorbidity	3.283 (0.520-20.725)	0.206
Immunosuppression at ICU admission	1.650 (0.315-8.634)	0.553
Concomitant shock in septic patients	5.085 (1.147-22.535)	**0.032**
AKI according to AKIN classification	10.015 (1.435-69.909)	**0.02**
CCI	1,038 (,784-1,374)	0.795

ICU: Intensive Care Unit, **AKI:** Acute Kidney Injury, **AKIN:** Acute Kidney Injury Network, **CCI**: Charlson Comorbidity Index

ROC curve analysis revealed that SOFA score had superior discriminative ability for predicting ICU mortality (AUC: 0.916, 95% CI 0.856-0.976) compared to APACHE-II (AUC: 0.873, 95% CI 0.784-0.962). The optimal cutoffs were 6.6 for SOFA (sensitivity 87.0%, specificity 84.5%) and 18.5 for APACHE II (sensitivity 82.6%, specificity 75.9%) (Figure 1, Table 3).

Discussion

This study focused on fully active critically ill patients with good functional performance before acute deterioration, as indicated by ECOG 0–1 or CSF Score of 3 or below. While performance status has been extensively studied in populations with advanced cancer, elderly, or high comorbidity burden, data specifically addressing the determinants of outcomes among fully active individuals in the ICU remain limited. (3,13,14). Our findings underscore that even patients considered “resilient” may face a considerable risk of adverse outcomes once critical illness develops. Among the 139 patients analyzed, 16.5% died during their ICU stay, a rate notably lower than higher-risk populations, but still clinically meaningful given their preserved baseline function. Specifically, ICU mortality was independently associated with several early clinical features, including admission from inpatient wards, cardiac arrest prior to ICU admission, pulmonary comorbidities, sepsis-related shock, and acute kidney injury as defined by the AKIN classification.

The ICU mortality rate observed in this cohort (16.5%) aligns with contemporary reports from broad ICU populations. For instance, a large meta-analysis reported a pooled ICU mortality of approximately 38% (range 13–70%) across various settings, and a recent study on non-COVID ICU patients found rates around 29.6% (15,16). Previous studies have consistently shown that ECOG is the most potent independent predictor of in-hospital mortality in oncologic ICU patients, while the CFS independently predicts both short-term and long-term mortality in diverse ICU populations, including older adults and general critically ill patients, with adjusted effect sizes reaching hazard ratios around 1.26 per unit increase (17-19). Given that previously reported ICU mortality rates range from 19% to 59% in these higher-risk populations, such as cancer patients or the frail elderly, the 16.5% mortality observed in our fully active cohort, although seemingly modest, warrants attention (20-23). Unlike these higher-risk populations, fully active patients present with preserved physical function and are often perceived as resilient. Therefore, their risk of adverse outcomes may not be adequately captured by conventional predictors, underscoring the need for alternative markers tailored to this unique and less-studied physiological baseline.

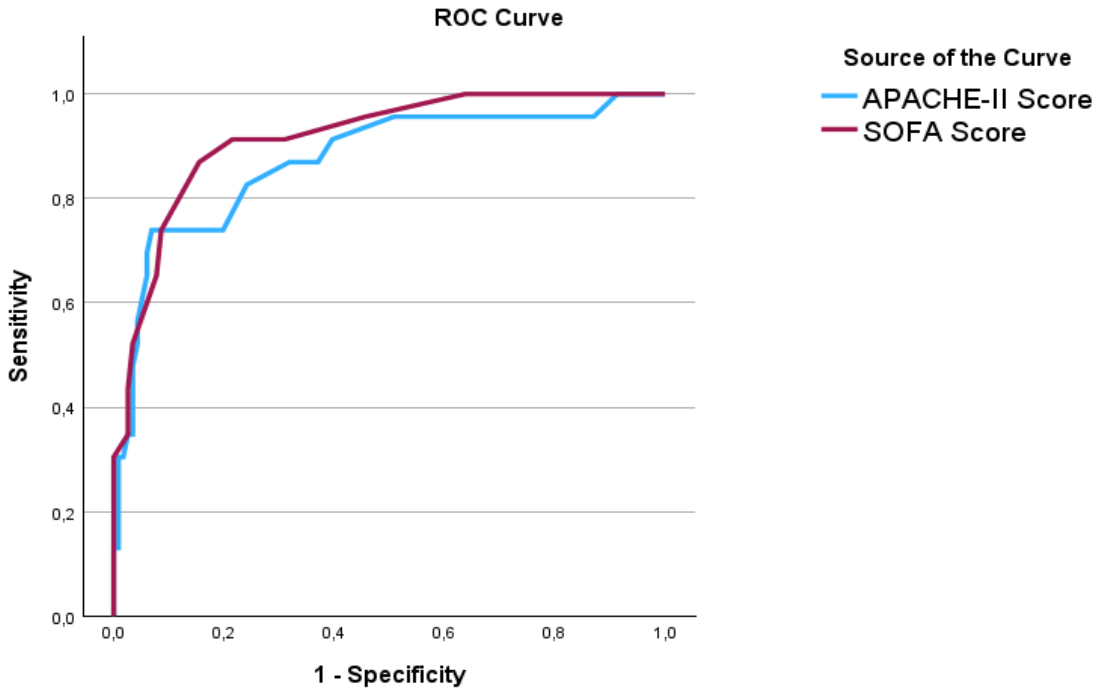

Figure 1. ROC curves of acute illness severity and organ failure scores (**APACHE:** Acute Physiology and Chronic Health Evaluation, **SOFA:** Sequential Organ Failure Assessment, **AUC:** Area Under the Curve, **ROC:** Receiver Operating Characteristic**)**

Table 3. Sensitivity, specificity, and ROC curve analysis of APACHE II and SOFA scores in fully active critically ill patients

	AUC	P Value	Cut-Off	Sensitivity (%)	Specificity (%)
APACHE II	0.873	**0.000**	18.5	82.6	75.9
SOFA	0.916	**0.000**	6.6	87.0	84.5

ROC: Receiver Operating Characteristic, **APACHE:** Acute Physiology and Chronic Health Evaluation, **SOFA:** Sequential Organ Failure Assessment, **AUC:** Area under the curve

In univariate analyses, indicators of organ failure, such as the presence of shock, need for IMV, requirement for RRT, low GCS, and laboratory markers of organ dysfunction, were significantly associated with ICU mortality, as were higher disease severity scores, including SOFA and APACHE II. While these variables are well-established prognostic markers, their predictive value often reflects downstream consequences of disease severity in both general ICU populations and specific patient groups (24-28). Therefore, in our multivariate model, we intentionally excluded overt severity indicators like SOFA and APACHE II scores, IMV, RRT, and markers of advanced organ failure to focus on admission-level features that may facilitate earlier risk stratification. Accordingly, ICU admission from inpatient wards, cardiac arrest prior to ICU admission, pulmonary disease as a comorbidity, concomitant shock in septic patients, and AKI defined by the AKIN classification emerged as independent predictors of ICU mortality. These variables represent clinical conditions that are typically identifiable at the time of ICU referral, prior to the onset of irreversible organ dysfunction.

Importantly, our ROC analysis supports this approach. Both SOFA and APACHE-II scores demonstrated strong discriminative ability for ICU mortality in our cohort, with AUC values exceeding 0.85, consistent with current literature where admission SOFA AUC ranges between 0.80 and 0.92 (27,29). In fact, SOFA emerged as the best single predictor, likely because its components reflect both the extent of organ dysfunction across six organ systems and the need

for corresponding organ support interventions, such as MV, vasopressor use, and neurologic assesment (27,30). Rather than relying solely on broadly validated illness severity scores, which are well-established across nearly all ICU populations, we sought to enhance early and proactive risk stratification in this clinically significant yet understudied subgroup by narrowing our focus to admission-level characteristics. These findings carry notable clinical implications, suggesting that part of the mortality observed in functionally independent patients may stem from delays in recognizing early signs of deterioration in non-ICU settings. Our study revealed that non-survivors were more frequently admitted from inpatient wards, had a higher incidence of cardiac arrest prior to ICU admission, and more often presented with shock in the context of sepsis, underscoring the need for prompt triage and ICU referral (31). Previous studies have demonstrated that structured early warning systems, such as the Modified Early Warning Score (MEWS) and the National Early Warning Score (NEWS), can facilitate timely escalation of care and improve outcomes by identifying deterioration before it becomes critical (32-33). Our results emphasize that even patients with preserved baseline function may be prone to sudden deterioration and therefore require appropriate monitoring and timely clinical response (34).

Several limitations warrant consideration. First, this was a single-center, retrospective study, which may limit generalizability and introduce selection bias. Second, the sample size, although sufficient for preliminary inference, remains modest, particularly for subgroup comparisons and multivariate modeling, which may affect statistical power and confidence intervals. Third, despite our deliberate exclusion of downstream severity indicators from the multivariate model, residual confounding cannot be entirely ruled out, especially given the inherent complexity of critical illness trajectories. Additionally, we did not include long-term functional or survival outcomes beyond ICU discharge, which may underestimate the broader prognostic implications for this patient group and further limits the assessment of the long-term prognostic significance of baseline functional independence. Lastly, some relevant variables, such as frailty indices beyond CFS, biomarker data, or time to ICU referral, were not systematically captured and could provide further insight in future prospective designs. Despite several limitations, this study provides novel insight into an often-overlooked subgroup of ICU patients, those who are functionally independent at baseline. By strictly defining inclusion criteria based on ECOG and CFS scores, we ensured a homogenous population with preserved performance status prior to ICU admission.

Conclusion

Fully active patients, despite preserved baseline performance, are not immune to poor ICU outcomes, particularly when high-risk features such as admission from inpatient wards, septic shock, cardiac arrest prior to ICU admission, and acute kidney injury are present. These findings challenge the assumption that good pre-ICU functional status ensures favorable prognosis. Instead, they highlight the need for early recognition of physiological deterioration using admission-level indicators and support risk-based triage strategies that go beyond traditional performance metrics.

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Research Article / Araştırma Makalesi

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Predictive Performance of Admission Hematological Parameters for Adverse Clinical Outcomes in Acute Cholangitis

Akut Kolanjit Hastalarında Olumsuz Klinik Sonuçları Öngörmede Başvuru Hematolojik Parametrelerinin Tahmin Gücü

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ABSTRACT

Objectives: This study aimed to evaluate the prognostic performance of hematological parameters, including red cell distribution width (RDW) and neutrophil-to-lymphocyte ratio (NLR), in patients with acute cholangitis (AC).

Materials and Methods: In this prospective study, 202 patients diagnosed with AC between December 2023 and August 2024 were included. The predictive performance of admission hematological parameters for clinical outcomes, including in-hospital mortality, bacteremia, need for inotropic support, prolonged hospital stay, intensive care unit (ICU) admission, and prolonged ICU stay, was assessed and compared.

Results: Among the 202 patients, 16 (7.9%) died during hospitalization. Multivariate regression analysis identified RDW as an independent risk factor for in-hospital mortality (odds ratio [OR]: 2.25, 95% confidence interval [CI]: 1.48–3.42, p<0.001). For the composite outcome, both NLR (OR: 1.04, 95% CI: 1.01–1.07, p=0.009) and RDW (OR: 1.61, 95% CI: 1.26–2.10, p<0.001) were independent risk factors. Receiver operating characteristic (ROC) analysis showed that RDW had the highest predictive accuracy for both in-hospital mortality (AUC [95% CI]: 0.826 [0.711–0.941]) and the composite outcome (AUC [95% CI]: 0.761 [0.681–0.842]) At a cut-off value of 15.6, RDW yielded a sensitivity of 75% and specificity of 88.2% for predicting in-hospital mortality. Patients with RDW >15.6 had a 21.3-fold higher risk of in-hospital mortality compared to those with lower RDW values (OR: 21.3, 95% CI: 6.3-71.5).

Conclusion: RDW demonstrated the strongest prognostic value among hematological parameters and may serve as a practical and reliable marker for early risk stratification in patients with AC.

Keywords: acute cholangitis, hematological parameters, mortality

ÖZET

Amaç: Bu çalışma, akut kolanjit (AC) hastalarında **eritrosit dağılım genişliği (RDW)** ve **nötrofil-lenfosit oranı (NLR)** dahil olmak üzere hematolojik parametrelerin prognostik performansını değerlendirmeyi amaçlamıştır.

Gereç ve Yöntem: Bu prospektif çalışmaya, Aralık 2023 ile Ağustos 2024 tarihleri arasında AC tanısı almış **202 hasta** dâhil edildi. Başvuru anındaki hematolojik parametrelerin, **hastane içi mortalite, bakteriyemi, inotrop ihtiyacı, uzamış hastane yatışı, yoğun bakım (ICU) yatışı ve uzamış ICU yatışı** gibi klinik sonuçları öngörme gücü değerlendirildi ve karşılaştırıldı.

Bulgular: Çalışmaya dâhil edilen **202 hastanın 16'sı (%7.9) hayatını kaybetti**. Çok değişkenli regresyon analizinde **RDW**, hastane içi mortalite için bağımsız bir risk faktörü olarak bulundu (**odds ratio [OR]: 2.25, %95 güven aralığı [GA]: 1.48–3.42, p<0.001**). **Kompozit sonlanım** açısından hem **NLR (OR: 1.04, %95 GA: 1.01–1.07, p=0.009)** hem de **RDW (OR: 1.61, %95 GA: 1.26–2.10, p<0.001)** bağımsız risk faktörleri olarak bulundu. **ROC analizinde**, hematolojik parametreler arasında **hastane içi mortaliteyi ve kompozit sonlanımı en iyi öngören parametrenin RDW olduğu** belirlendi (sırasıyla, **AUC [%95 GA]: 0.826 [0.711–0.941] ve AUC [%95 GA]: 0.761 [0.681–0.842]**). **15.6 kesme değeri** için **RDW'nin mortalite için duyarlılığı %75, özgüllüğü ise %88.2** olarak hesaplandı. **RDW >15.6** olan hastaların, diğer hastalara kıyasla **21.3 kat daha fazla hastane içi mortalite riski taşıdığı** saptandı (**OR: 21.3, %95 GA: 6.3–71.5**).

Sonuç: RDW, hematolojik parametreler arasında en güçlü prognostik değeri göstermiştir ve AC hastalarında erken risk sınıflandırması için pratik ve güvenilir bir belirteç olarak hizmet edebilir.

Anahtar kelimeler: akut kolanjit, hematolojik parametreler, mortalite

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Introduction

Acute cholangitis (AC) is a condition that occurs due to biliary obstruction and subsequent infection of the biliary tree (1). Although there have been advances in the diagnosis and treatment of AC, if appropriate and timely management is not provided, it may lead to severe implications, such as sepsis and multiorgan failure (2). It is very important to recognize patients who are at high risk for poor outcome in order to direct the management and determine the appropriate timing of biliary drainage (3).

Hematological parameters have emerged as easy to use and cost-effective biomarkers for predicting the severity of disease and the outcome in different inflammatory and infectious diseases (4). Parameters such as white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), platelet count, and red cell distribution width (RDW) have demonstrated prognostic value in conditions ranging from sepsis to acute pancreatitis (5-7). In recent years, several studies have highlighted the prognostic value of hematological indices, particularly RDW and NLR, in infection-related emergencies such as sepsis, community-acquired pneumonia, and biliary tract infections (8-10). These parameters have been associated with mortality, ICU admission, and prolonged hospitalization in various acute care settings. Nevertheless, their prognostic utility in acute cholangitis has not been fully investigated. Incorporating insights from these recent studies may help improve early risk stratification, particularly in emergency department settings.

The Tokyo guidelines, especially the latest version Tokyo 2018 (TG18) offers a guideline for the management and diagnosis of AC (11). However, the present guidelines do not emphasize the prognostic value of hematological parameters despite presenting clinical, laboratory, and imaging criteria for disease severity (11). Assessing the application of these prominent parameters in AC, which have gained significance in many inflammatory diseases today, will be valuable in understanding their role and providing guidance for future guidelines. In this study, we aim to evaluate the prognostic significance of hematological parameters in patients with AC.

Materials And Methods

Study design and clinical outcomes

This prospective study was conducted between December 2023 and August 2024, including 202 patients. Ethical approval was obtained from the Hospital Ethics Committee (Approval number: E2-22-2101), and written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki. Patients aged 18 years or older who met the diagnostic criteria for AC according to the TG18 were included in the study. Patients diagnosed with AC at their initial presentation to the emergency department were enrolled, while those who developed AC during hospitalization were excluded. Additionally, patients with a suspected but unconfirmed diagnosis of AC and those with malignancy were excluded.

The prognostic role of hematological parameters, including WBC count, hemoglobin, platelet count, NLR, and RDW, at the time of presentation was investigated in patients with AC. The primary outcome was in-hospital mortality. Secondary outcomes included prolonged hospital stay, intensive care unit (ICU) admission, bacteremia, and the development of inotropic support requirements. Furthermore, patients were also evaluated for composite outcomes, including in-hospital mortality, inotropic support requirement, prolonged ICU stay, and bacteremia.

Definitions and data collection

The diagnosis and severity classification of AC were based on TG18 criteria (11). Patients meeting all three of the following criteria were diagnosed with AC: (1) evidence of systemic inflammation, such as body temperature >38°C, WBC count >10 × 10^9/L, or C reactive protein (CRP) >10 mg/L; (2) evidence of cholestasis, such as jaundice (total bilirubin ≥2 mg/dL) or abnormal liver enzymes (more than 1.5 times the upper limit of normal); and (3) imaging evidence of biliary dilatation or underlying etiology (e.g., stricture, stones, or stent). Patients were classified as having severe AC (Grade III) in the presence of at

least one organ/system dysfunction, including cardiovascular, neurological, respiratory, renal, hepatic, or hematological dysfunction. Moderate AC (Grade II) was defined by the presence of two or more of the following criteria: abnormal WBC count (<4 × 10^9/L or >12 × 10^9/L), fever ≥39°C, age ≥75 years, total bilirubin ≥5 mg/dL, and hypoalbuminemia (<70% of the lower normal limit). Mild AC was diagnosed in cases not meeting the criteria for moderate or severe AC.

Data collected included demographic characteristics, vital signs, comorbid conditions, detailed hematological and biochemical parameters at admission, the presence of bacteremia, length of hospital stay, radiological findings, ICU admission status and duration, and inotropic support requirements. Bacteremia was defined as blood culture positivity deemed clinically significant by the infectious diseases team; contaminants and clinically irrelevant findings were excluded. The time from hospital admission to biliary drainage was recorded in hours. Endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangiography (PTC) were performed as biliary drainage methods in the study cohort. Patients with hospital or ICU stays exceeding the 75th percentile of the overall study population were classified as having prolonged hospital or ICU stays.

Statistical analysis

IBM SPSS software version 26.0 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analyses. The normality of data distribution was checked with the Kolmogorov–Smirnov test. Continuous variables were presented as mean ± standard deviation or median (interquartile range) and compared with Student's *t*-test or Mann–Whitney *U* test, while categorical variables were presented as number (%) and compared with Pearson's Chi-square or Fisher's exact test. Parameters associated with mortality and composite outcome at $P < 0.1$ level were included in the univariate logistic regression analysis. Parameters found to be associated with mortality and composite outcome at $P < 0.1$ level in the univariate analysis were included in the forward stepwise multivariate logistic regression analysis to determine independent risk factors for in-hospital mortality and composite outcome. The area under the curve (AUC) values of the predictors for primary and secondary endpoints were calculated using the receiver operating characteristic (ROC) curve analysis. The appropriate cut-off values of the independent risk factors were determined based on Youden's index using the ROC curve (12). At the appropriate cut-off values of the predictors, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated. A $P < 0.05$ was considered statistically significant.

Results

Comparison of baseline characteristics according to mortality

In the total population, 16 patients (7.9%) died. The median age of deceased patients was 84 (76-87) years, compared to 69 (55-79) years for survivors (p<0.001). The proportion of females was higher in the deceased group (p=0.034). The deceased group had lower mean arterial pressure and oxygen saturation (p < 0.001), while heart rate and respiratory rate were higher (p = 0.002 and p < 0.001, respectively). Altered mental status at admission was present in 12 (75%) deceased patients (p < 0.001). The Charlson comorbidity index score was significantly higher in the deceased group (p < 0.001). RDW and NLR values were higher in the deceased group, whereas hemoglobin and platelet levels were lower (p<0.001, p=0.004, p=0.022, and p=0.027, respectively). Comparisons of other baseline characteristics and laboratory parameters are shown in Table 1.

Comparison of clinical outcomes according to mortality

In the deceased group, 13 patients (81.3%) had grade 3 AC according to TG18, compared to 30 patients (16.1%) in the survivor group (p < 0.001). Systemic inflammatory response syndrome (SIRS) score was significantly higher in the deceased group (p < 0.001). Length of hospital stay was similar between the deceased and survivor groups (p = 0.412). The duration from admission to biliary drainage was also comparable between the groups (p = 0.968). Of the 28 pa-

Table 1. Comparison of clinical characteristics and laboratory parameters

Parameter	Overall n = 202	Survivors n = 186	Non-survivors n = 16	p
Age, years	69 (56-80)	69 (55-79)	84 (76-87)	<0.001
Female gender	100 (49.5)	88 (47.3)	12 (75)	0.034
Comorbidities				
Cardiovascular disease	45 (22.3)	37 (19.9)	8 (50)	0.010
Hypertension	117 (57.9)	107 (57.5)	10 (62.5)	0.699
Diabetes mellitus	64 (31.7)	57 (30.6)	7 (43.8)	0.280
Main complaint at admission				
Abdominal pain	198 (98)	184 (98.9)	14 (87.5)	0.032
Jaundice	54 (26.7)	49 (26.3)	5 (31.3)	0.769
Fever	35 (17.3)	29 (15.6)	6 (37.5)	0.026
Vital signs				
Mean arterial pressure	90 (83-99)	92 (84-99)	69 (59-79)	<0.001
Heart rate per minute	85 (79-96)	84 (78-95)	99 (91-106)	0.002
Respiratory rate per minute	15 (14-18)	15 (14-17)	20 (18-24)	<0.001
Oxygen saturation, %	95 (92-98)	96 (93-98)	90 (87-92)	<0.001
Altered mental status	20 (9.9)	8 (4.3)	12 (75)	<0.001
Concomitant acute pancreatitis	88 (43.6)	82 (44.1)	6 (37.5)	0.610
Concomitant acute cholecystitis	47 (23.3)	44 (23.7)	3 (18.8)	0.656
History of cholecystectomy	35 (17.3)	34 (18.3)	1 (6.3)	0.316
Charlson comorbidity index	1 (0-2)	1 (0-2)	4 (2-6)	<0.001
Laboratory parameters				
White blood cell count, 10^9/L	10.3 (7.4-13.9)	10.2 (7.4-13.8)	12.4 (8.4-25.3)	0.175
Neutrophil count, 10^9/L	8.3 (5.5-11.8)	8.3 (5.5-11.8)	8.5 (6-22)	0.435
Lymphocyte count, 10^9/L	0.9 (0.6-1.4)	0.9 (0.6-1.4)	0.5 (0.4-1.2)	0.093
Hemoglobin, g/dL	13.5 (12.1-14.7)	13.5 (12.1-14.9)	12.5 (11.2-13.5)	0.022
Platelet count, 10^9/L	225 (176-288)	226 (183-288)	169 (124-268)	0.027
RDW	14.2 (13.5-14.9)	14.1 (13.5-14.8)	16.8 (15.1-18)	<0.001
NLR	8.9 (4.9-18)	8.4 (4.6-16.6)	19.8 (9.2-33.3)	0.004
Total bilirubin, mg/dL	3.1 (1.7-5.1)	3.2 (1.7-5.1)	2.8 (1.3-7.3)	0.925
Creatinine, mg/dL	0.9 (0.7-1.1)	0.9 (0.7-1)	1.3 (1.1-2.5)	<0.001
Albumin, g/dL	4 (3.7-4.3)	4.1 (3.7-4.3)	3.3 (2.6-3.6)	<0.001
C-reactive protein, mg/L	41 (16-125)	37 (15-114)	98 (44-266)	0.009
Procalcitonin, µg/L	0.5 (0.14-4.06)	0.47 (0.13-3.51)	4.29 (0.35-35)	0.006

Categorical variables are presented as n (%), non-normally distributed numerical variables as median (first quartile, third quartile), and normally distributed numerical variables as mean ± standard deviation. Abbreviations: RDW; red cell distribution width, NLR; neutrophil to lymphocyte ratio,

Table 2. Comparison of disease severity and clinical outcomes

Parameter	Overall n = 202	Survivors n = 186	Non-survivors n = 16	p
TG18 severity grading				<0.001
Grade 1 (mild)	110 (54.5)	109 (58.6)	1 (6.3)	
Grade 2 (moderate)	49 (24.3)	47 (25.3)	2 (12.5)	
Grade 3 (severe)	43 (21.3)	30 (16.1)	13 (81.3)	
SIRS score, ≥2	53 (26.2)	40 (21.5)	13 (81.3)	<0.001
Duration from admission to biliary drainage, hours	96 (34-168)	96 (36-168)	76 (28-252)	0.968
Biliary drainage method				0.145
ERCP	127 (62.9)	124 (66.7)	3 (18.8)	
PTC	5 (2.5)	4 (2.2)	1 (6.4)	
Length of hospital stay, day	9 (7-13)	9 (7-13)	6 (3-25)	0.412
Prolonged hospitalization	42 (20.8)	36 (19.4)	6 (37.5)	0.107
ICU admission	51 (25.2)	36 (19.4)	15 (93.8)	<0.001
Length of ICU stay, day	5 (3-14)	6 (3-12)	4 (2-25)	0.640
Prolonged ICU stay	12 (5.9)	7 (3.8)	5 (31.3)	0.287
Inotrope requirement	20 (9.9)	6 (3.2)	14 (87.5)	<0.001
Bacteremia	28 (13.9)	17 (9.1)	11 (68.8)	<0.001
Gram-negative	19 (9.4)	14 (7.5)	5 (31.3)	
Gram-positive	9 (4.5)	3 (1.6)	6 (37.5)	

TG18; Tokyo 2018 guidelines, SIRS; systemic inflammatory response syndrome, ERCP; endoscopic retrograde cholangiopancreatography, PTC; percutaneous transhepatic cholangiography, ICU; intensive care unit

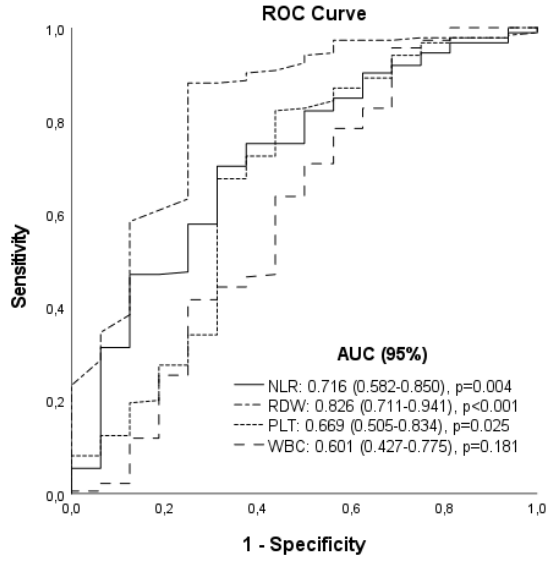

Figure 1. Predictive performance of hematologic parameters for in-hospital mortality.

NLR: Neutrophil-to-Lymphocyte Ratio; PLT: Platelet count; RDW: Red Cell Distribution Width; WBC: White Blood Cell count

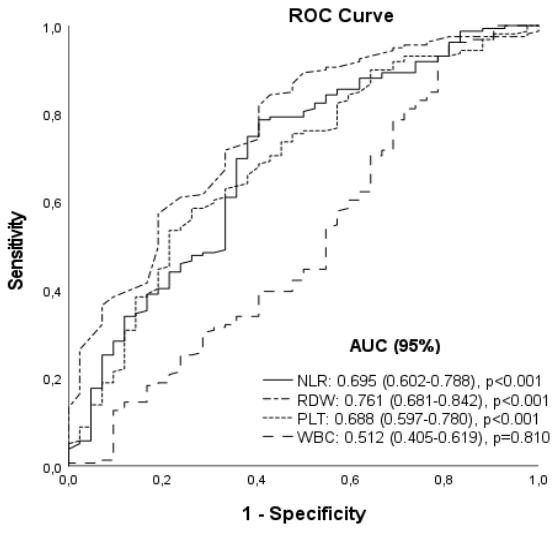

Figure 2. Predictive performance of hematologic parameters for composite outcome.

NLR: Neutrophil-to-Lymphocyte Ratio; PLT: Platelet count; RDW: Red Cell Distribution Width; WBC: White Blood Cell count

Table 3. Univariate and ROC analysis results of hematological parameters for clinical outcomes

		Prolonged hospitalization	ICU admission	In-hospital mortality	Bacteremia
WBC	*AUC*	0.402 (0.300-0.505) p=0.052	0.530 (0.435-0.626) p=0.516	0.601 (0.427-0.775) p=0.181	0.517 (0.385-0.649) p=0.0775
	OR	0.97 (0.92-1.03) p=0.336	1.02 (0.98-1.08) p=0.307	1.09 (1.03-1.16) p=0.006	1.05 (0.99-1.11) p=0.058
NEU	*AUC*	0.401 (0.299-0.504) p=0.050	0.538 (0.443-0.632) p=0.422	0.557 (0.378-0.737) p=0.446	0.532 (0.401-0.663) p=0.586
	OR	0.97 (9.91-1.03) p=0.352	1.03 (0.98-1.09) p=0.252	1.08 (1.01-1.16) p=0.018	1.06 (1.00-1.12) p=0.047
LYM	*AUC*	0.605 (0.514-0.696) p=0.036	0.682 (0.596-0.767) p=<0.001	0.627 (0.463-0.790) p=0.093	0.726 (0.618-0.834) p<0.001
	OR	0.49 (0.25-0.95) p=0.036	0.37 (0.19-0.71) p=0.003	0.79 (0.34-1.87) p=0.602	0.17 (0.06-0.482) p<0.001
HB	*AUC*	0.611 (0.504-0.717) p=0.027	0.639 (0.550-0.728) p=0.003	0.672 (0.551-0.794) p=0.022	0.624 (0.519-0.728) 0.036
	OR	0.79 (0.68-0.95) p=0.012	0.76 (0.64-0.90) p=0.002	0.68 (0.52-0.89) p=0.005	0.75 (0.61-0.93) p=0.008
PLT	*AUC*	0.604 (0.502-0.707) p=0.038	0.686 (0.600-0.773) p<0.001	0.667 (0.503-0.831) p=0.027	0.711 (0.609-0.813) p<0.001
	OR	0.99 (0.99-1.00) p=0.560	0.99 (0.98-0.99) p=0.002	0.99 (0.98-0.99) p=0.027	0.99 (0.98-0.99) p=0.001
RDW	*AUC*	0.642 (0.556-0729) p=0.005	0.697 (0.612-0.781) p<0.001	0.826 (0.711-0.941) p<0.001	0.744 (0.652-0.837) p<0.001
	OR	1.27 (1.05-1.56) p=0.016	1.49 (1.21-1.83) p<0.001	1.90 (1.43-2.51) p<0.001	1.51 (1.20-1.89) P<0.001
NLR	*AUC*	0.538 (0.445-0.631) p=0.538	0.675 (0.586-0.764) p<0.001	0.716 (0.582-0.850) p=0.004	0.708 (0.600-0.816) p<0.001
	OR	0.99 (0.97-1.02) p=0.780	1.04 (1.02-1.06) p<0.001	1.04 (1.01-1.07) p=0.005	1.05 (1.02-1.07) p<0.001

WBC; white blood cells, NEU; neutrophil, LYM; lymphocyte, HB; hemoglobin, PLT; platelet, RDW; red cell distribution width, NLR; neutrophil to lymphocyte ratio, ICU; intensive care unit, ROC; receiver operating characteristic.

tients with bacteremia, the most frequently isolated pathogen was Escherichia coli (n=14), followed by Enterococcus spp. (n=6), Klebsiella spp. (n=3), Pseudomonas spp. (n=2), and Acinetobacter spp. (n=3). Bacteremia was significantly more common in the deceased group (68.8% vs. 9.1%, p < 0.001). Comparative clinical outcomes are summarized in Table 2.

Predictive performance of hematologic parameters

Univariate regression and ROC analyses were conducted to evaluate the predictive performance of WBC, neutrophil (NEU), lymphocyte (LYM), hemoglobin (HB), platelet (PLT), RDW, and NLR for prolonged hospital stay, ICU admission, in-hospital mortality, and bacteremia. RDW demonstrated the highest predictive accuracy for in-hospital mortality, with an AUC (95% CI) of 0.826 (0.711–0.941) (Figure 1). For the composite outcome, which included the need for inotropes, mortality, prolonged ICU stay, and bacteremia, RDW was also the best predictor, with an AUC (95% CI) of 0.761 (0.681–0.842) (Figure 2). The predictive performances of hematologic parameters for

Table 4. Parameters predicting in-hospital mortality and composite outcome

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	**OR (95% CI)**	**p**
In-hospital mortality				
Age	1.12 (1.04-1.17)	0.001		
Mean arterial pressure	0.91 (0.87-0.95)	<0.001		
White blood cell count	1.09 (1.03-1.16)	0.006		
Hemoglobin	0.68 (0.52-0.89)	0.005		
Platelet count	0.99 (0.98-0.99)	0.027		
RDW	1.90 (1.43-2.52)	<0.001	2.27 (1.42-3.62)	**<0.001**
NLR	1.04 (1.01-1.07)	0.005		
Albumin	0.07 (0.03-0.21)	<0.001		
C-reactive protein	1.01 (1.00-1.01)	0.002		
Procalcitonin	1.01 (0.99-1.02)	0.137		
TG18 severity grading	7.83 (2.98-20.6)	<0.001	4.80 (1.41-16.32)	**0.012**
Charlson comorbidity index	1.40 (1.19-1.65)	<0.001		
SIRS score ≥ 2	15.8 (4.3-58.2)	<0.001		
Altered mental status	66.75 (17.56-253.6)	<0.001	42.31 (6.91-259.27)	**<0.001**
Composite outcome				
Age	1.07 (1.04-1.11)	<0.001		
Mean arterial pressure	0.94 (0.92-0.97)	<0.001		
White blood cell count	1.04 (0.99-1.09)	0.087		
Hemoglobin	0.75 (0.63-0.90)	0.002		
Platelet count	0.99 (0.98-0.99)	<0.001		
RDW	1.71 (1.36-2.14)	<0.001	1.61 (1.26-2.1)	**<0.001**
NLR	1.06 (1.03-1.08)	<0.001	1.04 (1.01-1.07)	**0.009**
Albumin	0.16 (0.08-0.33)	<0.001		
C-reactive protein	1.01 (1.00-1.01)	<0.001		
Procalcitonin	1.04 (1.02-1.07)	<0.001		
TG18 severity grading	4.89 (2.96-8.01)	<0.001	3.80 (2.21-6.54)	**<0.001**
Charlson comorbidity index	1.28 (1.12-1.47)	<0.001		
SIRS score ≥ 2	6.93 (3.31-14.51)	<0.001		

Composite outcome includes in-hospital mortality, need for inotropes, prolonged ICU stay, and bacteremia. SIRS; systemic inflammatory response syndrome, RDW; red cell distribution width, NLR; neutrophil to lymphocyte ratio, CRP; C-reactive protein, TG18; Tokyo 2018 guidelines, GCS; Glasgow Coma Scale

prolonged hospital stay, ICU admission, in-hospital mortality, and bacteremia are presented in Table 3.

Predictors of in-hospital mortality

Univariate and multivariate regression analyses were performed to identify predictors of in-hospital mortality and the composite outcome. While many parameters were associated with in-hospital mortality in the univariate analysis, multivariate analysis identified TG18 severity grading ($p = 0.012$), RDW ($p < 0.001$), and altered mental status ($p < 0.001$) as independent predictors of in-hospital mortality. For the composite outcome, multivariate analysis revealed NLR ($p =$

Table 5. Predictive abilities of hematologic parameters for clinical outcomes at different cut-off values

		Cut-off value	Number of patients*	OR (95% CI)	Sens	Spec	PPV	NPV
RDW	Composite outcome	15	46 (22.8%)	6.2 (2.9-13.1)	52.4%	85%	47.8%	87.2%
	In-hospital mortality	15.6	34 (16.8%)	21.3 (6.3-71.5)	75%	88.2%	35.3%	97.6%
NLR	Composite outcome	15.7	59 (29.2%)	5.4 (2.6-11.2)	59.5%	78.8%	42.4%	88.1%
	In-hospital mortality	14.6	66 (32.7%)	5.2 (1.7-15.8)	68.8%	70.4%	16.7%	96.3%
PLT	Composite outcome	150.000	28 (13.9%)	4.3 (1.9-10.1)	52.9%	90.7%	52.9%	80.8%
	In-hospital mortality	150.000	28 (13.9%)	4.5 (1.5-13.5)	31%	90.6%	46.4%	83.3%

*Composite outcome includes in-hospital mortality, need for inotropes, prolonged ICU stay, and bacteremia. PLT; platelet, RDW; red cell distribution width, NLR; neutrophil to lymphocyte ratio, Sens; sensitivity, Spec; specificity, PPV; positive predictive value, NPV; negative predictive value, *Number (%) of patients with values above or below the given cut-off values*

0.009), RDW ($p < 0.001$), and TG18 severity grading ($p < 0.001$) as independent predictors (Table 4).

The optimal cut-off values for independent predictors were determined using ROC analysis with Youden's index. For in-hospital mortality, the optimal RDW cut-off value was 15.6, with a sensitivity of 75% and a specificity of 88.2%. For the composite outcome, the RDW cut-off value was 15, with a sensitivity of 52.4% and a specificity of 85%. Patients with an RDW value > 15.6 had a 21.3-fold increased risk of in-hospital mortality (OR = 21.3, 95% CI: 6.3-71.5) (Table 5).

Discussion

In this prospective study, we evaluated the prognostic significance of routinely available hematological parameters in patients with AC, a condition associated with substantial morbidity and mortality. Our findings revealed that RDW was the most powerful hematological marker for predicting in-hospital mortality. Furthermore, RDW also served as a reliable predictor of adverse composite outcomes, including bacteremia, prolonged ICU stay, in-hospital mortality, need for inotropic support, and prolonged hospital stay.

AC is a critical condition requiring timely intervention, particularly in severe cases, as delays in treatment can lead to poor outcomes such as sepsis and mortality (13, 14). Therefore, the early identification of high-risk patients at presentation is crucial for patient triage and determining the timing of interventional procedures such as ERCP. Currently, the primary guideline for risk stratification and assessing disease severity is the TG18 (11). According to TG18, AC severity is classified into three grades (11, 15). For Grade 3 patients, in addition to appropriate fluid resuscitation and antibiotic therapy, urgent ERCP is recommended, whereas Grade 1 patients are advised to receive more conservative treatment approaches (15). Among hematological parameters, WBC and PLT are the two markers included in the TG18 criteria, classified as Grade 2 and Grade 3 severity markers, respectively (11).

WBC count is widely used as a marker of infection and inflammation in clinical practice due to its rapid and accessible measurement (16). Elevated WBC levels are often indicative of systemic inflammatory responses, such as those observed in sepsis, AC, or other infectious processes (17). In a study by Murayama et al., a WBC count exceeding 20,000 was identified as a poor prognostic factor in AC patients (18). Similar findings have been reported in other studies, where a WBC count above 20,000 was associated with worse outcomes (19, 20). However, the diagnostic specificity of WBC is limited, as elevated levels may also occur in non-infectious inflammatory conditions or due to physiological stress (21, 22). In our study, although WBC was associated with in-hospital mortality in univariate analysis, its prognostic value was lower than other hematological parameters based on ROC analysis.

Another widely used hematological parameter is the PLT count. Beyond its role in hemostasis, PLT count serves as an inflammatory marker in various clinical conditions (23). Thrombocytopenia is frequently associated with severe infections, systemic inflammation, or disseminated intravascular coagulation, reflecting disease severity (24). It is also recognized as a poor prognostic factor in numerous conditions, including AC, where it may indicate advanced disease, systemic involvement, or increased risk of complications (25). In a study by Chen et al., PLT was shown to predict bacteremia, with an AUC of 0.649, a finding consistent with our study (26). TG18 also includes thrombocytopenia (PLT < 100,000/μL) as a marker of poor prognosis (11). Similarly, our findings support the predictive value of PLT for adverse outcomes, in alignment with the TG18 criteria.

In recent years, alongside traditional hematological parameters, novel markers such as the NLR and RDW have been shown to predict poor prognosis, particularly in inflammatory conditions (5, 7, 8). RDW primarily reflects heterogeneity in erythrocyte size and serves as an indicator of the systemic effects of inflammation (27). In states of chronic inflammation and oxidative stress, erythropoiesis is suppressed, iron metabolism is disrupted, and erythrocyte lifespan is shortened (28). These mechanisms lead to increased RDW, which has been associated with the severity of inflammatory diseases and worse clinical outcomes (29). Additionally, elevated RDW may reflect endothelial dysfunction and microvascular injury, further linking it to adverse prognostic outcomes (30).

NLR, on the other hand, is regarded as a marker of the balance between pro-inflammatory and anti-inflammatory responses in the immune system (31). During inflammation, neutrophil mobilization increases, while lymphocyte counts decrease, resulting in an elevated NLR (32). A high NLR indicates an exaggerated immune response, as observed in conditions such as SIRS and sepsis, where immune dysregulation can lead to organ dysfunction or multi-organ failure. NLR is also associated with severe inflammatory responses, such as cytokine storms, and serves as an indirect measure of inflammatory burden (10, 32-34). The distinct mechanisms by which RDW and NLR reflect different aspects of the inflammatory response suggest that these parameters could play complementary roles in assessing disease severity in conditions like AC.

In a study by Yesil et al., NLR was found to predict AC severity (35). Similar findings from other studies have demonstrated NLR as a strong predictor of adverse outcomes in AC (36, 37). In our study, while NLR was a reliable predictor of composite outcomes, its ability to predict in-hospital mortality was lower in multivariate analysis. Moreover, NLR's prognostic value for both in-hospital mortality and composite outcomes was inferior to RDW based on ROC analysis. Our study highlights that RDW outperformed all other hematological parameters in predicting poor prognosis. These findings suggest that incorporating RDW into AC guidelines and severity classifications could enhance patient prognosis and triage decisions. To the best of our knowledge, this is the first prospective study of this scale to demonstrate the superior prognostic value of RDW in AC, emphasizing its potential role in future clinical decision-making.

Our study has some limitations. The primary limitations are the small sample size and the single-center design. However, despite being a single-center study, it is noteworthy that our hospital is one of the largest healthcare institutions in Türkiye, serving a heterogeneous patient population referred from various healthcare facilities. Additionally, our study evaluated hematological parameters obtained at the time of hospital admission. As a result, these values may not fully reflect the initial stages of the disease. Nevertheless, given that studies on AC can only be conducted in advanced tertiary care hospitals, this limitation is inevitable.

In conclusion, our study, which thoroughly investigated the prognostic value of hematological parameters in AC, demonstrated that RDW reflects AC prognosis significantly better than traditionally used parameters such as PLT and WBC. However, for RDW to be incorporated into clinical guidelines and

gain widespread clinical utility in assessing AC severity, future multicenter studies with larger patient cohorts are needed.

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Ethics Committee Approval: This study was conducted with the approval of the Hospital Ethics Committee (Number: E2-22-2101).

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Diagnostic Utility of PRAME in Differentiating Melanocytic Lesions, Especially Spitzoid Tumors*

Melanositik Tümörlerin Tanısında PRAME'in Tanısal Değeri

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ABSTRACT

Objectives: Differential diagnosis of melanoma and nevi is usually straightforward. However, there can be difficult cases, and additional reliable methods are needed for differentiating benign melanocytic lesions from malignant ones. We aimed to investigate the role of PRAME in challenging melanocytic lesions.

Materials and Methods: The study group included 27 atypical Spitz tumors, 12 Spitzoid melanomas, 20 conventional melanomas, and 10 high-grade dysplastic nevi. The control group included 20 melanocytic nevi, 20 Spitz nevi, and 10 low-grade dysplastic nevi. Immunohistochemistry for PRAME, BRAF, p16, BAP1, MelanA, and Ki67 was performed using automated systems. A staining percentage of ≥75% was considered PRAME positive. Data were analyzed using SPSS v11.5, and a p-value <0.05 was accepted as significant.

Results: PRAME positivity (≥75%) was observed in 60% of melanomas and 25% of Spitzoid melanomas, but not in any type of nevi. Mean PRAME staining percentages: conventional melanoma (58.1%), Spitzoid melanoma (28%), atypical Spitz tumor (2%), Spitz nevus (3.3%), dysplastic nevi (0.75%), and 0% in melanocytic nevi. PRAME showed 60% sensitivity and 100% specificity for melanoma, and 25% sensitivity and 100% specificity for Spitzoid melanoma. A 55% threshold increased sensitivity to 70% and 33.3%, respectively, without reducing specificity. PRAME expression correlated with Clark level in atypical Spitz tumors, tumor-infiltrating lymphocytes in Spitzoid melanomas, but not with Breslow thickness or dysplasia grade.

Conclusion: PRAME shows high specificity in distinguishing melanoma from benign lesions. Lowering the positivity threshold to 55–60% may improve sensitivity, especially in Spitzoid variants, without compromising specificity.

Key Words: PRAME, melanoma, atypical Spitz tumor, Spitzoid melanoma, nevi

ÖZET

Amaç: Melanositik lezyonların ayırıcı tanısında bazı vakalar zorlayıcı olabilmekte ve benign melanositik lezyonları malign olanlardan ayırt edebilmek için ek güvenilir yöntemlere ihtiyaç duyulmaktadır. Bu çalışmada tanı güçlüğü yaşanan melanositik lezyonlarda PRAME'in rolünü araştırmak amaçlanmıştır.

Gereç ve Yöntem: Çalışma grubuna 27 atipik Spitz tümör, 12 Spitzoid melanom, 20 konvansiyonel melanom ve 10 yüksek dereceli displazili displastik nevüs dahil edilmiştir. Kontrol grubunu 20 melanositik nevüs, 20 Spitz nevüs ve 10 düşük dereceli displazili displastik nevüs oluşturmuştur. PRAME, BRAF, p16, BAP1, MelanA ve Ki67 immünohistokimya boyamaları otomatik boyama cihazında gerçekleştirilmiştir. %75 ve üzeri boyanma PRAME pozitif olarak tanımlandı. Veriler SPSS v11.5 programı ile analiz edilerek, p<0,05 değeri istatistiksel anlamlı kabul edilmiştir.

Bulgular: PRAME pozitifliği (≥%75), konvansiyonel melanomların %60'ında ve Spitzoid melanomların %25'inde gözlenmiştir. Nevüslerde boyanma saptanmamıştır. Ortalama PRAME boyanma oranları: konvansiyonel melanomda %58,1; Spitzoid melanomda %28; atipik Spitz tümörde %2; Spitz nevüste %3,3; displastik nevüslerde %0,75 ve melanositik nevüslerde %0 olarak bulunmuştur. PRAME; melanom tanısı için %60 duyarlılık ve %100 özgüllük; Spitzoid melanomlar için %25 duyarlılık ve %100 özgüllük göstermiştir. Pozitiflik için eşik değer %55'e indirildiğinde, melanom için duyarlılık %70'e, Spitzoid melanomlar için ise %33,3'e yükselmiş; özgüllük ise değişmemiştir. PRAME ekspresyonu, atipik Spitz tümörlerde Clark seviyesi, Spitzoid melanomlarda tümörü infiltre eden lenfositler ile anlamlı ilişki göstermiştir. Ancak Breslow kalınlığı veya displazi derecesiyle ilişki saptanmamıştır.

Sonuç: PRAME, melanomun benign lezyonlardan ayırt edilmesinde yüksek özgüllük göstermektedir. Pozitiflik için eşik değerin %55–60'a düşürülmesi, özellikle Spitzoid varyantlarda duyarlılığı yükseltip ve özgüllükten ödün vermeden tanısal doğruluğu artırabilmektedir.

Anahtar Kelimeler: PRAME, melanom, atipik Spitz tümör, Spitzoid melanom, nevüs

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Introduction

Melanocytic lesions encompass a broad spectrum ranging from benign nevi to melanoma. These lesions exhibit diverse histopathological features, genetic alterations, and clinical behaviours (1). Although most melanocytic tumors can be classified as benign or malignant based on histopathologic morphological features on hematoxylin and eosin (H&E) sections, a significant number pose diagnostic challenges (2,3). Immunohistochemistry (IHC) is often employed in challenging cases. IHC markers such as S100, SOX10, Melan-A, MITF, HMB45, and tyrosinase are routinely used to confirm melanocytic differentiation. Evaluation of MelanA+Ki67 dual immunostaining, HMB45 expression pattern (for investigating nevus maturation), loss of p16 expression provides valuable information in distinguishing benign from malignant melanocytic lesions. (4,5).

PRAME (PReferentially expressed Antigen in MElanoma) is a tumor-associated antigen initially identified in metastatic melanoma through autologous T-cell analysis. It has since been recognized as a useful marker due to its frequent expression in melanomas and absence in most benign melanocytic lesions, offering diagnostic value in challenging cases (6-8).

Spitz tumors represent a distinct subset of melanocytic proliferations characterized by unique cytological and molecular features. Differentiating Spitz nevi from their malignant counterparts, particularly Spitzoid melanoma, remains a well-known diagnostic difficulty due to overlapping histologic features (9,10).

Our study aimed to investigate the expression of PRAME antibody, which has gained importance in distinguishing melanoma from benign lesions in recent years, and it's importance especially in Spitzoid lesions in the diagnostic approach with BRAF(VE1), p16, BAP1, and MelanA+Ki67 dual stains.

Material and Methods

The study was approved by Ankara University Human Research Ethics Committee under decision number I11-684-21 on 9 December 2021 and was conducted in full compliance with the principles of the Declaration of Helsinki. Human Research Ethics Committee under decision number I11-684-21 on 9 December 2021 and was conducted in full compliance with the principles of the Declaration of Helsinki.

Study Design and Case Selection

A total number of 119 melanocytic lesion cases diagnosed at Ankara University Faculty of Medicine, Department of Pathology, between 2005 and 2022 were included in the study. The study group consisted of 27 atypical Spitz tumors (AST), 12 Spitzoid melanoma, 20 conventional melanoma, and 10 dysplastic nevi showing high-grade dysplasia. The control group consisted of patients diagnosed with 20 melanocytic dermal nevi, 20 Spitz nevi, and 10 dysplastic nevi showing low-grade dysplasia (Table 1).

The conventional melanomas were categorized into 3 nodular, 4 lentigo malignant melanoma (LMM), 4 superficial spreading, 3 acral, 1 desmoplastic, 1 nevoid melanoma, and 4 melanomas developing on a nevus.

The melanocytic nevi group was divided into four groups: 5 congenital nevi, 5 junctional, 5 compound, and 5 dermal nevi. H&E-stained sections were re-evaluated by two pathologists.

Table 1. Distribution of study and control groups

Group	Subtype	(n)
Study Group	Atypical Spitz Tumor	27
	Spitzoid Melanoma	12
	Conventional Melanoma	20
	Dysplastic Nevus (High-grade)	10
Control Group	Melanocytic Dermal Nevus	20
	Spitz Nevus	20
	Dysplastic Nevus (Low-grade)	10
Total		119

Histopathological evaluation

In melanoma cases, Breslow tumor thickness, Clark level, mitotic rate (per mm^2), lymphovascular invasion (LVI), ulceration, regression, and tumor-infiltrating lymphocytes (TIL) were evaluated. In dysplastic nevi, the degree of dysplasia was assessed by the presence of both cellular and structural dysplasia.

Table 2. Antibodies, Clones, and Staining Conditions Used for Immunohistochemical Evaluation in Melanocytic Lesions

Antibody	Clone	Host Species	Dilution	Incubation Time	Antigen Retrieval
PRAME	Cell Marque	Rabbit	1:100	92 min	Extended
BRAF	VE1	Mouse	RTU	120 min + AMP	Extended
p16	NK1	Mouse	RTU	120 min	Extended
BAP1	C-4	Mouse	1:50	92 min + AMP	Standard
Melan-A	Mart-1 A103	Mouse	RTU	120 min	Standard
Ki-67	30-9	Rabbit	RTU	48 min	Standard

All immunohistochemical stains were performed using automated immunostainers (Ventana, Roche, USA) with the Streptavidin-Biotin Peroxidase method. OptiView and UltraView DAB detection kits were used.

Immunohistochemical evaluation

PRAME, BRAF, p16, BAP1, MelanA, and Ki67 antibodies were applied to the sections using the Streptavidin-Biotin Peroxidase method on automatic immunohistochemistry staining devices (Roche-Ventana) (Table 2). Evaluation of immunohistochemical examinations (PRAME, BRAF, p16, BAP1, MelanA, Ki67) was performed under light microscopy. The evaluation of these markers was as follows:

PRAME expression was scored according to both the intensity and the percentage of stained cells (0%-100%). Diffuse moderate to strong membranous staining for the BRAF VE1 antibody was considered positive. Focal staining or complete absence of nuclear expression was defined as loss of p16 expression. Only diffuse BAP1 loss was considered positive. MelanA and Ki67 dual staining was performed to show the Ki-67 proliferation index at MelanA-positive melanocytic cells.

Statistical analysis

SPSS version 11.5 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. Categorical variables were presented with frequencies and percentages, while metric variables were presented with mean ± SD or median with minimum and maximum values. The chi-square test was used to compare categorical variables, while the Kruskal–Wallis variance analysis was employed for comparisons among more than two independent groups. Spearman's rank correlation coefficient (Spearman's rho) was used to assess the relationship between PRAME expression and histopathological parameters, including Clark level, Breslow thickness, tumor-infiltrating lymphocytes (TILs), and dysplasia grade. Additionally, receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of PRAME expression and determine the optimal cut-off point using the Youden index. A p-value of <0.05 was considered statistically significant.

Results

Among the 119 cases included in the study, 66 (55.5%) were female and 53 (44.5%) were male. The mean age of patients was 36.84 ± 20.16 years, with a range of 1 to 94 years.

The microscopic images of some immunohistochemical stainings are illustrated in Figures 1-3.

Mean staining percentages for PRAME were as follows in the groups; Conventional melanoma: 58.05% ±38.76 (ranging between 0% to 95%), Spitzoid melanoma: 28% ±37.72 (ranging between 0% and 85%), AST: 2% ±9.60 (ranging between 0% and 50%), Spitz nevi: 3.25% ±8.31 (ranging between 0% and 30%), and dysplastic nevi 0.75% ±2.44 (ranging between 0% and 30%). Melanocytic nevi did not show any nuclear staining (Figure 4).

When a threshold of >75% staining was used to define PRAME positivity, 12/20 (60%) cases in the conventional melanoma group and 3/12 (25%) cases in the Spitzoid melanoma group were positive. Nuclear staining with PRAME below the threshold value was seen in 1 of 27 cases (3.7%) in AST, in 3 (15%) of 20 cases in Spitz nevi, and 2 (10%) of 20 cases in dysplastic nevi.

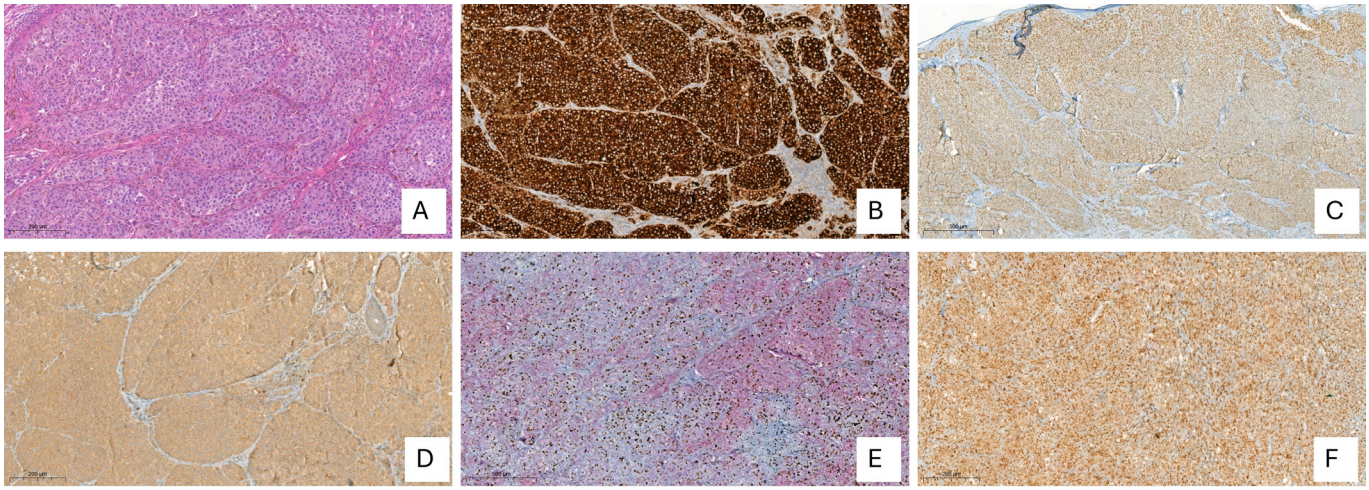

Figure 1. Histopathologic and immunohistochemical features of conventional melanoma **(A)** Hematoxylin and eosin (H&E) stain reveals a dermal-based atypical melanocytic proliferation, x200 **(B)** BRAF V600E shows diffuse, strong cytoplasmic positivity, x200 **(C)** PRAME is strongly and diffuse positive (score:3+, 90%) ,x200 **(D)** p16 demonstrates complete loss of nuclear expression in tumor cells, x200 **(E)** Melan A**+**Ki-67 double stain demonstrates a high (20%) proliferative index in tumor cells, x200 **(F)** BAP1 shows retained nuclear expression in tumor cells, x 500.

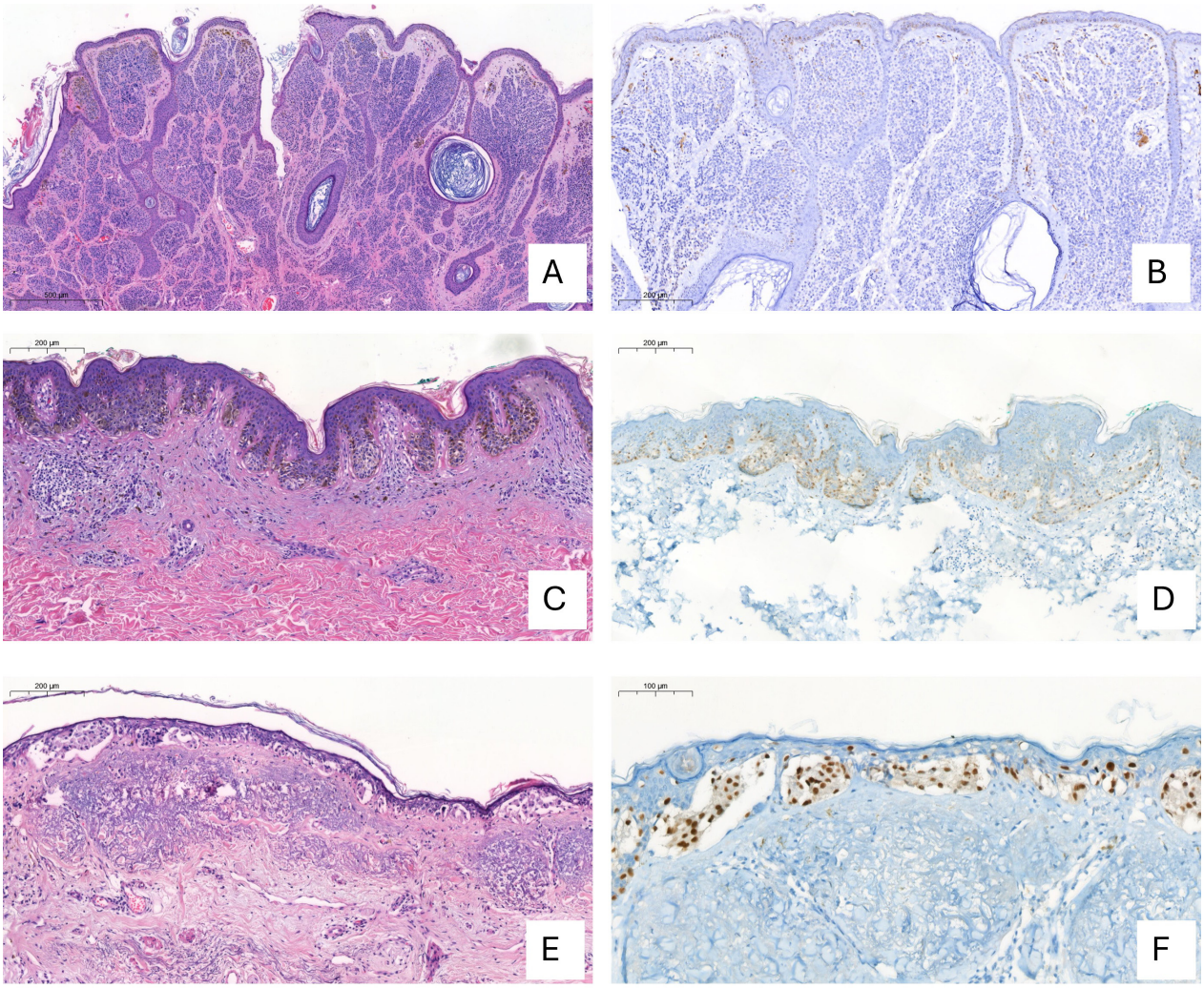

Figure 2. PRAME expression in benign and dysplastic melanocytic lesions. **(A-B)** H&E shows a compound nevus which is PRAME negative, x200 **(C-D)** H&E shows high-grade dysplastic nevus with weak to moderate PRAME positivity (score: 3+, 10%), x200. **(E–F)** H&E reveals lentigo maligna and the tumor is diffusely PRAME positive (score: +3, 90%), x200.

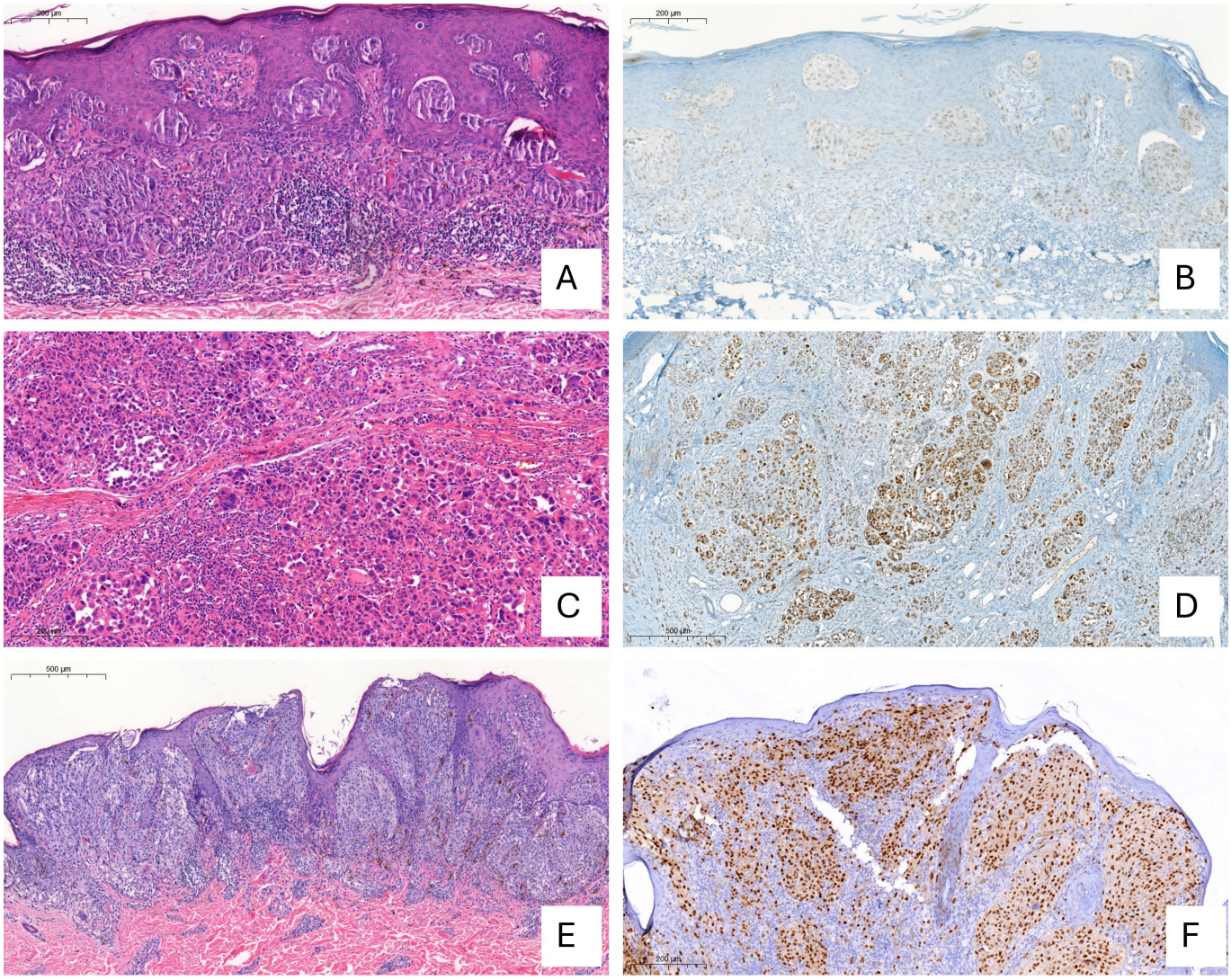

Figure 3: PRAME expression in the spectrum of Spitzoid lesions. **(A–B)** H&E shows Spitz nevus with PRAME negativity, x200 **(C–D)** H&E shows atypical Spitz tumor and PRAME is patchy nuclear positive (score: 3+, 50%), x500. **(E–F)** H&E shows Spitzoid melanoma with diffuse PRAME positivity (score: 3+, 80%), x200.

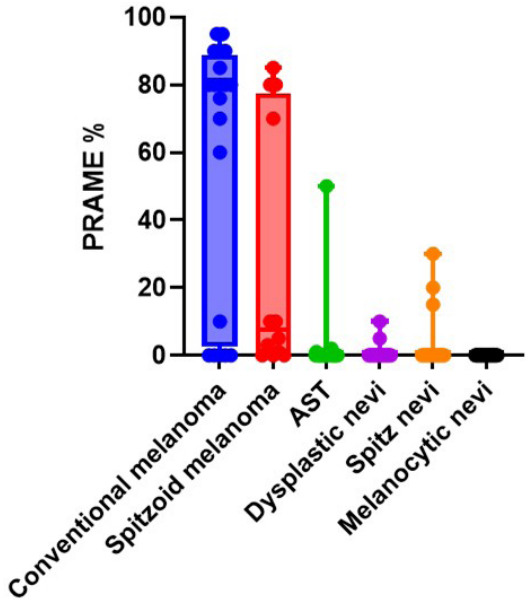

Figure 4. PRAME expression percentages in the study and control groups. Significantly higher PRAME percentages were seen in the melanoma groups (Kruskal–Wallis test, $p < 0.0001$).

A statistically significant difference was found for PRAME staining between conventional melanoma and melanocytic nevi, dysplastic nevi, AST, and Spitz nevi ($p<0.05$). When staining over 75% was considered the threshold for PRAME, the sensitivity was 60%, and the specificity was 100% in conventional melanomas. For Spitzoid melanomas, the sensitivity was 25%, and the specificity was 100%.

As a result of ROC analysis, the highest Youden index was found at 55%. When 55% was considered the positivity threshold for PRAME, sensitivity increased to 70% in conventional melanomas and 33.3% in Spitzoid melanomas without any change in specificity.

There was no statistically significant relationship between PRAME expression and the degree of dys-

plasia. However, the percentage and the intensity of PRAME expression increased with the severity of dysplasia.

In AST cases, there was a statistically significant relationship between PRAME expression and Clark level ($p<0.05$). However, there was no significant correlation between Clark level or Breslow thickness and PRAME expression in Spitzoid or conventional melanoma cases ($p>0.05$).

We found a statistically significant relationship between TILs and PRAME expression in Spitzoid melanoma cases ($p<0.05$). However, in conventional melanoma cases, no statistically significant association was found ($p=0.5$).

BRAF VE1 antibody immunoreactivity was observed in 9 of 20 conventional melanoma (45%), 6 of 12 Spitzoid melanoma (50%), 1 of 27 AST (3.7%), 9 of 20 Spitz nevi (45%), 11 of 20 dysplastic nevi (55%), and 19 of 20 melanocytic nevi (95%).

Loss of **p16** expression was not observed in melanocytic nevi or dysplastic nevi cases. Focal loss of p16 expression was detected in 2 of 20 Spitz nevi (10%) and in 1 of 12 Spitzoid melanomas (8.3%). Among 27 AST, diffuse or focal loss of p16 expression was identified in 3 cases (11.1%). Loss of p16 expression was observed in 4 of 20 conventional melanomas (3 nodular and 1 nevoid melanoma) (20%). Statistical analysis revealed a significant difference in p16 expression loss between ASTs and conventional melanomas ($p = 0.009$), as well as between conventional melanomas and melanocytic nevi ($p = 0.001$).

None of the cases exhibited diffuse loss of **BAP1** expression. BAP1 staining was generally observed as diffuse nuclear staining. In some cases, focal and scattered nuclear staining was accompanied by cytoplasmic and perinuclear staining patterns.

Using **MelanA + Ki-67** dual immunostaining, the Ki-67 proliferation index was observed to range between 10–40% in conventional melanomas, 4–50% in Spitzoid melanomas, 1–20% in ASTs, 0–10% in Spitz nevi, 0–3% in dysplastic nevi, and 0–5% in melanocytic nevi.

Discussion

This study supports the diagnostic utility of PRAME immunohistochemistry in distinguishing melanoma from benign melanocytic lesions. Our findings are in concordance with Lezcano et al, who reported diffuse PRAME expression in melanoma and minimal staining in benign nevi (6). Similarly, we observed high and widespread PRAME expression in malignant lesions and no nuclear staining in conventional melanocytic nevi. Notably, we identified that adjusting the positivity threshold from 75% to 55% enhanced sensitivity in detecting melanoma without compromising specificity, suggesting that a 55% cut-off may be more appropriate for diagnostic purposes.

Lohman et al reported a sensitivity of 67% and specificity of 100% using a +4 PRAME score for melanoma diagnosis, similar to our sensitivity rates of 60% at the 75% cut-off and 70% at the 55% cut-off (11).

Previous studies have demonstrated that PRAME expression complements other melanocytic markers such as Melan-A and HMB-45, and is particularly valuable in challenging cases (12). In lentigo maligna and lentigo malignant melanoma, Tio et al, showed variable PRAME expression, with stronger expression in LMM (13) . Our study supports these findings; in one LMM case, PRAME positive cells were observed at the surgical margin, although no visible lesion was detected in H&E-stained sections.

The interpretation of PRAME expression in Spitzoid lesions remains challenging. Our results were consistent with Raghavan et al., who reported diffuse PRAME staining in only a minority of Spitzoid melanomas and emphasized its rarity in Spitz nevi and ASTs. Only 4 of 12 Spitzoid melanomas (33.3%) showed positivity at the 55% threshold, and most ASTs exhibited weak, heterogeneous staining below 50%. Another point to emphasize is that Raghavan et al reported PRAME-positive ASTs harbored CDKN2A deletions (7). In parallel with this, we observed that one of our AST cases, having no 9p21 deletion, had a low (under 50%) PRAME expression. These results underline the importance of integrating PRAME IHC with cytogenetic and morphological assessments.

The diagnostic relevance of PRAME in dysplastic nevi remains limited. Although PRAME positivity is rare in these lesions, our study noted focal but strong staining in a small subset of high-grade dysplastic nevi. Koch et al proposed quantitative digital thresholds for PRAME positivity in distinguishing dysplastic nevi from thin superficial spreading melanomas (14). Olds et al similarly emphasized the importance of quantifying PRAME-positive cells per mm^2 to differentiate early melanoma in situ from benign lesions (15). Our findings suggest a potential association between the severity of dysplasia and increased PRAME expression, although the correlation did not reach statistical significance.

Lezcano et al cautioned that focal PRAME staining may occur in junctional melanocytes or sun-damaged skin, advising careful interpretation in small biopsies (6,16). Raghavan et al further recommended against malignancy diagnosis based solely on PRAME positivity in small or traumatized specimens (7). Finally, while rare, PRAME expression may be observed in proliferative nodules of congenital melanocytic nevi. Gill et al demonstrated its utility in evaluating such proliferations when molecular tests are unavailable (17).

One of the main strengths of our study was the evaluation of Spitzoid and non-Spitzoid melanocytic lesions in a diagnostic spectrum from benign to borderline to malignant ones. Another strong aspect of our study was the presence of different histopathological subtypes of lesions in both benign and malignant non-Spitzoid melanocytic groups.

Study Limitations

This study has several limitations. Firstly, the number of cases in each different histopathological subtype of conventional melanoma groups, as well as Spitzoid melanoma cases, could be expanded. Secondly, immunohistochemical markers such as ALK, NTRK, and ROS could be added to the panel in the evaluation of Spitzoid lesions.

Conclusion

In conclusion, our study supports the role of PRAME IHC in distinguishing benign from malignant melanocytic tumors, although a limited help in Spitzoid lesions. Lowering the diagnostic threshold to 55% improves sensitivity without decreasing specificity. While PRAME is not an independent marker, its expression (particularly when combined with morphology and genetic findings) can significantly aid diagnostic precision.

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Research Article / Araştırma Makalesi

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Evaluation of Toxoplasmosis Serology Results and Test Request Dynamics in Ankara University Medical Faculty Hospitals*

Ankara Üniversitesi Tıp Fakültesi Hastanelerinde Toksoplazmoz Seroloji Sonuçlarının ve Test İsteme Dinamiklerinin Değerlendirilmesi

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ABSTRACT

Background: Toxoplasmosis, caused by *Toxoplasma gondii*, is one of the most common parasitic diseases in the world and in our country. Serological methods are mainly used for diagnosis; however, there are some difficulties in the diagnosis of acute infection.

Aim: The aim of this study was to evaluate the anti-*Toxoplasma* IgM and anti-*Toxoplasma* IgG results of serum samples sent to Ankara University İbni Sina Research and Training Hospital from various clinics, as well as the sociodemographic characteristics of the patients.

Materials and Methods: Blood sample results from January 1, 2021, to September 1, 2024, were assessed. Anti-*Toxoplasma* antibodies were examined using the Elecsys kit in the Cobas device with the electrochemiluminescence immunoassay technique.

Results: Anti-*Toxoplasma* IgG and IgM positivity rates were found to be 23.95% and 1.30%, respectively, between the specified dates. IgG results were positive in 21.54% of women and 30.48% of men. There was a statistically significant difference between the two genders (p<0.001). It was discovered that 1.19% of males and 1.34% of women had reactive anti-*Toxoplasma* IgM results; however, the difference was not statistically significant between the two genders (p=0.667). Most patients who tested positive for anti-*Toxoplasma* IgM and IgG were 80 years of age or above. Anti-*Toxoplasma* IgG positivity was highest in immunocompromised adult and pediatric clinics (44.06% and 42.21%, respectively); however, the adult infection service had the highest percentage (1.59%) for IgM positivity.

Conclusion: Studies conducted in Türkiye revealed that the range of anti-*Toxoplasma* IgG positivity varied between 20.3% and 69.6%. Our study's anti-*Toxoplasma* IgG positivity rate was between this range near the lower limit and consistent with prior studies conducted in Ankara. Studies in Türkiye have shown that IgM seropositivity varied from 0% to 9.7%. The anti-*Toxoplasma* IgM positivity ratio of our study was in this range and close to the lower limit similar to the anti-*Toxoplasma* IgG.

Keywords: Anti-*Toxoplasma* IgM, anti-*Toxoplasma* IgG, positivity rate, immunocompromised, pregnant

ÖZET

Giriş: *Toxoplasma gondii*'nin neden olduğu toksoplazmoz dünyada ve ülkemizde yaygın olarak görülen paraziter hastalıklardan biridir. Tanısında başlıca serolojik yöntemlerden faydalanılmasına rağmen akut enfeksiyon tanısında birtakım güçlükler yaşanmaktadır.

Amaç: Bu çalışmanın amacı Ankara Üniversitesi İbni Sina Uygulama ve Araştırma Hastanesi'ne çeşitli kliniklerden gönderilen serum örneklerinin anti-*Toxoplasma* IgM ve anti-*Toxoplasma* IgG sonuçlarını ve hastaların sosyodemografik özelliklerini değerlendirmektir.

Gereç ve yöntem: Çalışma kapsamında 1 Ocak 2021 ile 1 Eylül 2024 tarihleri arasında alınan kan örneklerinin sonuçları değerlendirildi. Anti-*Toxoplasma* antikorları Elecsys kiti ile Cobas cihazında elektrokemilüminesan immünoassay tekniği kullanılarak incelendi.

Bulgular: Anti-*Toxoplasma* IgG ve IgM pozitiflik oranları sırasıyla %23,95 ve %1,30 olarak bulundu. Anti-*Toxoplasma* IgG kadınların %21,54'ünde ve erkeklerin %30,48'inde pozitifti. İki cinsiyet arasında istatistiksel olarak anlamlı bir fark vardı (p<0,001). Erkeklerin %1,19'unun ve kadınların %1,34'ünün anti-*Toxoplasma* IgM sonuçları reaktif olarak saptandı, ancak fark istatistiksel olarak anlamlı değildi (p=0,667). Anti-*Toxoplasma* IgM ve IgG sonuçları pozitif olan hastaların çoğu 80 yaş ve üzeriydi. Anti-*Toxoplasma* IgG pozitiflik yüzdesi en yüksek, immün sistemi baskılanmış yetişkin ve çocuk kliniklerinde (sırasıyla %44,06 ve %42,21) saptanırken; yetişkin enfeksiyon servisi anti-*Toxoplasma* IgM pozitifliği bakımından en yüksek yüzdeye sahipti (%1,59).

Sonuç: Türkiye'de yapılan çalışmalar anti-*Toxoplasma* IgG pozitifliğinin %20,3 ile %69,6 arasında değiştiğini göstermektedir. Çalışmamızın anti-*Toxoplasma* IgG pozitiflik oranı bu aralığın içinde alt sınıra yakın olup, Ankara'da yapılan önceki çalışmalarla uyumlu bulunmuştur. Türkiye'deki çalışmalarda IgM seropozitifliğinin %0 ile %9,7 arasında değiştiği gösterilmiştir. Çalışmamızın anti-*Toxoplasma* IgM pozitiflik oranı anti-*Toxoplasma* IgG ile benzer olarak bu aralığın içinde alt sınıra yakın olarak saptanmıştır.

Anahtar kelimeler: Anti-*Toxoplasma* IgG, anti-*Toxoplasma* IgG, pozitiflik oranı, bağışıklığı baskılanmış, gebe

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* The study has not been presented previously as an abstract in any congress or symposium

Introduction

One of the most prevalent protozoa in the world and in Türkiye is *Toxoplasma gondii* [1, 2]. Whereas felines are the agent's definitive host, mammals, including humans, are intermediate hosts. The protozoa is primarily spread via eating undercooked meat harboring tissue cysts, consuming food contaminated with oocysts excreted in cat feces, organ and blood transplantation, and transplacental transmission from mother to fetus. In an individual with a competent immune system, the disease proceeds asymptomatically or with mild symptoms resembling those of infectious mononucleosis, but it can be fatal in a patient with a compromised immune system. Moreover, congenital abnormalities, stillbirth, and miscarriage may also occur as a result of transmission of the protozoan from mother to fetus during pregnancy. Therefore, toxoplasmosis is an urgent threat for immunocompromised patients and pregnant women, and the approach to diagnostic testing and treatment must be carefully considered [2-4].

The majority of toxoplasmosis diagnoses are made by serological testing. Anti-*Toxoplasma* IgM turns positive in the first week of infection, peaks in the 1st-3rd months, begins to decline thereafter, and usually turns negative six months later, or within a year [5]. Positive results, however, may sometimes last for years [6]. Anti-***Toxoplasma***a IgG turns positive in the second week, peaks in the 3rd-6th months, then starts to decline and stabilizes after a while. It usually remains positive for the rest of the life [7]. It can be challenging to differentiate between acute and past infections since anti-***Toxoplasma*** IgM positivity may last for a long time [5]. The time of infection could be ascertained using the avidity test. High avidity definitely rules out the diagnosis of acute infection and suggests that the infection occurred at least 16 weeks earlier. Low avidity, on the other hand, could indicate either an acute infection or a past infection with inadequate avidity maturation. In this case, the dynamics of the anti-***Toxoplasma*** IgG test must be assessed in conjunction with the avidity index. The procedure of assessing the findings of serological tests used to diagnose toxoplasmosis is intricate [7, 8]. For the tests to be used effectively, it would be advantageous for the centers to ascertain their own test request and interpretation dynamics.

The aim of this study was to evaluate the results of serum samples sent to Ankara University İbni Sina Research and Training Hospital for anti-*Toxoplasma* IgM and anti-*Toxoplasma* IgG requests from various clinics, together with the sociodemographic characteristics of the patients.

Materials and Methods

In the course of the study, the results of blood samples sent for anti-*Toxoplasma* IgM and anti-*Toxoplasma* IgG testing to İbni Sina Research and Training Hospital at Ankara University Faculty of Medicine between January 1, 2021, and September 1, 2024, were assessed retrospectively. The patient's age and gender, clinics and outpatient clinics where the sample was sent, anti-*Toxoplasma* IgM, anti-*Toxoplasma* IgG, and avidity test results were recorded. The patients were evaluated in nine age-based groups: 0–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80 and above. When a patient gave multiple samples over the course of specified years, only the initial result—if any, the first positive result—was included.

The Elecsys Anti-***Toxoplasma*** kit (Roche Diagnostics, Rotkreuz, Switzerland), which detects anti-***Toxoplasma*** antibodies using the electrochemiluminescence immunoassay (ECLIA) method, was used on a Cobas system (Mannheim, Baden-Württemberg: Roche Diagnostics GmbH, Germany). Test results for IgM with a score of less than 0.8 were evaluated as negative, 0.8–1 as borderline, and 1 and higher as positive. Test results for IgG, which were less than 1 IU/ml, were reported as negative; those that were 1-30 were reported as intermediate, and those that were 30 and higher as positive. The avidity index had three categories, such as low avidity (less than 70), grey zone (70–79), and high avidity (80 and above). The study was approved by the Human Research Ethics Committee of Ankara University (date: 07.10.2024, desicion number: İ08-650-24).

Statistical analysis

The statistical analysis was conducted with IBM Statistical Package for the Social Sciences for Windows

Table 1. Distribution of anti-*Toxoplasma* IgG positivity rate according to age groups and gender

Age groups	Gender	Anti-*Toxoplasma IgG*							
		Negative		Intermediate		Positive		Total	
		n	%	n	%	n	%	n	%
0-9	**Women**	422	80.38	6	1.14	97	18.48	525	100
	Men	530	81.54	8	1.23	112	17.23	650	100
	Total	952	81.02	14	1.19	209	17.79	1175	100
10-19	**Women**	298	86.38	5	1.45	42	12.17	345	100
	Men	268	83.23	7	2.17	47	14.60	322	100
	Total	566	84.86	12	1.80	89	13.34	667	100
20-29	**Women**	2356	88.70	3	0.11	297	11.18	2656	100
	Men	270	82.32	6	1.83	52	15.85	328	100
	Total	2626	88.00	9	0.30	349	11.70	2984	100
30-39	**Women**	2022	81.53	2	0.08	456	18.39	2480	100
	Men	273	75.62	3	0.83	85	23.55	361	100
	Total	2295	80.78	5	0.18	541	19.04	2841	100
40-49	**Women**	412	65.09	1	0.16	220	34.76	633	100
	Men	200	58.31	4	1.17	139	40.52	343	100
	Total	612	62.70	5	0.51	359	36.78	976	100
50-59	**Women**	199	47.84	4	0.96	213	51.20	416	100
	Men	164	51.41	4	1.25	151	47.34	319	100
	Total	363	49.39	8	1.09	364	49.52	735	100
60-69	**Women**	115	36.62	1	0.32	198	63.06	314	100
	Men	121	42.46	5	1.75	159	55.79	285	100
	Total	236	39.40	6	1.00	357	59.60	599	100
70-79	**Women**	47	39.83	0	0.00	71	60.17	118	100
	Men	47	37.30	0	0.00	79	62.70	126	100
	Total	94	38.52	0	0.00	150	61.48	244	100
80 and above	**Women**	7	20.59	1	2.94	26	76.47	34	100
	Men	13	37.14	2	5.71	20	57.14	35	100
	Total	20	28.99	3	4.35	46	66.67	69	100
All ages	**Women**	5878	78.15	23	0.31	1620	21.54	7521	100
	Men	1886	68.11	39	1.41	844	30.48	2769	100
	Total	7764	75.45	62	0.6	2464	23.95	10290	100

(version 23.0; Armonk, NY: IBM Corp., USA). The chi-square test and Fisher's exact test were used to compare qualitative variables. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 10,290 patient samples were examined for anti-*Toxoplasma* IgG and 10,685 samples for anti-*Toxoplasma* IgM between January 1, 2021, and September 1, 2024. Anti-*Toxoplasma* IgG and IgM

positivity rates were detected as 23.95% and 1.30%, respectively. There were 2769 men and 7521 women whose anti-*Toxoplasma* IgG was tested. Among these patients, 30.48% of men had IgG-positive results and 1.41% had intermediate values, whereas 21.54% of women were IgG positive and 0.31% were within the intermediate values. There was a statistically significant difference between anti-*Toxoplasma* IgG seropositivity of men and women (p<0.001). Anti-*Toxoplasma* IgM was investigated in 2952 men and 7733 women. Anti-*Toxoplasma* IgM was found to be positive in 1.34% of women and borderline in 0.65% of them, while 1.19% of men tested positive and 0.54% of them were borderline. There was no statistically significant difference between the anti-*Toxoplasma* IgM positivity rate of men and women (p=0.667).

When the anti-*Toxoplasma* IgG positivity rates were evaluated according to the age groups of the patients, it was seen that the positivity rate, which was 17.79% in the 0-9 age group, decreased until the 20-29 age group (11.7%) and increased from the 30-39 age group onwards, reaching its highest point in patients aged 80 and above (66.67%) (Table 1). The differences between the anti-*Toxoplasma* IgG positivity rates of the age groups were found to be statistically significant (p<0.001). Analysis of the anti-*Toxoplasma* IgM positivity rates by age groups revealed that, resembling the IgG test results, the highest positivity rate was 5.33% in patients 80 years of age and above, and followed by the 40–49 (2.14%), 50–59 (1.73%), and 10–19 (1.54%) age groups (Table 2). The differences were also found to be statistically significant between the anti-*Toxoplasma* IgM seropositivity rates of age groups (p<0.001).

Immunocompromised adult and pediatric clinics showed the greatest percentages of anti-*Toxoplasma* IgG positivity (44.06% and 42.21%, respectively), while pediatric infection clinics had the lowest rate (7.55%). For ophthalmology services, the seropositivity rate was 28.02%, and for newborn units, it was 26.25%; however, for obstetrics and gynecology clinics, it was 14.83%. The differences between the positivity rates of departments were found to be statistically significant (p<0.001). For anti-*Toxoplasma* IgM positivity, adult infection clinics led with 1.99%, followed by adult immunocompromised with 1.69% and ophthalmology clinics with 1.59%; however, the differences between clinics were not found to be statistically significant (p=0.158). The distribution of anti-*Toxoplasma* IgG and IgM positivity rates according to clinics was given in Table 3.

Sixty-five patients, 18 of whom were men and 47 of whom were women, had anti-*Toxoplasma* IgG avidity test results during the study period. Six men had low avidity, three had grey zone avidity, and nine had high avidity, while 15 women had low avidity, 10 had grey zone avidity, and 22 had high avidity (Table 4). There was no statistically significant difference between the avidity indexes of men and women (p=0.917). Of the 23 patients who visited the obstetrics and gynecology services, six had low avidity, five had grey zone, and 12 had high avidity. Of the patients in whom avidity testing was performed, 64 were anti-*Toxoplasma* IgM positive, while one was negative. The sample with negative anti-*Toxoplasma* IgM came from the neonatal intensive care unit, and the avidity testing was reported as a grey zone.

Discussion

Toxoplasmosis is a neglected parasitic disease caused by the obligate intracellular protozoan *Toxoplasma gondii*. The disease is common all over the world and significant, especially in pregnant women and immunocompromised patients. The average of various studies undertaken globally indicates that approximately one-third of the population has had toxoplasmosis at a time in their lives [9]. The studies conducted in various regions of Türkiye reveal a prevalence ranging from 20.3% to 69.6%, as shown in Table 5 [10-32]. Anti-*Toxoplasma* IgG positivity in our study was found to be 23.95%, which is around the lower limit of the range of the studies conducted in our country. In Ankara, three studies investigating *Toxoplasma* seroprevalence in pregnant women reported similar ratios of 21.85%, 22.3%, and 25.5% [19, 20, 23]. Contrary to the studies conducted in Ankara, the ratios reported from Şanlıurfa city and the southeastern region were found to be the highest among the local studies in Türkiye. The high positivity ratio in this region was asso-

Table 2. Distribution of anti-*Toxoplasma* IgM positivity rate according to age groups and gender

Age groups	Gender	Anti-*Toxoplasma* IgM							
		Negative		Borderline		Positive		Total	
		n	%	n	%	n	%	n	%
0-9	**Women**	595	99.50	3	0.50	0	0.00	598	100
	Men	719	99.58	2	0.28	1	0.14	722	100
	Total	1314	99.55	5	0.38	1	0.08	1320	100
10-19	**Women**	354	97.52	2	0.55	7	1.93	363	100
	Men	343	97.72	4	1.14	4	1.14	351	100
	Total	697	97.62	6	0.84	11	1.54	714	100
20-29	**Women**	2606	98.27	12	0.45	34	1.28	2652	100
	Men	325	98.48	1	0.30	4	1.21	330	100
	Total	2931	98.29	13	0.44	38	1.27	2982	100
30-39	**Women**	2418	97.66	24	0.97	34	1.37	2476	100
	Men	349	97.21	5	1.39	5	1.39	359	100
	Total	2767	97.60	29	1.02	39	1.38	2835	100
40-49	**Women**	640	96.82	5	0.76	16	2.42	661	100
	Men	359	98.09	1	0.27	6	1.64	366	100
	Total	999	97.27	6	0.58	22	2.14	1027	100
50-59	**Women**	457	98.07	1	0.21	8	1.72	466	100
	Men	338	97.97	1	0.29	6	1.74	345	100
	Total	795	98.03	2	0.25	14	1.73	811	100
60-69	**Women**	345	98.57	2	0.57	3	0.86	350	100
	Men	294	98.33	2	0.67	3	1.00	299	100
	Total	639	98.46	4	0.62	6	0.92	649	100
70-79	**Women**	129	99.23	0	0.00	1	0.77	130	100
	Men	139	97.89	0	0.00	3	2.11	142	100
	Total	268	98.53	0	0.00	4	1.47	272	100
80 and above	**Women**	35	94.59	1	2.70	1	2.70	37	100
	Men	35	92.11	0	0.00	3	7.89	38	100
	Total	70	93.33	1	1.33	4	5.33	75	100
All ages	**Women**	7579	98.01	50	0.65	104	1.34	7733	100
	Men	2901	98.27	16	0.54	35	1.19	2952	100
	Total	10480	98.08	66	0.62	139	1.30	10685	100

ciated with conditions such as warm and humid climate, unhygienic eating habits, eating raw meatballs, and drinking contaminated water [12, 16, 26].

In the current study, there was a statistically significant difference in the IgG positivity ratio of men and women ($p<0.001$). This may be due to a couple of reasons. Firstly, far more tests were requested from women than men, and especially from gynecology clinics accounted for around half of all women's test requests as preconceptional and pregnancy screening tests. One possible explanation for the low positivity rate among women may be the large quan-

Table 3. Distribution of anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM positivity rates according to departments

Departments		Anti-*Toxoplasma* IgG				Anti-*Toxoplasma* IgM			
		Negative	**Intermediate**	**Positive**	**Total**	**Negative**	**Borderline**	**Positive**	**Total**
Obstetrics and Gynecology	n	3714	3	647	4364	4266	34	56	4356
	%	85.11	0.07	14.83	100	97.93	0.78	1.29	100
Adult immunocompromised*	n	595	17	482	1094	1157	4	20	1181
	%	54.39	1.55	44.06	100	97.97	0.34	1.69	100
Pediatric immunocompromised**	n	133	8	103	244	251	1	2	254
	%	54.51	3.28	42.21	100	98.82	0.39	0.79	100
Adult infection	n	359	0	143	502	488	5	10	503
	%	71.51	0.00	28.49	100	97.02	0.99	1.99	100
Pediatric infection	n	195	1	16	212	215	0	1	216
	%	91.98	0.47	7.55	100	99.54	0.00	0.46	100
Adult other	n	1553	18	817	2388	2433	13	38	2484
	%	65.03	0.75	34.21	100	97.95	0.52	1.53	100
Pediatrics (other)	n	971	15	163	1149	1303	9	8	1320
	%	84.51	1.31	14.19	100	98.71	0.68	0.61	100
Ophtalmology	n	185	0	72	257	247	0	4	251
	%	71.98	0.00	28.02	100	98.41	0.00	1.59	100
Newborn	n	59	0	21	80	120	0	0	120
	%	73.75	0.00	26.25	100	100.00	0.00	0.00	100
Total	n	7764	62	2464	10290	10480	66	139	10685
	%	75.45	0.60	23.95	100	98.08	0.62	1.30	100

*Adult immunocompromised=hematology, medical oncology, transplantation unit

**Pediatric immunocompromised=pediatric hematology, pediatric oncology, pediatric immunology, pediatric bone marrow transplantation

tity of screening tests that were ordered. Secondly, the positivity rate of men may be significantly higher because anti-*Toxoplasma* antibody tests are mostly requested from men suspected to have toxoplasmosis. In most of the studies, the anti-*Toxoplasma* IgG positivity rate was found to be higher in women than in men [10, 17, 21, 25, 27]. However, a single study reported higher seropositivity among men, which was similar to our study [28]. This finding may also change based on patients' demographic properties and test-request patterns of the hospital. Anti-*Toxoplasma* IgM positivity was found to be 1.30% in our study. According to studies conducted in Türkiye, IgM seropositivity ranged from 0% to 9.7% (Table 5). The finding of our investigation falls within this range and is around the lower limit, similar to that of anti-*Toxoplasma* IgG. Anti-*Toxoplasma* IgM positivity was 1.34% among women, and it was 1.19% among men. However, the difference between two genders was not deemed statistically significant (p=0.667).

The majority of patients with anti-*Toxoplasma* IgM and IgG positivity were 80 years of age and above. Similarly, the study by Alver et al. in 2019 indicated that patients aged 60 and above had the greatest prevalence of both anti-*Toxoplasma* IgG and *anti-Toxoplasma* IgM positivity [21]. Anti-*Toxoplasma* IgM and anti-*Toxoplasma* IgG positivity were shown to be highest in the 20–39 age range in two further investigations published in the literature [22, 28]. Comparing our study with these two studies, we found lower positivity rates of anti-*Toxoplasma* IgG and IgM among women in the childbearing age group of 20–39 years.

Table 4. Distribution of the avidity index results of 65 patients according to gender

		Avidity			
Gender		Low	Grey-zone	High	Total
Women	n	15	10	22	47
	%	31.91	21.28	46.81	100
Men	n	6	3	9	18
	%	33.33	16.67	50	100
Total	n	21	13	31	65
	%	32.31	20	47.69	100

Both adult and pediatric immunocompromised individuals had the highest anti-*Toxoplasma* IgG positivity rates (Table 3). In a study by Taşbent et al. that evaluated *Toxoplasma* serology in different patient groups, in contrast to our results, immunocompromised patients exhibited lower anti-*Toxoplasma* IgG and IgM positivity rates by 14.7% and 0.5%, respectively [27]. The IgG positivity rate among patients referred from obstetrics and gynecology services was found to be 14.83% in our study. This rate seems to be lower than the positivity rates reported in other published studies in the literature [11-15, 18-20, 23, 24, 23, 24, 23, 24, 23,24,28-32]. In our study, the adult infection service led in IgM positivity rate with 1.99%. The patients whose samples were detected positive for anti-*Toxoplasma* IgM in the adult infection units may be the patients who were tested positive in other healthcare facilities and were referred to our hospital for further investigation. When *Toxoplasma* seropositivity distribution according to the clinics was examined, 26.25% of the newborns' samples tested positive for anti-*Toxoplasma* IgG, while none tested positive for anti-*Toxoplasma* IgM. This indicates that around one in four newborns was detected as IgG positive. *Toxoplasma* serology is typically requested from newborns who exhibit symptoms suggestive of congenital toxoplasmosis or whose mothers have been diagnosed or suspected of having acute toxoplasmosis, which may be the cause of the high seropositivity rate. During pregnancy, IgG can passively diffuse from mother to fetus. In this case, the diagnostic exclusion of congenital toxoplasmosis could be made by either using an immunoblot test to compare the IgG patterns of the mother's and the newborn's sera or by monitoring the decline in IgG levels at specific intervals [33].

Low avidity was detected in six of the samples sent from the obstetrics and gynecology service. These results may indicate acute toxoplasmosis in these pregnant women, but not enough for a definite diagnosis. Due to the retrospective nature of the study and the restriction in patient data within the hospital information management system, information about the subsequent processes of the pregnant women could not be obtained.

Study limitations

The study's limitations include its retrospective design, the fact that it only included data from one center, and the fact that patient data was restricted to information found in the hospital information management system. Prospectively planned studies are required in which the results of the avidity, anti-*Toxoplasma* IgM, and anti-*Toxoplasma* IgG tests are assessed together within clinical and serological follow-up to find out how the avidity test contributes to the diagnosis of acute toxoplasmosis, which is not within the scope of our study.

Conclusion

Our study is expected to contribute to the literature by highlighting the results of patients whose *Toxoplasma* serology was requested according to age groups, gender, and the clinics they applied to during the 44-month period. The anti-*Toxoplasma* IgG positivity rate was determined to be 23.95%, and the results of the study are similar to the results of other studies conducted in our province.

Table 5. Summary of studies conducted in Türkiye investigating *Toxoplasma* serology

Study population	The number of serum samples (n)	The positivity rate of anti-*Toxoplasma* IgG (%)	The positivity rate of anti-*Toxoplasma* IgM (%)	The test method	City	Time Interval	References
All patients	4908	31.01	0.77	ELISA	Elazığ	1999-2003	10
Pregnant women	4651	39.9	2.5	ELISA	İzmir	2003-2008	11
Pregnant women	1652	52.1	0.54	ELISA	Hatay	2004-2006	12
Women (childbearing age)	17751	24.61	1.34	ELFA	İstanbul, Bursa, Adana, Kayseri, Kocaeli	2004-2010	13
Pregnant women	389	30.1	0	ELISA, IFA, DA	Aydın	-	14
Pregnant women	1972	48.3	0.4	ELISA	Kocaeli	2005-2007	15
Women	2586	69.6	3	CLIA	Şanlıurfa	2006	16
All patients	8095	31.5	1.6	ELISA, IFA	Aydın	2007-2017	17
Pregnant women	1102	37	1.4	-	Denizli	2008-2009	18
Pregnant women	30863	25.5	0.3	CLIA	Ankara	2008-2010	19
Pregnant women	84587	22.3	0.64	CLIA	Ankara	2008-2017	20
All patients	25251	30.7	9.7	ELFA	Bursa	2009-2016	21
All patients	2778	47.1	1.19	ELISA	Reference laboratory	2009-2019	22
Syrian pregnant women Turkish pregnant women	752 76835	47 21.85	0.4 0.56	CLIA	Ankara	2010-2018	23
Pregnant women	9809	37.6	1.1	ELISA	Van	2012-2013	24
All patients	3899	32.4	2.7	ELISA	İzmir	2012-2013	25
Farmworker women	684	58.3	1	EIA	Southeastern region	2013	26
All patients	20875	24.1	2.4	ELFA	Konya	2015-2019	27
All patients Turkish pregnant women Syrian pregnant women	12694 3798 264	29.58 25.88 47.10	0.94 0.49 1.83	CMIA	İzmir	2017-2021	28
Pregnant women	3141	23	0.6	CLIA	İstanbul	2018-2019	29
Pregnant women	1294	20.3	1.1	CLIA	Kastamonu	2018-2022	30
Pregnant women	1844	28.7	0.7	ELFA	Mersin	2019	31
Pregnant women	256612	36.76	2.91	-	Türkiye	Last 30 years	32

ELISA: Enzyme-Linked Immunosorbent Assay
ELFA: Enzyme-Linked Fluorescent Assay
IFA: Immunofluorescent Antibody DA: Direct Agglutination
CLIA: Chemiluminescent Immunoassay
EIA: Enzyme Immonoassay
CMIA: Chemiluminescent Microparticle Immunoassay
DA: Direct Agglutination

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Research Article / Araştırma Makalesi

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Impact of Obesity and Prognostic Nutritional Index on Outcomes of Ventricular Tachycardia Ablation in Patients with Structural Heart Disease

Yapısal Kalp Hastalığı Olan Hastalarda Ventriküler Taşikardi Ablasyonunun Sonuçları Üzerinde Obezite ve Prognostik Beslenme İndeksinin Etkisi

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ABSTRACT

Objectives: Previous studies have demonstrated that nutritional disorders such as obesity and malnutrition are associated with adverse events in patients with a structural heart disease. The impact of these conditions in patients undergoing ventricular tachycardia (VT) ablation is not known. In this study we sought to investigate the effect of obesity and malnutrition on procedural and post-procedural midterm outcomes in patients undergoing VT ablation.

Materials and methods: This is a single center, retrospective study which enrolled patients undergoing structural VT ablation. Patients were classified as normal weight, overweight and obese by body mass index (BMI). Malnutrition was defined as prognostic nutritional score (PNI) < 38. Outcomes between groups were compared.

Results: A total of 203 patients were enrolled to this study. Among these patients, 54 (25.6%) were normal weight, 102 patients (50.2%) were overweight, and 47 patients (23.1%) were obese. 18 patients (8.8%) were classified as malnourished. No significant difference in VT recurrence was observed across BMI (p=0.335) or nutritional status (p=0.556). However, mortality during follow-up was significantly higher in the malnourished group (50% vs. 7%, p<0.001), while BMI showed no significant association with mortality (p=0.202). On multivariate analysis, only PNI <38 (HR 6.659, 95% CI 2.553–17.369, p<0.001) and electrical storm (HR 2.848, 95% CI 1.133–7.160, p=0.026) remained independent predictors of mortality.

Conclusion: This study demonstrated that malnourished patients, as classified by PNI, had significantly worse acute success and higher long-term mortality with similar VT recurrence rate, compared to non-malnourished patients. Obesity status, as defined by BMI, did not lead to different outcomes.

Keywords: catheter ablation; heart failure; malnutrition; obesity; ventricular tachycardia

ÖZET

Amaç: Önceki çalışmalar, obezite ve malnütrisyon gibi beslenme bozukluklarının yapısal kalp hastalığı olan hastalarda olumsuz sonuçlarla ilişkili olduğunu göstermiştir. Bu durumların ventriküler taşikardi (VT) ablasyonu geçiren hastalardaki etkisi ise bilinmemektedir. Bu çalışmada, VT ablasyonu geçiren hastalarda obezite ve malnütrisyonun işlem sırasındaki ve işlem sonrası orta vadeli sonuçlar üzerindeki etkisini araştırmayı amaçladık.

Gereç ve yöntem: Bu tek merkezli, retrospektif çalışmaya yapısal VT ablasyonu uygulanan hastalar dahil edilmiştir. Hastalar beden kitle indeksi (BKİ) kullanılarak normal kilolu, fazla kilolu ve obez olarak sınıflandırılmıştır. Malnütrisyon, prognostik beslenme indeksi (PNI) < 38 olarak tanımlanmıştır. Gruplar arasındaki sonuçlar karşılaştırılmıştır.

Bulgular: Çalışmaya toplam 203 hasta dahil edilmiştir. Bu hastaların 54'ü (%25,6) normal kilolu, 102'si (%50,2) fazla kilolu ve 47'si (%23,1) obez olarak sınıflandırılmıştır. Öte yandan, 18 hasta (%8,8) malnütrisyonlu olarak tanımlanmıştır. BKİ (p=0,335) veya beslenme durumu (p=0,556) ile VT nüksü arasında anlamlı bir ilişki gözlemlenmemiştir. Ancak takip sürecinde mortalite, malnütrisyonlu grupta anlamlı şekilde daha yüksek bulunmuştur (%50 ve %7, p<0,001); buna karşın BKİ ile mortalite arasında anlamlı bir ilişki saptanmamıştır (p=0,202). Çok değişkenli analizde, yalnızca PNI <38 (HR 6,659, %95 GA 2,553–17,369, p<0,001) ve elektriksel fırtına (HR 2,848, %95 GA 1,133–7,160, p=0,026) mortalitenin bağımsız belirleyicileri olduğu saptanmıştır.

Sonuç: Bu çalışma, PNI ile malnütrisyonlu olarak sınıflandırılan hastaların, malnütrisyonlu olmayan hastalara kıyasla benzer VT nüks oranına rağmen anlamlı şekilde daha düşük akut başarı ve daha yüksek uzun vadeli mortaliteye sahip olduğunu göstermiştir. BKİ ile tanımlanan obezite durumu ise sonlanım noktalarında farklılığa yol açmamıştır.

Anahtar kelimeler: kateter ablasyon; kalp yetersizliği; malnutrisyon; obezite; ventriküler taşikardi

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Introduction

Ventricular tachycardias (VTs) impose a significant burden on patients with reduced ejection fraction (EF) in terms of increased mortality rates and worsening heart failure (HF).(1) Although recent studies have reported improvement in outcomes in patients undergoing VT catheter ablation(2,3), studies identifying factors that affect clinical outcomes remain scarce. Identification of optimal clinical settings as well as modifiable factors associated with adverse outcomes is crucial for patient selection and preprocedural optimization of the clinical condition.

Previous studies have demonstrated that nutritional disorders such as obesity and malnutrition are associated with adverse events in HF patients.(4,5) Furthermore, there is an established relation between obesity and ventricular arrhythmias (including sudden cardiac death).(6,7) Limited data suggest that obesity is associated with prolonged hospitalization and higher rate of pericardial effusion/hemopericardium following VT ablation, however, long-term outcomes are not known.(8) Currently, no data are present assessing outcomes of VT ablation in malnourished patients. Comparably, malnourished patients have higher recurrence risk after atrial fibrillation (AF) ablation compared to non-malnourished patients.(9)

The question of whether aforementioned nutritional disorders affect outcomes of patients undergoing VT ablation is of great clinical importance. Accordingly, in this study we sought to investigate the effect of obesity and malnutrition on procedural and post-procedural midterm outcomes in patients undergoing VT ablation.

Methods

This is a single center retrospective observational study. Ethical approval was obtained from the Ankara University Clinical Research Ethical Committee. The study was conducted in accordance with the Declaration of Helsinki. All participants or their legal guardians (when consent could not be obtained from the patient) provided written informed consent for participation.

Patient selection and subgroups

The study was conducted between 1st February 2022 and 1st April 2025. All patients undergoing VT or ventricular fibrillation (VF) ablation in the setting of structural heart disease by one of the first three authors (EB, TA, OA) were screened for inclusion to this study. Usual indications for ablation were in accordance with the current clinical practice guidelines (including electrical storm, an ICD shock, three or more VT episodes treated by anti-tachycardia pacing).(10) A minimum follow-up of two months (except for the patients who died prior to this period) was required for the enrollment to the study. Patients with known liver disease, severe chronic kidney disease (estimated glomerular filtration rate < 30 mL/min/1.73 m^2), known hematologic disease, known malignancy and those with chronical inflammatory/infections disease were excluded from the study.

Patients were classified as normal weight, overweight and obese by body mass index (BMI) as previously defined (BMI<25 kg/m^2, BMI ≥25-<30 kg/m^2, and BMI ≥30 kg/m^2, respectively).(5) The malnutrition status was assessed by prognostic nutritional index (PNI), a well-validated index calculated by 10 × serum albumin (g/dl) + 0.005 × total lymphocyte count (mm^3).(11) Patients with PNI < 38 were assigned to the malnourished group, with all other patients being assigned to the control group.(11)

The procedure

Multiple techniques and protocols exist for VT/VF catheter ablation and the procedure may vary among centers. Detailed presentation of our ablation approach has been previously published.(12,13) Briefly, we perform a mixture of substrate and activation mapping (when patient has a hemodynamically and clinically tolerable VT) under conscious sedation or general anesthesia using 3D mapping systems and multipolar mapping catheters. All targeted areas (based on the substrate and VT isthmi) are then ablated with radiofrequency ablation catheters. Acute success was defined as non-inducibility by multisite, multiple programmed extra-stimulus deliveries depending on the initial VT induction protocol and/or patients' hemodynamic status.

Table 1. Baseline characteristics

	Classification by body mass index				Classification by presence of malnutrition		
	Normal weight (n=54)	Overweight (n=102)	Obese (n=47)	Statistics	Malnutriotion(-) n=185	Malnutrition (+) n=18	Statistics
Age, years median (IQR)	67.5(74.2-58)	66(72-58)	61.5(68-44.3)	0.038	65(71-57)	72(76-68)	0.007
Sex Male, -n(%)	44(18.5)	89(87.3)	39(83)	0.59	157(84.9)	15(83.3)	0.863
EF, % median (IQR)	25(40-20)	25(35-20)	30(40-20)	0.568	25(40-20)	20(28.5-15)	0.214
EF < 25% -n(%)		47(46.1)	22(46.8)	0.944	87(47.0)	6(33.3)	0.257
Diabetes Mellitus, -n(%)	15(27.8)	32(31.4)	17(36.2)	0.614	60(32.4)	4(22.2)	0.366
Hypertension, -n(%)	36(66.7)	74(72.5)	36(76.6)	0.624	131(70.8)	15(83.3)	0.272
CABG, -n(%)	16(29.6)	24(23.5)	10(21.3)	0.583	40(21.6)	10(55.6)	0.001
PCI, -n(%)	25(46.3)	60(58.8)	23(48.9)	0.263	96(51.9)	12(66.7)	0.230
Atrial fibrillation,-n(%)	17(31.5)	44(43.1)	17(36.2)	0.34	70(37.8)	8(44.4)	0.582
Prosthetic valve, -n(%) -MVR -AVR	4(7.4)	5(4.9)	3(6.4) 1(2.1)	0.438	11(5.9) 1(0.5)	1(5.6)	0.950
Previous history of VT ablation, -n(%)	2(3.7)	10(9.8)	5(10.6)	0.346	15(8.1)	2(11.1)	0.661
Beta blocker usage, -n(%)	47(87)	99(97.1)	45(95.7)	0.035	173(93.5)	18(100)	0.265
Ischemic etiology, -n(%)	32(59.3)	72(70.6)	25(53.2)	0.1	115(62.5)	14(77.8)	0.198
Implantable cardioverted defibrillator, -n(%)							
Electrical storm	21(38.9)	43(42.2)	20(42.6)	0.909	75(40.5)	9(50)	0.437

AVR: aortic valve replacement, CABG: Coronary artery bypass grafting, EF: ejection fraction, IQR: interquartile range, MVR: mitral valve replacement, VT: ventricular tachycardia

Data gathering and study endpoints

Clinical data of participants was collected from electronic patient record system. Patient height and weight was measured and recorded prior to the procedure. Blood panels were measured utilizing Beckman Coulter Analyzer, an automatic blood cell counter. Follow-up data were obtained from patient files.

Ablation success was defined as VT non-inducibility at the end of the procedure. Periprocedural death was defined as death from any cause occurring during the same hospitalization as for ablation. Recurrence of VT was identified by any therapy delivered by an ICD device (either shocks or anti-tachycardia pacing) or if the patient presented with sus-

Figure 1. Kaplan Meier curves displaying death-free (A) and VT-free (B) survival across all body mass index (BMI) categories.

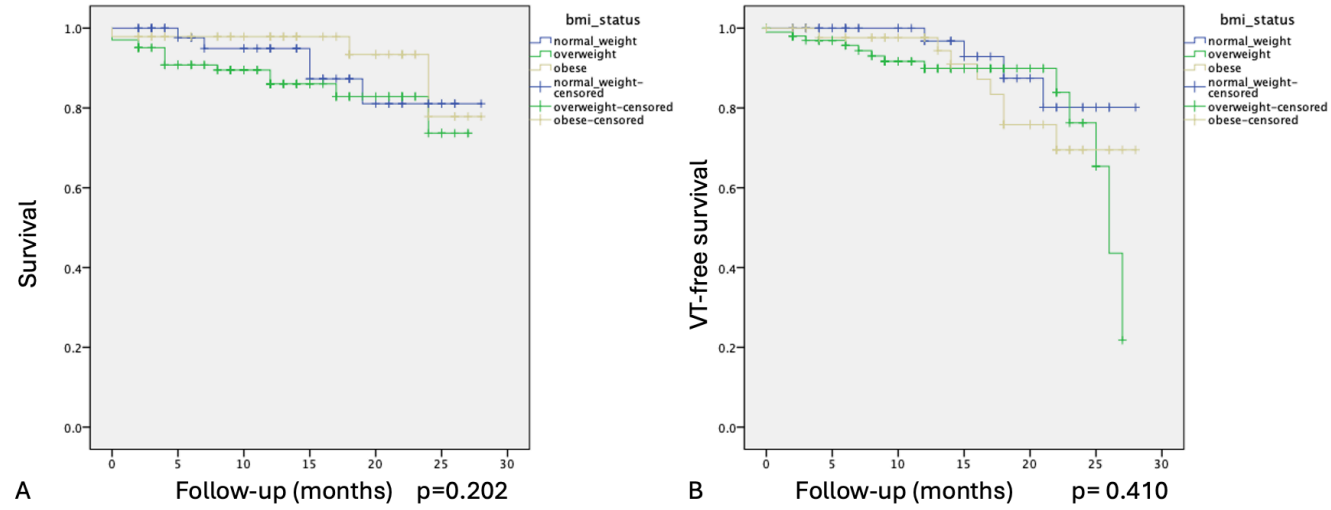
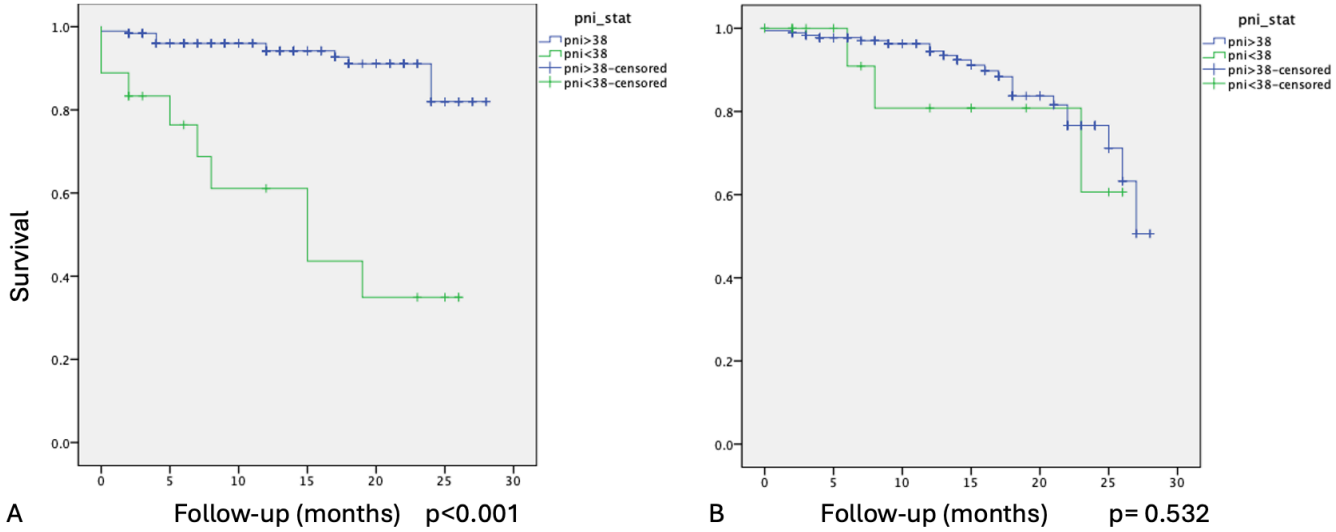


Figure 2. Kaplan Meier curves displaying death-free (A) and VT-free (B) survival in malnourished (prognostic nutritional index (PMI) <38) and non-malnourished (PNI ≥ 38) groups.



tained, hemodynamically stable VT. Mid-term death was defined as death from any cause occurring during the follow-up period. The standard follow-up schedule included an initial visit one month after the procedure, followed by visits every three months. Patients who could not present for a device interrogation were contacted by a telephone call. Patients with missing procedural of follow-up data were excluded from the study.

Statistical analysis

Statistical analysis in this study was conducted using SPSS Statistics version 23 (IBM, New York, NY, USA). Categorical variables are presented as numbers and percentages, while continuous variables are expressed as mean ± standard deviation (SD) for normally distributed data and as median (interquartile range [IQR]) for non-normally distributed data. The Kolmogorov–Smirnov test was used to assess normality Parametric data were analyzed using the unpaired Student's t-test, whereas non-parametric data were compared using the Mann-Whitney U test. Categorical variables were evaluated using the chi-square (χ^2) test. Survival analysis was performed with Kaplan-Meier graphs. Univariate and multivariate Cox regression analyses were performed to explore the relationship between VT ablation outcomes and obesity/malnutrition. All probabilities are 2-tailed and $p<0.05$ was considered statistically significant.

Table 2. Procedural and follow-up data

	Classification by body mass index				Classification by presence of malnutrition		
	Normal weight (n=54)	Overweight (n=102)	Obese (n=47)	Statistics	Malnutrition(-) n=185	Malnutrition (+) n=18	Statistics
Procedure duration, median (IQR)	135(168-90)	132(160-100)	145(184-103.7)	0.391	130(170-100)	157(208-135)	0.029
Rf time, minutes median (IQR)	30(41-20)	31(41.2-20)	36.5(48.5-24.1)	0.203	32(43-20.1)	32.6(53.5-29)	0.284
Fluoro duration, minutes median (IQR)	4,7(16-1.7)	4,4(10-1.3)	5.6(9.6-3)	0.313	4.4(10.5-2.3)	7.3(14.6-3.5)	0.197
ECMO, -n(%)	1(1.9)	0	1(2.2)	0.352	2(1.1)	0(0)	0.656
General anesthesia, -n(%)	32(59.3)	61(59.8)	29(61.7)		107(57.8)	15(83.3)	0.035
Intraaortic balloon counterpulsation, -n(%)	0	4(3.9)	3(6.4)	0.201	7(3.8)	0(0)	0.401
Epicardial access, -n(%)	6(11.1)	18(17.6)	11(23.4)	0.261	30(16.2)	5(27.8)	0.215
Early death, -n(%)	0	3(2.9)	1(2.1)	0.452	2(1.1)	2(11.1)	0.04
Acute Success Non-inducible, -n(%)	51(94.4)	100(98)	44(93.8)	0.338	180(97.8)	15(83.3)	0.035
Acute kidney injury, -n(%) Tamponade Respiratory Failure, -n(%)	2(3.7) 0 0	2(2.0) 0 3(2.9)	4(8.5) 2(4.3) 2(4.3)		6(3.2) 2(1.1) 3(1.6)	2(11.1) 0 2(11.1)	
Follow-up duration, median months (IQR)	13(19.25-5)	12(17-5.5)	15(22-8.7)	0.059	12(19-6)	7.5(20-2)	0.214
Follow-up recurrence, -n(%)	4(7.4)	13(12.7)	8(17)	0.335	22(11.9)	3(16.7)	0.556
Follow-up death, -n(%)	5(9.3)	14(13.7)	3(6.4)	0.371	13(7)	9(50)	<0.001

ECMO: extracorporeal membrane oxygenation, IQR: interquartile range

Results

A total of 203 patients were enrolled to this study. Among these patients, 54 (25.6%) were normal weight, 102 patients (50.2%) were overweight, and 47 patients (23.1%) were obese. One patient had BMI > 40, and one patient had BMI < 18 . Conversely, 18 patients (8.8%) were classified as malnourished by PNI < 38. All baseline characteristics of study groups are present at Table 1. Obese patients were significantly younger compared to normal-weight patients (median age: 61.5 [IQR 68–44.3] vs. 67.5 [74.2–58] years, p=0.038). Malnourished patients

were older than those without malnutrition (median age: 72 [76–68] vs. 65 [71–57] years, p=0.007). No significant differences were observed in terms of sex distribution, EF, diabetes, hypertension, AF, or ischemic etiology between BMI or nutritional subgroups.

Comparison of procedural and follow-up data

Comparison of procedural and follow-up data is presented at Table 2. Procedure durations were similar across all BMI groups. Conversely, malnourished patients had longer median procedure duration compared to non-malnourished patients (157 [208–135] vs. 130 [170–100] minutes, p=0.029). Additionally, although there was no early mortality difference across various BMI groups, early mortality was higher in the malnourished group (11.1% vs. 1.1%, p=0.04).

The median follow-up duration was similar among BMI groups but trended shorter in malnourished patients (7.5 [20–2] months vs. 12 [19–6], p=0.214). No significant difference in VT recurrence was observed across BMI (p=0.335) or nutritional status (p=0.556). However, mortality during follow-up was significantly higher in the malnourished group (50% vs. 7%, p<0.001), while BMI showed no significant association with mortality (p=0.202). Acute kidney injury following the ablation was numerically higher in both obese and malnourished patients (Table 2).

Kaplan-Meier curves depicted similar long-term death and VT-free survival among various BMI groups (p=0.202 and p=0.410, respectively) (Figure 1). Malnourished patients had significantly worse long-term mortality but similar VT-free survival when compared to non-malnourished patients (p<0.001 and p=0.532, respectively) (Figure 2).

Predictors of death during follow-up (Table 3)

In univariate Cox regression, age >70 years (HR 2.688, 95% CI 1.161–6.266, p=0.021), presence of electrical storm (HR 2.566, 95% CI 1.076–6.121, p=0.034), and PNI <38 (HR 7.820, 95% CI 3.327–18.380, p<0.001) were significantly associated with increased mortality. On multivariate analysis, only PNI <38 (HR 6.659, 95% CI 2.553–17.369, p<0.001) and electrical storm (HR 2.848, 95% CI 1.133–7.160, p=0.026) remained independent predictors of mortality. BMI >30 was not significantly associated with long-term mortality.

Discussion

This retrospective study investigated how obesity and malnutrition affect procedural outcomes, long-term mortality, and VT-free survival. Our findings indicate that although being overweight or obese was common among participants, the acute procedural and long-term follow-up outcomes were similar regardless of the BMI group. In contrast, we observed a significantly higher rate of early death, lower procedure success and importantly, substantially greater long-term mortality in malnourished patients compared to their non-malnourished counterparts.

Obesity and ventricular arrhythmias in structural heart disease

Similar to the acute outcomes after VT catheter ablation reported by Tan et al.(8), as well as similar acute outcomes in AF ablation patients(14), we have confirmed that obesity has no impact on early mortality implying that overall safety of VT ablation procedure is similar to non-obese patients. Previous data have shown that vascular complications are most common in extreme BMI (underweight or extreme obesity) patients(15,16). We did not observe this trend, likely due to a low number of patients with extreme obesity (i.e. BMI>40) and no routine post-procedural imaging which may have led to underdiagnosis of femoral pseudoaneurysms.

The similar long-term mortality in obese vs non-obese patients observed in this study can be partly explained by the 'obesity paradox' that has been previously observed in both heart failure and cardiovascular diseases(4). Secondly, the prognostic weight of advanced heart failure may dominate in this clinical setting, rendering obesity non-influential. Thirdly, although BMI is a well validated tool for classification, it may be imperfect due to failure of identification of increased body fat. Finally, similar VT-free survival among groups may be an additional factor contributing to long-term mortality. Obesity has been shown to negatively impact arrhythmia free survival in AF ablation patients, which contrasts with our findings in VT ablation(14,16). This discrepancy may be attributed to the higher prevalence of advanced atrial fibrillation (AF) substrate in obese individuals, while

Table 3. Univariate and multivariate analyses for death prediction

	Univariate			Multivariate		
	HR	Confidence intervals	Statistics	HR	Confidence intervals	Statistics
Age > 70 years	**2.688**	**1.161-6.266**	**0.021**	1.229	0.478-3.158	0.668
Male sex	0.984	0.290-3.332	0.979			
Electrical storm	**2.566**	**1.076-6.121**	**0.034**	**2.848**	**1.133-7.160**	**0.026**
Ischemic etiology	0.653	0.255-1.668	0.373			
Coronary artery bypass grafting	2.007	0.885-4.875	0.093			
Atrial fibrillation	1.455	0.630-3.359	0.380			
BMI > 25	1.292	0.476-3.508	0.615			
BMI >30	0.335	0.099-1.134	0.079	0.586	0.156-2.065	0.390
PNI < 38	**7.820**	**3.327-18.380**	**<0.001**	**6.659**	**2.553-17.369**	**<0.001**
EF < 25%	0.569	0.232-1.397	0.219			
Epicardial access	1.363	0.502-3.700	3.700			
General anesthesia	0.841	0.359-1.971	0.690			

obesity appears to have a limited effect on the ventricular arrhythmogenic substrate.

Malnutrition, prognostic nutritional index and VT ablation

Contrary to our observation that obesity has no prognostic impact in patient undergoing VT ablation, malnutrition has been identified as a strong predictor of both acute and long-term mortality. Previous studies have demonstrated that acute complications are more common in malnourished AF patients, and they may have worse long-term arrhythmia-free survival(9,17). Therefore, worse acute outcomes in terms of success and complications (including death) in VT population is not surprising. Interestingly, although there is a hypothetical association between arrhythmogenesis and malnutrition, long-term recurrence was similar in malnourished patients most probably due to competing outcomes (i.e. death). It is well known that malnutrition is a predictor of death in advanced heart failure patients(18), with our study now extending the prognostic impact to those with ventricular arrhythmias. Overall, these findings suggest that non-arrhythmic death is predominant mechanism of mortality in malnourished population.

Recent studies have tried to identify predictors of mortality in patients undergoing VT ablation. PAINESD score is probably the best studied score with aim of predicting outcomes(19,20). In contrast to the PAINESD score, PNI incorporates serum albumin which may be potential therapeutic target. Whether a therapeutic intervention targeting serum albumin and improving nutritional status may improve outcomes should be studied. This hypothesis is particularly important in patients presenting for ablation in non-emergent settings, leaving sufficient window for therapeutic intervention.

In this study we observed that obese patients were found to be younger compared to normal-weight patients and malnourished patients were older than those without malnutrition. It is well known that sarcopenia is common in both advanced age and heart failure patients, therefore it is likely that this condition may have contributed to our observations.(21) Nevertheless, we could not diagnose sarcopenia due to lack of muscle strength, muscle quantity or quality and physical performance measurements, as per European Working Group on Sarcopenia in Older People, 2019 update.(22) This association and

prognostic impact of sarcopenia should be investigated in separate studies.

Limitations of our study

This study must be interpreted cautiously due to the single-center, retrospective nature. The sample size, particularly for the malnourished subgroup, was relatively small, which may limit the statistical power to detect smaller differences in outcomes. Additionally, given the modest sample size, the possibility that the mortality results are attributable to chance cannot be excluded. Furthermore, although PNI is a well validated score, it is imperfect particularly due to potential influence of systemic inflammation and hepatic dysfunction. Waist circumference measurements were not available which is an additional limitation. Finally, despite multivariate analysis, we cannot exclude the possibility of residual unmeasured confounders, such as frailty, sarcopenia, or the severity of non-cardiac comorbidities, which may be correlated with both PNI and mortality.

Conclusion

This study demonstrated that malnourished patients, as classified by PNI, had significantly worse acute success and higher long-term mortality with similar VT recurrence rate, compared to non-malnourished patients. These findings suggest that PNI may be used as a pre-procedural screening tool, pending approval from larger studies. Additionally, studies are needed to show whether preprocedural intervention targeting PNI>38 can improve acute and long-term outcomes. Finally, obesity status as defined by BMI did not lead to different outcomes.

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Clinical Trial Registration: N/A No materials from other sources have been reproduced.

Data Availability Statement: Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Patterns of Organ Involvement and Mortality in Extrapulmonary Tuberculosis: A Twelve-Year Retrospective Study

Tüberkülozda Organ Tutulum ve Mortalite Modelleri: On İki Yıllık Retrospektif Çalışma

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ABSTRACT

Background: Extrapulmonary tuberculosis (EPTB) constitutes a significant proportion of the tuberculosis (TB) burden and has diagnostically challenging manifestations. This study aimed to evaluate the demographic, clinical, diagnostic, and treatment characteristics of adult patients with EPTB in a tertiary care setting in Türkiye.

Methods: This retrospective cohort study included adult patients (≥18 years) diagnosed with EPTB between June 2010 and March 2022 at two tertiary hospitals in Ankara, Türkiye.Demographic, clinical, diagnostic, treatment, and outcome data were also collected.Comparative analyses between survivors and non-survivors were performed using the chi-square test, Fisher's exact test, and Mann–Whitney U test, with significance set at $p < 0.05$.

Results: Ninety-three patients were included (51.6% men; median age, 55 y).The most frequent sites of involvement were the vertebral bones and joints (32.3%), lymph nodes (26.9%), and central nervous system (16.1%).Histopathological confirmation was obtained in 60.2% of patients, culture positivity in 30.1% of patients, and PCR positivity in 31.2% of patients.Standard quadruple anti-TB therapy was initiated in 95.7% of the cases, with a median total treatment duration of 6 months (range, 1–13 months).Surgical intervention was required in 22.6% of the patients.During the follow-up period, 9 patients (9.7%) died.Immunosuppressive conditions were significantly more frequent among non-survivors than among survivors (44.4% vs. 11.9%, $p = 0.027$).The median duration of dual therapy (1 vs. 4 months, $p < 0.001$) and total therapy (3 vs. 6 months, $p < 0.001$) were significantly shorter in non-survivors.

Conclusions: Adult EPTB presents with diverse and often severe manifestations.Vertebral and central nervous system involvement predominated in this tertiary-care cohort.Mortality was associated with immunosuppression and shorter treatment duration.Comprehensive diagnostic strategies and tailored management of high-risk patients are essential to improve outcomes in EPTB.

Keywords: Extrapulmonary tuberculosis; spinal TB; TB meningitis; histopathology; mortality

ÖZET

Giriş: Ekstrapulmoner tüberküloz (EPTB), küresel tüberküloz yükünün artan bir kısmını oluşturmaktadır ve Türkiye dâhil birçok ülkede insidansı yükselmektedir. Bu çalışmada, üçüncü basamak bir sağlık kuruluşunda EPTB tanısı alan erişkin hastaların demografik, klinik, tanısal ve tedaviye ilişkin özelliklerinin değerlendirilmesi amaçlanmıştır.

Yöntem: Bu retrospektif kohort çalışmaya, Haziran 2010 – Mart 2022 tarihleri arasında Ankara'daki iki üçüncü basamak hastanede EPTB tanısı alan 18 yaş ve üzeri erişkin hastalar dâhil edilmiştir. Tanımlayıcı ve karşılaştırmalı istatistiksel analizler yapılmıştır.

Bulgular: Toplam 93 hasta dâhil edilmiştir (%51,6 erkek; medyan yaş: 55 yıl). En sık tutulan bölgeler omurga/eklemler (%32,3), lenf nodları (%26,9) ve merkezi sinir sistemi (%16,1) idi. Histopatolojik doğrulama %60,2, kültür pozitifliği %30,1, PCR pozitifliği %31,2 oranında sağlandı. AFB boyası %25,8 pozitifti. İlaç duyarlılık testi %27,9 hastada yapılmış ve iki olguda direnç saptanmıştır. Hastaların %95,7'sine standart dörtlü anti-TB tedavisi uygulanmış, medyan tedavi süresi 6 ay (1–13 ay) idi. %22,6 hastada cerrahi müdahale gerekmiştir. Takipte 9 hasta (%9,7) hayatını kaybetti. Bağışıklık baskılanması ölenlerde anlamlı düzeyde daha fazlaydı (%44,4 vs. %11,9, $p = 0,027$) ve bu hastalarda tedavi süresi daha kısaydı ($p < 0,001$).

Sonuç: EPTB erişkinlerde ciddi tablolarla seyredebilir. Vertebra ve merkezi sinir sistemi tutulumu sık olup, tanıda histopatoloji belirleyicidir. Bağışıklık baskılanması ve yetersiz tedavi süresi mortaliteyle ilişkilidir. Erken tanı ve bireyselleştirilmiş tedavi yaklaşımları önem taşımaktadır.

Anahtar kelimeler: Ekstrapulmoner tüberküloz, spinal TB, TB menenjiti, mortalite, histopatoloji

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Introduction

Despite being preventable and curable, tuberculosis (TB) remains a significant global health challenge and ranks among the top causes of death worldwide. According to World Health Organization (WHO), approximately 10.6 million individuals fell ill with TB in 2021, resulting in 1.6 million deaths, including 187,000 among people with Human Immunodeficiency Virus (HIV) (1, 2). According to the 2024 WHO Global Tuberculosis Report, TB remains the second leading infectious killer worldwide, with approximately 10.8 million new cases and 1.25 million deaths in 2023(3).Although pulmonary tuberculosis (PTB) is the most common form, extrapulmonary tuberculosis (EPTB), characterized by Mycobacterium tuberculosis infection outside the lung parenchyma, accounts for a substantial disease burden and presents unique clinical, diagnostic, and therapeutic challenges (4, 5).EPTB may involve any organ system, with the lymph nodes, pleura, bones, meninges, genitourinary tract, and gastrointestinal system being the commonly affected sites (6).The disease is insidious in onset and heterogeneous in presentation, often mimicking other conditions, leading to diagnostic delays and deferred treatment (7).Unlike PTB, which relies on sputum-based diagnostics, EPTB requires invasive procedures, histopathological confirmation, and advanced imaging, particularly when microbiological evidence is limited (8).The relative proportion of EPTB among patients with TB has increased in multiple settings, including high-burden countries and industrialized regions.This shift has been attributed to improved pulmonary TB control, better access to imaging, increased awareness of EPTB manifestations, and the rising prevalence of immunosuppressive conditions such as HIV infection, malignancies, and immunomodulatory therapies (9, 10).Data from the Turkish Ministry of Health show that extrapulmonary involvement accounts for over one-third of new TB cases, with lymphadenitis and pleural and skeletal system involvement being the most common (11-13).

Given the heterogeneity of EPTB and the paucity of long-term institutional data in Türkiye, we conducted a 12-year retrospective cohort study of adult patients with extrapulmonary tuberculosis across two tertiary hospitals. The primary objective was to characterize the demographic and clinical features, diagnostic modalities, and treatment strategies, with a particular focus on organ-specific patterns and factors associated with mortality.This study is expected to contribute to the evidence base needed for improved diagnostic and therapeutic strategies in similar, high-burden healthcare settings.

Materials and Methods

Study Design and Setting

This retrospective observational study was conducted at two tertiary care centers in Turkey. Adult patients (aged ≥18 years) diagnosed with EPTB between June 2010 and March 2022 were included. The study population consisted of patients evaluated at the Infectious Diseases and Clinical Microbiology outpatient clinics and inpatient services of Ankara Numune Training and Research Hospital (June 2014 – May 2019) and Ankara Bilkent City Hospital (June 2019 – March 2022).

Inclusion and Exclusion Criteria

Patients were eligible for inclusion if they had a clinical, histopathological, or microbiological diagnosis of EPTB, defined as a TB infection involving anatomical sites other than the lung parenchyma.Patients with isolated pulmonary TB or concomitant pulmonary and extrapulmonary involvement were excluded unless the extrapulmonary manifestations were the primary diagnostic component.Only cases with complete clinical and diagnostic records were included in the study.

Data Collection

Patient data were obtained from the institutional electronic medical record systems (FONET® and HICAMP®) and the national MEDULLA physician platform. The following parameters were collected: demographics (age, sex, nationality, and province of residence).Medical history: presence of comorbidities (diabetes mellitus, malignancy, cardiovascular disease, chronic kidney disease, and HIV), immunosuppressive conditions, and family history of TB.Diagnostic methods: Purified Protein Derivative (PPD), interferon-gamma release assay (IGRA),

acid-fast bacilli (AFB) smear, TB polymerase chain reaction (PCR), culture, and histopathology results. Site of involvement: classified by organ system involvement (lymph nodes, central nervous system, peritoneum, bone and joints, skin, and genitourinary tract).Treatment data included standard first- or second-line anti-TB regimens, treatment duration, surgical intervention, and resistance testing. All included patients were newly diagnosed with extrapulmonary tuberculosis and had no prior history of TB.Immunosuppression was defined as the presence of HIV infection, ongoing chemotherapy, long-term corticosteroid use (>20 mg/day prednisone equivalent for >4 weeks), or treatment with other immunosuppressive drugs (methotrexate, cyclosporine, and biologics).Mortality was defined as all-cause mortality during hospitalization or follow-up, regardless of the direct attribution of TB.

Ethical Approval

The study protocol was approved by the Ethics Committee of Ankara Bilkent City Hospital (Decision No: E2-22-1952, Date: 08/06/2022). This study was conducted in accordance with the Declaration of Hel-

Table 1. Demographic and Clinical Characteristics of the Patients Diagnosed with Extrapulmonary Tuberculosis

Parameter	n (%)
Sex	
– Male	48 (51.61)
– Female	45 (48.39)
Age, Median (Min–Max)/Mean ± SD	55 (18–88) (52.20 ± 18.90)
Nationality	
– Turkish citizen	84 (90.32)
– Foreign national	9 (9.68)
Place of residence	
– Ankara	56 (60.22)
– Outside Ankara	37 (39.78)
Family history of tuberculosis	
– Absent	85 (91.40)
– Present	6 (6.45)
– Unknown	2 (2.15)
Comorbidity status	
– No comorbidity	52 (55.91)
– At least one comorbidity	41 (44.09)
• Diabetes mellitus	17 (18.28)
• Malignancy	12 (12.90)
• Cardiovascular disease	20 (21.51)
• Chronic kidney disease	3 (3.23)
• HIV infection	2 (2.15)
Immunosuppression	
– Absent	79 (84.95)
– Present	14 (15.05)

SD: Standard deviation. HIV: Human immunodeficiency virus. Percentages are column percentages, unless otherwise specified.

Table 2. Diagnostic Evaluation of Patients with Extrapulmonary Tuberculosis

Diagnostic Parameter	n (%)
PPD	
– Negative	35 (37.63)
– Positive	58 (62.37)
IGRA	
– Negative	14 (15.05)
– Positive	38 (40.86)
– Not performed	41 (44.09)
Acid-Fast Bacilli Smear	
– Negative	51 (54.84)
– Positive	24 (25.81)
– Not performed	18 (19.35)
TB PCR	
– Negative	22 (23.66)
– Positive	29 (31.18)
– Not performed	42 (45.16)
Histopathology	
– Compatible with TB	56 (60.22)
– Not compatible with TB	3 (3.23)
– Not available	34 (36.56)
Culture	
– Negative	40 (43.01)
– Positive	28 (30.11)
– Not performed	25 (26.88)
Diagnostic approach	
– Diagnosis prior to treatment	61 (65.59)
– Diagnosis confirmed after treatment initiation	32 (34.41)

PPD: purified protein derivative; IGRA: interferon-gamma release assay; AFB: acid-fast bacilli; PCR: polymerase chain reaction.The percentages represent the proportion of total patients (n = 93).

sinki and the institutional data protection regulations.The need for individual informed consent was waived because of the retrospective nature of the study.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows version 20.0 (Armonk, NY: IBM Corp).Continuous variables are expressed as mean ± standard deviation (SD) and median (minimum–maximum), depending on the distribution. Categorical variables are presented as frequencies and percentages.

Table 3. Distribution of Extrapulmonary Tuberculosis Sites of Involvement

Site of Involvement	n (%)
Vertebral bone and joint	30 (32.26)
Peripheral lymphadenitis (non-thoracic)	25 (26.88)
Meningitis	15 (16.13)
Peritoneal / gastrointestinal system	11 (11.83)
Genitourinary system	7 (7.53)
Cutaneous	3 (3.23)
Non-vertebral bone and joint	2 (2.15)

Percentages are based on the total number of patients (n = 93). A single patient may present with more than one site of involvement, although primary sites are prioritized for treatment.

Comparisons between survivors and non-survivors were performed using the chi-square or Fisher's exact test for categorical variables and independent-samples t-test or Mann–Whitney U test for continuous variables, depending on the distribution. Statistical significance was set at $p < 0.05$.

Results

A total of 93 adult patients were included, comprising 48 men (51.6%) and 45 women (48.4%), with a median age of 55 years (range, 18–88 years).Most patients were Turkish citizens (90.3%), while nine (9.7%) were foreign nationals (six Syrian, two Afghan, and one Iraqi). Comorbidities were present in 44.1% of patients, most frequently cardiovascular disease (21.5%) and diabetes mellitus (18.3%). Immunosuppression was documented in 15.1% of the cases (Table 1).

Diagnostic evaluation revealed a positive PPD result in 62.4% of patients and a positive IGRA result in 40.9% of patients.The AFB smear, TB PCR, and culture positivity rates were 25.8 %, 31.2%, and 30.1 %, respectively.Histopathological confirmation was obtained in 60.2% of the cases.The diagnosis was established before treatment initiation in 65.6% of patients (Table 2).

The most common sites of involvement were the vertebral bones and joints (32.3%), lymph nodes (26.9%), and central nervous system (16.1%).Less frequent sites included the peritoneal/gastrointestinal (11.8%), genitourinary (7.5%), cutaneous (3.2%), and non-vertebral skeletal (2.2%) localizations (Table 3).

First-line quadruple therapy was administered to 95.7% of the patients.The median duration of dual therapy was 4 months (range, 0–10 months), and the median total treatment duration was 6 months (range, 1–13 months).Surgical intervention was performed in 22.6% of the cases.Drug susceptibility testing was performed in 27.9% of patients, of whom 2.2% showed resistance (Table 4).

During the follow-up period, 9 patients (9.7%) died. No significant differences between survivors and non-survivors were observed in age, sex, nationality, place of residence, family history of TB, AFB or culture positivity, or surgical intervention. Immunosuppression was significantly more frequent among non-survivors than among survivors (44.4% vs. 11.9%, $p = 0.027$).The median duration of dual therapy (1 vs. 4 months, $p < 0.001$) and total therapy (3 vs. 6 months, $p < 0.001$) were significantly shorter in non-survivors.No association with drug resistance was identified, although the subgroup numbers were small (Table 5).

Discussion

National and Global Perspectives Our 12-year retrospective cohort highlights the growing burden of EPTB in Turkey and worldwide. Globally, EPTB accounts for 15–20% of all tuberculosis cases(14). According to the WHO, 16% of the 7.1 million TB cases notified in 2019 were extrapulmonary, ranging

Table 4. Anti-Tuberculosis Treatment Characteristics and Drug Resistance Status

Treatment Parameter	n (%)
Initial anti-TB regimen	
– INH, RIF, PZ, ETB (first-line quadruple)	89 (95.70)
– Moxifloxacin, Cycloserine, ETB, Streptomycin	3 (3.23)
– INH, RIF, ETB, Streptomycin	1 (1.08)
Duration of quadruple therapy, Median (Min–Max)/ Mean ± SD	2 (1–3) (2.10 ± 0.30)
Duration of dual therapy, Median (Min–Max)/ Mean ± SD	4 (0–10) (5.40 ± 2.60)
Total treatment duration, Median (Min–Max)/ Mean ± SD	6 (1–13) (7.40 ± 2.80)
Surgical intervention	
– Not performed	72 (77.42)
– Performed	21 (22.58)
Drug susceptibility testing performed	
– Not performed	67 (72.04)
– Performed	26 (27.96)
• Susceptible	24 (25.81)
• Resistant	2 (2.15)

INH: Isoniazid; RIF: Rifampicin; PZ: Pyrazinamide; ETB: Ethambutol. SD: Standard deviation. The duration values are expressed in months.

from 8% in the Western Pacific to 24% in the Eastern Mediterranean (15). In high-income, low-incidence settings, the proportion is higher; EPTB constitutes 27% of TB cases in the United States and 38% in the Netherlands(14). Turkey has one of the highest EPTB proportions; before the COVID-19 pandemic, over one-third of Turkish TB cases were extrapulmonary. National data show that the share rising from 28.6% in 2005 to 35–36% by 2018(15).Ulusoy et al. reported that immunosuppressive medication use was significantly more frequent among patients with PTB than among those with EPTB in a Turkish cohort (16).

Recent data show that this proportion increased to 41.6% in 2020, with 3,710 extrapulmonary cases among 8,925 new TB cases(17).This increase is partly due to the declining pulmonary TB notifications during the pandemic and better EPTB recognition. Improvements in pulmonary TB control have left a larger fraction of TB presenting at extrapulmonary sites(15). The rise in immunosuppressive comorbidities also contributes to the changing epidemiology. Our cohort reflects these trends: EPTB remains significant, with median patient age in the sixth decade and nearly equal sex distribution. Unlike pulmonary TB's male predominance, many EPTB series show a balanced sex ratio, as European studies noted women and older adults at higher risk for EPTB(15). The epidemiological profile of our patients aligns with national data and shows EPTB increasing where overall TB incidence is declining(15). This emphasizes the need for continued vigilance for extrapulmonary TB under national programs as we strive to meet End TB targets amid the COVID-19 pandemic.

Patterns of Organ Involvement and Clinical Comparisons

The spectrum of organ involvement in our 93 patients demonstrated both concordance with and distinctive differences from patterns reported in the literature.In

Table 5. Comparison of Demographic and Clinical Characteristics Between Survivors and Non-Survivors

	Parameter	Survivors	Non-survivors 9)	p
Sex	Male	43 (51.19)	5 (55.56)	1.000
	Female	41 (48.81)	4 (44.44)	
Age	Mean ± SD	51.40 ± 18.40	60.60 ± 22.10	0.096
	Median (Min–Max)	54 (18–88)	71 (22–82)	
Nationality	Turkish citizen	75 (89.29)	9 (100.00)	0.592
	Foreign national	9 (10.71)	0 (0.00)	
Place of residence	Ankara	52 (61.90)	4 (44.44)	0.475
	Outside Ankara	32 (38.10)	5 (55.56)	
Family history of TB (n = 91)	Absent	77 (91.67)	8 (88.89)	0.572
	Present	5 (6.00)	1 (11.11)	
Immunosuppressive condition	Absent	74 (88.10)	5 (55.56)	0.027
	Present	10 (11.90)	4 (44.44)	
AFB smear	Negative	48 (57.14)	3 (33.33)	0.201
	Positive	20 (23.81)	4 (44.44)	
Culture result	Negative	35 (41.67)	5 (55.56)	0.691
	Positive	26 (30.95)	2 (22.22)	
Site of involvement	Vertebral bone and joint	27 (32.14)	3 (33.33)	–
	Lymphadenitis	23 (27.38)	2 (22.22)	
	Meningitis	12 (14.29)	3 (33.33)	
	Peritoneal / GI system	11 (13.10)	0 (0.00)	
	Genitourinary system	7 (8.33)	0 (0.00)	
	Cutaneous	2 (2.38)	1 (11.11)	
	Non-vertebral bone and joint	2 (2.38)	0 (0.00)	
Initial anti-TB regimen	INH, RIF, PZ, ETB	80 (95.24)	9 (100.00)	–*
	Moxifloxacin, Cycloserine, ETB, SM	3 (3.57)	0 (0.00)	
	INH, RIF, ETB, SM	1 (1.19)	0 (0.00)	
Duration of quadruple therapy	Mean ± SD	2.10 ± 0.30	1.90 ± 0.60	0.108
	Median (Min–Max)	2 (2–3)	2 (1–3)	
Duration of dual therapy	Mean ± SD	5.80 ± 2.30	1.30 ± 1.70	<0.001
	Median (Min–Max)	4 (4–10)	1 (0–4)	
Total treatment duration	Mean ± SD	7.90 ± 2.40	3.20 ± 2.10	
	Median (Min–Max)	6 (6–13)	3 (1–7)	<0.001
Surgical intervention	Not performed	64 (76.19)	8 (88.89)	0.678
	Performed	20 (23.81)	1 (11.11)	
Drug resistance testing	Not performed	60 (71.43)	7 (77.78)	–*
	Performed	24 (28.57)	2 (22.22)	
	• Susceptible	22 (26.19)	2 (22.22)	
	• Resistant	2 (2.38)	0 (0.00)	

Pyrazinamide; ETB: Ethambutol; SM: Streptomycin; SD: Standard deviation. AFB: Acid-Fast Bacilli; TB: Tuberculosis; INH: Isoniazid; RIF: Rifampicin; PZ: Pyrazinamide

**Pearson's chi-square test could not be applied due to expected frequencies <5 in some cells. The duration values are expressed in months. Survivors (n = 84), Non-survivors (n = 9)*

general, TB lymphadenitis is the most common form of EPTB across many populations (13).For example, Turkish Ministry of Health data indicate that extrathoracic lymph node TB comprises approximately one-third of EPTB cases nationally (31.6%), followed by pleural TB (22–23%) as the next leading site (17). Our cohort also had a high frequency of lymph node TB (26.9% of cases), consistent with the notion that lymphatic involvement is the dominant clinical form of EPTB.However, the most frequent site in our series was skeletal TB, particularly vertebral disease, which accounted for 32.3% of cases.This is a noticeably higher share than that generally reported; typically, bone and joint TB constitutes approximately 10–15% of EPTB cases in large cohorts (13).Similarly, central nervous system (CNS) TB accounted for 16.1% of our cases, whereas population-based data often show CNS TB to be relatively rare, at approximately 3–7% of EPTB (13).The prominence of spinal and CNS TB in our study likely reflects a referral bias; being tertiary care centers, our hospitals receive complicated EPTB cases that might be managed at specialty centers, whereas more straightforward EPTB cases are often handled by general pulmonary services.Indeed, pleural TB was notably under-represented in our cohort; none of our patients had isolated pleural involvement, even though pleural TB is traditionally among the top two extrapulmonary sites in national statistics (17).This anomaly reinforces that our hospital-based series is enriched for severe, multi-system, or diagnostically challenging EPTB presentations rather than the full spectrum seen in the community setting.

Despite differences in distribution, our findings mirror the global diversity of EPTB manifestations. Consistent with published observations, virtually any organ system can be affected. In addition to lymphatic, osteoarticular, and CNS TB, we observed intra-abdominal TB in 12% of patients, genitourinary TB in 8%, and rare cutaneous TB (3%). Such heterogeneity is documented; Baykan et al. reported that the most common sites globally are peripheral lymph nodes (50% of EPTB cases), pleura (18%), and genitourinary, bone/joint, gastrointestinal, and CNS in descending order (13). A recent national survey in China found lymphatic TB most frequent but highlighted regional variations and showed no single site predominates universally (18). Our cohort's profile shows tertiary centers may see cases skewed toward severe forms, which is important when comparing clinical studies. It emphasizes the need for clinical suspicion across specialties; patients often presented to neurology, orthopedics, or gastroenterology before TB was recognized. As noted previously, EPTB can mimic other diseases and presents insidiously. The diverse organ involvement reinforces the importance of maintaining TB in the differential diagnosis for persistent lymphadenopathy, chronic back pain, unexplained meningitic syndromes, or fever of unknown origin, especially in TB-endemic regions.

Diagnostic Challenges and Advances

Our experience highlights diagnostic challenges of EPTB. Unlike pulmonary TB, where sputum microscopy and Xpert testing can rapidly confirm cases, extrapulmonary disease requires invasive sampling of inaccessible sites, with modest yields. In our cohort, 25.8% of patients had positive acid-fast bacilli smears, and 30.1% had cultures that grew M. tuberculosis. These figures align with other series; a Turkish study reported microbiological confirmation in roughly one-third of EPTB patients (15). The paucibacillary nature of EPTB means many patients are microbiologically negative despite active disease. In our study, most cases relied on clinical, radiological, and histopathological evidence. Histopathology showing caseating granulomas supported TB in 60% of patients, highlighting that tissue biopsy remains crucial when direct bacteriological proof is elusive. In 34% of patients, diagnosis was confirmed only after empiric treatment began, showing that clinicians often make therapeutic decisions based on presumptive evidence. This scenario is common; as Yıldız et al. noted, invasive procedures are required for EPTB diagnosis, and delays or misdiagnoses remain problematic (15). Critical forms like TB meningitis or miliary TB can be fatal if not recognized early, yet these forms are most difficult to diagnose definitively (15). Our findings support initiating empiric

therapy for life-threatening EPTB while awaiting results, given reasonable clinical suspicion, as recent guidelines emphasize.

Advances in diagnostics have improved our ability to confirm EPTB. The WHO now recommends rapid molecular tests as the initial diagnostic for suspected EPTB, showing higher sensitivity than smear microscopy in extrapulmonary specimens (15). During our study, Xpert became widely available with a positive yield in 31% of patients, contributing to the high rate of microbiological confirmation. Studies show good sensitivity of Xpert in tissue and pus samples, though lower in paucicellular fluids like pleural fluid or CSF (19, 20). Molecular diagnostics has narrowed this gap. Tools like adenosine deaminase (ADA) assays for pleural or peritoneal fluid and IGRA support diagnoses (21). In our cohort, IGRA tests were positive in 41% of those tested, though many patients did not undergo IGRA. While positive IGRA or tuberculin skin tests support EPTB diagnosis, they lack specificity and often show negative in immunosuppressed patients. Our experience confirms that diagnosing EPTB requires a multimodal approach, combining clinical judgment with imaging, biopsy/histology, and microbiologic tests. The high rate of empiric treatment reflects the need to treat presumptively when diagnostic certainty is difficult. This must balance with obtaining specimens for culture and drug susceptibility testing (DST) when possible, as WHO emphasizes for managing drug-resistant TB. We performed DST in 28% of patients, finding two cases of drug-resistant TB. This low resistance rate matches the national data on multidrug-resistant TB in Turkey but suggests possible undetected resistance in culture-negative EPTB (15). New tools like next-generation sequencing and improved imaging may enhance EPTB diagnosis. Our findings highlight the need for better diagnostics and early sample collection, as delays link to worse outcomes.

Treatment Outcomes and Clinical Outcomes in Context

Despite the challenges in diagnosis, once EPTB is recognized and treated, the outcomes are generally favorable, varying by disease form and patient factors. All patients received standard combination anti-TB therapy under the National TB Program. Treatment aligned with international and local guidelines: 96% started the conventional four-drug first-line regimen(3, 22). Exceptions were patients requiring second-line drugs or those unable to use pyrazinamide. The median therapy duration was 6 months, reflecting the recommended 2-month intensive phase + 4-month continuation phase for drug-susceptible TB. However, many patients, especially those with severe disease, were treated longer at their physicians' discretion. Mean treatment length was 7.4 ± 2.8 months, with longest courses reaching 12–13 months in some cases. Extended treatments were for TB meningitis and skeletal TB patients, where clinicians often prolong therapy. WHO and Turkish guidelines recommend 6-month regimens for most extrapulmonary TB forms, similar to pulmonary TB, but acknowledge CNS or bones/joints TB may need 9–12 months (23). Tuberculous meningitis typically requires 12 months with adjunctive corticosteroids initially due to risks of inadequate treatment. In our series, all CNS TB cases received adjuvant high-dose steroids and were treated for ≥12 months. Our management aligns with WHO's latest TB guidelines (2022–2023), which emphasize individualized treatment duration for extrapulmonary disease severity, while recommending at least 6 months of rifamycin-based therapy for drug-susceptible cases (24). Ongoing trials have explored shorter regimens for limited EPTB, but such strategies remain unadopted in adults.

Treatment outcomes in our cohort were positive with important caveats. Among the 93 patients, most achieved cure or treatment completion, as documented by the national TB registry. We observed no treatment failures in drug-susceptible patients, and few defaulted or were lost to follow-up, due to robust follow-up through TB dispensaries. This reflects Turkey's TB control program strength, which employs directly observed therapy and systematic monitoring (15). Comparable success rates have been reported elsewhere; a recent study from India noted treatment success of 91% among EPTB patients, ex-

ceeding the WHO target of 90% (14). Our real-world cohort of complex cases suggests high success when patients adhere to therapy. However, mortality emerged as significant, with a 9.7% overall rate. This aligns with the 8–10% early mortality in other Turkish hospital-based EPTB series (15)and shows that some EPTB patients, especially those with disseminated or CNS disease, succumb despite treatment. This in-hospital mortality rate exceeds the average TB mortality in national program data. The TB-related death rate among notified EPTB cases in many countries is approximately 3–5% (14), indicating many extrapulmonary cases have excellent prognosis. The higher mortality in our series reflects case severity and referral patterns; our hospitals treated high-risk EPTB cases. This is supported by profiles of deceased patients: their median age was higher than survivors, and most had CNS TB, miliary TB, or advanced malignancy comorbidities. Similarly, in the Acta Medica study from Gazi University, the most frequent EPTB types among deceased patients were CNS and miliary TB, with median age of 65 years versus 49 years in survivors (15). Our findings confirm that advanced age and severe disease drive mortality risk.

Mortality Risk Factors and Comparative Clinical Features

Comparing survivors and non-survivors in our cohort revealed clinical risk factors that mirror those reported elsewhere. Immunosuppressive conditions were significantly associated with mortality: 44% of patients who died had an immunosuppressive comorbidity versus 12% of survivors. This aligns with global observations that patients with TB and impaired immunity fare worse. Although only 2.1% of patients were HIV-positive, one had disseminated TB with poor outcomes. Worldwide, HIV coinfection drives extrapulmonary and disseminated TB; in 2021, 187,000 of the 1.6 million TB deaths were in people with HIV. These patients often present with atypical or multiple-site disease and high mortality if not promptly treated. Chronic kidney disease, diabetes, and malignancy were present in several patients, potentially contributing to worse outcomes (24). Diabetes was a common comorbidity, reflecting its high prevalence in TB cohorts and association with delayed sputum conversion and treatment complications. While diabetes wasn't an independent predictor of death in our small sample, its frequency reinforces the importance of managing comorbidities during TB therapy. In the Gazi University study's multivariate analysis, independent risk factors for early mortality were older age, high ESR (>50 mm/h), miliary TB, and CNS involvement (15). Our data, though underpowered for a multivariate model, agree; all fatal cases were elderly and/or had CNS or disseminated disease. Several non-survivors experienced diagnostic delays, highlighting the importance of timely detection of severe EPTB. Our cohort's median age of 55 years exceeds that in high-burden countries but matches Turkish and European case series (15), reflecting TB demographics in a middle-income country with an aging population. The near-equal sex distribution contrasts with male-predominant pulmonary TB, while some studies note slight female EPTB predominance. European surveys show females have higher EPTB odds, possibly due to biological or healthcare access factors. While our data cannot confirm sex differences, they show EPTB affects both sexes and all ages, and clinicians should consider TB in older adults or females without classic risk factors (24, 26).

When comparing outcomes, our results show most patients with EPTB can be cured with appropriate therapy. High treatment success is achievable for extrapulmonary disease when patients are diagnosed and supported through treatment (14). However, experience shows certain subgroups, such as the elderly, immunosuppressed, and those with CNS or disseminated TB, remain at high risk of poor outcomes. These findings call for targeted interventions, including closer monitoring of high-risk patients, managing comorbidities, and possibly novel therapies. Our findings align with national and international data, showing EPTB as an evolving challenge. It accounts for a growing proportion of TB cases in Turkey, presents with diverse manifestations, and requires a high degree of suspicion for

timely diagnosis.The distribution of organ involvement in our cohort, weighted towards severe forms such as skeletal and CNS TB, underscores the need for robust diagnostic strategies and specialist input. Our patients' outcomes were comparable to global figures, with high treatment completion but significant mortality in severe cases. These comparisons with studies and WHO reports highlight key areas for improvement: enhancing early detection, ensuring microbiological testing for EPTB patients, and sustaining TB care amid challenges such as pandemics. By integrating local insights with global evidence, we can better address the challenges of extrapulmonary TB and improve outcomes.

Study Strengths and Study Limitations

This study represents one of the few long-term institutional analyses in Türkiye that focused on adult EPTB.Its strengths include a large cohort spanning 12 years, systematic data collection across two tertiary care centers, and documentation of diagnostic modalities, organ-specific distribution, and mortality-associated factors.Multiple diagnostic test outcomes, treatment durations, and mortality comparisons provide comprehensive clinical and epidemiological insights relevant to national TB control efforts.However, as a retrospective, single-country, hospital-based study, the findings may be influenced by referral and selection bias, particularly for complex EPTB presentations such as CNS or vertebral involvement.The lack of universal microbiological confirmation limited molecular testing in the early years, and the absence of post-treatment follow-up data restricted generalizability.Small subgroup sizes also limited the power of the multivariate risk analysis of mortality predictors.

Conclusion

This 12-year retrospective cohort study demonstrated that extrapulmonary tuberculosis (EPTB) in Türkiye remains an important clinical and epidemiological challenge. Vertebral bone, lymph nodes, and central nervous system were the predominant sites of involvement, reflecting the referral profile of tertiary centers. Although most patients achieved successful outcomes with standard anti-tuberculosis therapy, mortality reached 9.7% and was strongly associated with immunosuppressive conditions and shortened treatment durations. These findings emphasize the necessity of early recognition, comprehensive diagnostic evaluation, and individualized therapeutic strategies, particularly for high-risk subgroups such as immunosuppressed patients and those with CNS disease. Sustained investment in rapid diagnostics, systematic drug susceptibility testing, and multidisciplinary management should remain priorities of national TB programs to reduce mortality and improve EPTB outcomes.

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Ethical Statement: The study protocol was approved by the Ethics Committee of Ankara Bilkent City Hospital (Decision No: E2-22-1952, Date: 08/06/2022). This study was conducted in accordance with the Declaration of Helsinki and the institutional data protection regulations.

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Desmopressin, Alarm, or Both? Evaluating Efficacy and Relapse in Children with Primary Monosymptomatic Nocturnal Enuresis

Primer Monosemptomatik Enürezis Nokturna'da Alarm Cihazı, Desmopressin ve Kombinasyon Tedavisinin Etkinliğinin Karşılaştırılması

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ABSTRACT

Background: Monosymptomatic nocturnal enuresis (MNE) is a common pediatric condition that affects a child's self-esteem and daily family life. Both enuresis alarm and desmopressin are recommended as first-line treatment options, but their comparative effectiveness and relapse rates remain subjects of discussion.

Aim: This study aimed to compare the short-term treatment outcomes and relapse rates of desmopressin, alarm therapy, and combination therapy in children with primary MNE.

Materials and Methods: This retrospective two-center study included 202 children diagnosed with MNE between September 2022 and January 2025. Patients were grouped into desmopressin monotherapy (n=124), alarm therapy (n=45), and combination therapy (n=33). Demographic data, treatment responses, and relapse rates were evaluated.

Results: Complete response rates were 39.5% for desmopressin, 42.2% for alarm therapy, and 63.6% for the combination group. Relapse occurred in 48.4% of the desmopressin group, 33.3% of the alarm group, and 30.3% of the combination group. Although the combination group showed higher response rates and lower relapse rates, these differences were not statistically significant (p>0.05).

Conclusion: Desmopressin, alarm therapy, and their combination are effective options for the treatment of primary MNE. Combination therapy may provide added benefit in selected patients, but no clear superiority was demonstrated in this study. Treatment plans should consider individual factors such as nocturnal urine output and family compliance.

Keywords: Monosymptomatic nocturnal enuresis, Desmopressin, Enuresis alarm

ÖZET

Giriş: Monosemptomatik noktürnal enürezis (MNE), çocuklarda özgüven ve aile düzenini olumsuz etkileyen yaygın bir durumdur. Enürezis alarmı ve desmopressin birinci basamak tedavi seçenekleri arasında yer almakta, ancak etkinlik ve nüks oranları hâlâ tartışılmaktadır.

Amaç: Bu çalışmanın amacı, primer MNE tanılı çocuklarda desmopressin, alarm tedavisi ve kombine tedavinin kısa dönem tedavi sonuçları ile nüks oranlarını karşılaştırmaktır.

Gereç ve Yöntem: Bu retrospektif iki merkezli çalışmaya, Eylül 2022 ile Ocak 2025 tarihleri arasında MNE tanısı alan 202 çocuk dahil edildi. Hastalar desmopressin monoterapisi (n=124), alarm tedavisi (n=45) ve kombine tedavi (n=33) gruplarına ayrıldı. Demografik veriler, tedavi yanıtları ve nüks oranları değerlendirildi.

Bulgular: Tam yanıt oranları desmopressin grubunda %39,5, alarm grubunda %42,2 ve kombine grupta %63,6 idi. Nüks oranı desmopressin grubunda %48,4, alarm grubunda %33,3 ve kombine grupta %30,3 olarak saptandı. Kombine tedavi sayısal olarak daha iyi sonuç verse de fark istatistiksel olarak anlamlı değildi (p>0,05).

Sonuç: Primer MNE tedavisinde desmopressin, alarm tedavisi ve kombine tedavi etkili seçeneklerdir. Kombine tedavi bazı hastalarda ek fayda sağlayabilir, ancak bu çalışmada belirgin bir üstünlük göstermemiştir. Tedavi planı, bireysel faktörler ve aile uyumu göz önünde bulundurularak yapılmalıdır.

Anahtar kelimeler: Monosemptomatik noktürnal enürezis, Desmopressin, Enürezis alarmı

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Introduction

Monosymptomatic nocturnal enuresis (MNE) is one of the most common pediatric urological conditions, characterized by involuntary bedwetting during sleep in children without other lower urinary tract symptoms(1). This condition can considerably impair a child's psychosocial well-being, self-esteem, and daily family life(2).

The International Children's Continence Society (ICCS) recommends both enuresis alarm therapy and desmopressin as first-line treatment options for MNE(2). Alarm devices aim to condition the child's arousal response to bladder filling, thereby training the child to wake up and void when necessary(3). Desmopressin, a synthetic analog of vasopressin, reduces nocturnal urine production by increasing renal water reabsorption(4). Despite their proven efficacy, each option has its limitations: alarm therapy requires strong family motivation and consistent compliance, whereas desmopressin is associated with higher relapse rates after treatment discontinuation(5).

Recent randomized controlled trials and meta-analyses have compared these modalities individually and have also begun to explore their combined use (5, 6). Some studies suggest that combination therapy may offer added benefits in children with high nocturnal urine production or poor response to single treatment(7).

Nevertheless, despite increasing evidence, data comparing these treatment approaches in routine clinical practice are still relatively limited. Therefore, this retrospective two-center study was designed to examine and compare the short-term effectiveness, relapse rates, and treatment adherence of alarm therapy, desmopressin monotherapy, and their combination in children with primary MNE. It is expected that combined therapy may provide higher treatment success and lower relapse rates than monotherapy alone, offering additional insight for clinical decision-making.

Methods

Study Design and Ethics

This retrospective, two-center cohort study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the local Non-Interventional Clinical Research Ethics Committee (Approval No: E-60116787-020-622659).

Patient Selection

Medical records of pediatric patients diagnosed with primary MNE were retrospectively reviewed. Eligible patients were those who attended the urology and pediatric urology outpatient clinics of two tertiary centers between September 2022 and January 2025.

Inclusion criteria were:

- Age ≥5 years,
- Diagnosis of MNE according to ICCS criteria,
- At least two wet nights per week before treatment,
- No prior pharmacological or surgical treatment for enuresis.

Children with non-monosymptomatic enuresis, daytime urinary tract symptoms, neurological or anatomical urinary tract abnormalities, urinary tract infections, or incomplete records were excluded.

Treatment Allocation

Patients were categorized into three groups based on their documented treatment strategy:

1. **Alarm Therapy Group:** Managed with enuresis alarm alone, with family guidance on daily use and device maintenance.
2. **Desmopressin Group:** Treated with oral desmopressin melt at standard pediatric doses (120 µg/day, adjusted if needed).
3. **Combination Group:** Received both alarm therapy and desmopressin melt concurrently, either as initial treatment or after insufficient response to monotherapy.

All patients received standard urotherapy advice, including healthy voiding habits, evening fluid restriction after dinner, and constipation management.

Table 1. Demographic and Clinical Characteristics

Variable	Desmopressin (n=124)	Alarm (n=45)	Combined (n=33)	p-value
Age (years), median [min–max]	9 [5–16]	10 [5–15]	9 [5–15]	0.458*
Baseline wet nights/week, median [min–max]	6 [3–7]	6 [4–7]	6 [4–7]	0.873*
Post-treatment wet nights/week, median [min–max]	2 [0–7]	2 [0–6]	1 [0–6]	0.061*
Male (n, %)	71 (57.3%)	25 (55.6%)	21 (63.6%)	0.753**
Family history of enuresis (n, %)	49 (39.5%)	19 (42.2%)	12 (36.4%)	0.872**

P*: P-values calculated using Kruskal-Wallis test for continuous variables

p**: P-values calculated using Chi-square test for categorical variables

Data Collection and Definitions

Demographic data (age, sex), baseline frequency of wet nights, family history of enuresis, and bladder diary records were extracted. Treatment duration, adherence notes, treatment response, and any relapse were documented.

Treatment response was defined according to ICCS standards as:

- *Complete Response:* Total dryness during follow-up.
- *Partial Response:* ≥50% reduction in wet nights per week compared to baseline.
- *No Response:* <50% reduction.

Relapse was defined as the recurrence of more than one wet night per month after treatment discontinuation or initial dryness.

Statistical Analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA), consistent with our previous methodology. Continuous variables were reported as mean ± standard deviation or median with interquartile ranges, based on distribution. Normality was tested using the Kolmogorov–Smirnov test. Between-group comparisons were made using one-way ANOVA or the Kruskal–Wallis test for continuous variables and chi-square or Fisher's exact test for categorical variables. A p-value of <0.05 was considered statistically significant.

Results

A total of 202 children with monosymptomatic nocturnal enuresis (MNE) were included in the study. Of these, 124 received desmopressin monotherapy, 45 received alarm therapy, and 33 received combined therapy. The mean age was 9.26 ± 2.49 years in the desmopressin group, 9.78 ± 2.58 years in the alarm group, and 9.30 ± 2.66 years in the combined group (p=0.458). The male proportion was 57.3% for desmopressin, 55.6% for alarm, and 63.6% for combined therapy (p=0.753). A positive family history of enuresis was present in 39.5%, 42.2%, and 36.4% of the desmopressin, alarm, and combined groups, respectively (p=0.872). The demographic and clinical characteristics of the study groups are summarized in Table 1. The median follow-up duration was 3 months (range 3–6) in the desmopressin group, 3 months (range 3–5) in the alarm group, and 3 months (range 3–6) in the combined therapy group. There was no statistically significant difference between the groups (p = 0.642).

The baseline mean number of wet nights per week was 5.90 ± 0.94 in the desmopressin group, 5.87 ± 0.97 in the alarm group, and 5.82 ± 0.95 in the combined group (p=0.873). After treatment, the mean number of wet nights per week decreased to 1.94 ± 1.82 for desmopressin, 1.76 ± 1.75 for alarm, and 1.12 ± 1.65 for combined therapy (p=0.061).

According to ICCS criteria, the complete response rate was 39.5% for desmopressin, 42.2% for alarm, and 63.6% for combined therapy. The partial response rate was 32.3%, 33.3%, and 18.2%, respectively. Non-response rates were 28.2% for desmopressin, 24.4% for alarm, and 18.2% for combined therapy (p=0.168).

Table 2. Treatment Response and Relapse

Outcome	Desmopressin (n=124)	Alarm (n=45)	Combined (n=33)	p-value
Complete Response (n, %)	49 (39.5%)	19 (42.2%)	21 (63.6%)	0.168*
Partial Response (n, %)	40 (32.3%)	15 (33.3%)	6 (18.2%)	
No Response (n, %)	35 (28.2%)	11 (24.4%)	6 (18.2%)	
Relapse (n, %)	60 (48.4%)	15 (33.3%)	10 (30.3%)	0.070*

P*: P-values calculated using Chi-square test for categorical variables

Relapse was observed in 48.4% of patients in the desmopressin group, 33.3% in the alarm group, and 30.3% in the combined therapy group (p=0.070). Treatment response and relapse rates are presented in Table 2.

Discussion

In this retrospective two-center study, we compared the treatment outcomes of desmopressin monotherapy, enuresis alarm therapy, and their combination in children with primary MNE. Our results showed that although the combined therapy group demonstrated numerically higher complete response rates and lower relapse rates compared to either monotherapy, these differences did not reach statistical significance. Similarly, while the relapse rate appeared lower for combined therapy than for desmopressin alone, this difference also failed to show a significant statistical effect. These findings indicate that the combined therapy did not demonstrate the strong benefit that might have been expected, highlighting the need for careful patient selection and realistic treatment expectations.

Several previous studies have similarly reported that while combination therapy intuitively seems promising due to its ability to target both the physiological (nocturnal polyuria) and behavioral (arousal training) components of enuresis, its superiority over monotherapy is not always evident in practice(7, 8). Randomized controlled trials and meta-analyses have shown that alarm therapy alone can achieve sustained dryness when families are well motivated(5, 9), whereas desmopressin provides rapid symptom relief but is associated with higher relapse rates when withdrawn abruptly(5). In this context, our results align with reports suggesting that adding desmopressin to alarm therapy does not consistently produce a statistically significant improvement in treatment success or relapse prevention, especially when alarm therapy is implemented effectively as a first-line intervention.

One possible explanation for the lack of a clear statistical advantage of combined therapy in our study may be related to the absence of detailed subgroup analyses rather than insufficient data collection. Although voiding diaries were routinely used to assess nocturnal urine output and bladder function, our study did not include separate analyses for children with specific risk factors such as pronounced nocturnal polyuria or reduced bladder capacity, which have been shown to influence response to desmopressin-based regimens(7, 8). Moreover, adherence to alarm use and family motivation, which are known to impact alarm therapy outcomes, were not quantitatively evaluated in our cohort(6, 9). These aspects may partly explain why the expected benefit of combination therapy did not clearly emerge and underline the importance of individualized treatment planning based on patient-specific characteristics.

Taken together, our findings support the view that all three treatment modalities, desmopressin, alarm therapy and their combination, are effective options for managing primary MNE and may be selected according to individual patient needs and family circumstances. Alarm therapy can be a good choice in families who are highly motivated and able to maintain consistent device use, while desmopressin may be preferable for children needing rapid symptom control, especially in the presence of nocturnal polyuria(7, 9). For patients with an insufficient response to either monotherapy, combined treatment remains a reasonable alternative, although its benefit may not

be as pronounced as expected in all cases(6, 10, 11). Future prospective studies with larger sample sizes and clear subgroup analyses are needed to better define which children benefit most from each approach.

This study has several limitations that should be acknowledged. First, its retrospective design may carry inherent biases related to data completeness and treatment documentation. Second, although voiding diaries were used for initial assessment, detailed subgroup analyses based on nocturnal polyuria or bladder capacity could not be performed due to the retrospective nature of the dataset. Third, treatment adherence and family motivation, which are known to influence outcomes especially in alarm therapy, were not quantitatively measured. Finally, the relatively small size of the combined therapy group may have limited the statistical power to detect significant differences. These factors should be considered when interpreting our results and highlight the need for future prospective studies with more rigorous subgroup evaluation.

Conclusion

This study adds to the existing evidence by demonstrating that although combined therapy may provide additional benefit for selected children with primary MNE, it did not show clear superiority over monotherapy in this cohort. Clinicians should carefully consider individual factors such as nocturnal urine production, bladder capacity, and family motivation when planning treatment. Well-designed prospective studies are still needed to clarify optimal patient selection and to identify the most effective sequencing or combination of available treatment options.

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Ethical Statement: This study was approved by the Non-Interventional Clinical Research Ethics Committee of Pamukkale University (Approval No: E-60116787-020-622659, Date: 10.12.2024). The study was conducted in accordance with the principles of the Declaration of Helsinki.

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Research Article / Araştırma Makalesi

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Post-ERCP Pancreatitis and Serum Asprosin: A Potential Marker Associated with Beta Cell Damage

ERCP Sonrası Pankreatit ve Serum Asprosini: Beta Hücre Hasarı ile İlişkili Potansiyel Bir Belirteç

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ABSTRACT

Aim: Asprosin, an adipose-derived protein, plays a key role in glucose regulation. Pancreatitis-related inflammation causes beta cell injury and functional decline. Recent studies have shown a relationship between asprosin and pancreatic beta cell damage. This research aimed to examine the association between pancreatitis developing after endoscopic retrograde cholangiopancreatography (ERCP) and asprosin levels, which may reflect beta cell damage.

Materials and Methods: The study was conducted with 25 patients who developed pancreatitis after ERCP and 21 participants as a control group at Ankara Bilkent City Hospital. Acute pancreatitis was diagnosed through correlation of clinical, imaging, and laboratory findings. In addition, the post-ERCP pancreatitis (PEP) group was classified as "mild" and "moderate-severe" according to disease severity, and asprosin levels were compared between the groups. Severity was classified as mild or moderate–severe because of the limited sample size.

Results: Serum asprosin concentrations were elevated in the PEP group ($p < 0,001$). Amylase, lipase, GGT, AST, total and direct bilirubin levels also increased significantly in the pancreatitis group. In the pancreatitis group, a negative correlation was observed between asprosin levels and leukocyte ($r = -0.433$; $p = 0.031$) and lymphocyte counts ($r = -0.440$; $p = 0.028$). No meaningful correlation was observed between asprosin concentrations and the severity of pancreatitis ($p = 0,347$).

Conclusion: In patients with PEP, asprosin levels are increased, which could potentially be associated with the inflammatory response. However, asprosin does not seem to directly reflect disease severity. Thus, asprosin may serve as a potential marker of pancreatic beta cell injury and inflammation.

Keywords: Asprosin; Endoscopic Retrograde Cholangiopancreatography; Pancreatic Beta Cells; Pancreatitis

ÖZET

Amaç: Asprosin, glukoz metabolizmasının düzenlenmesinde önemli bir role sahip olan yağ dokusu tarafından üretilen bir proteindir. Pankreatitteki inflamasyon, beta hücre hasarına ve beta hücre fonksiyonlarının bozulmasına yol açar. Son çalışmalarda asprosin ile pankreatik beta hücre hasarı arasında bir ilişki gösterilmiştir. Bu araştırmanın amacı, endoskopik retrograd kolanjiopankreatografi (ERCP) sonrası gelişen pankreatit ile beta hücre hasarını yansıtabileceği düşünülen asprosin seviyeleri arasındaki ilişkiyi incelemektir.

Yöntem: Çalışma Ankara Bilkent Şehir Hastanesinde ERCP sonrası pankreatit gelişen 25 hasta ve kontrol grubu olarak 21 katılımcı ile yürütüldü. Akut pankreatit tanısı klinik, görüntüleme ve laboratuvar bulgularının korelasyonu ile konuldu. Ayrıca, ERCP sonrası pankreatit grubu hastalık şiddetine göre "hafif" ve "orta-şiddetli" olarak sınıflandırıldı ve asprosin seviyeleri gruplar arasında karşılaştırıldı. Asprosin ile biyokimyasal ve hematolojik parametreler arasındaki korelasyonlar analiz edildi.

Bulgular: Serum asprosin konsantrasyonları ERCP sonrası pankreatit grubunda yüksekti ($p < 0,001$). Amilaz, lipaz, GGT, AST, toplam ve direkt bilirubin seviyeleri de pankreatit grubunda anlamlı şekilde arttı. Pankreatit grubunda, asprosin seviyeleri ile lökosit ($r = -0,433$; $p = 0,031$) ve lenfosit sayıları ($r = -0,440$; $p = 0,028$) arasında negatif bir korelasyon gözlendi. Asprosin konsantrasyonları ile pankreatit şiddeti arasında anlamlı bir korelasyon gözlenmedi ($p = 0,347$).

Sonuç: ERCP sonrssında pankreatit gelişen hastalarda asprosin düzeyleri artar ve bu potansiyel olarak inflamatuar yanıtla ilişkilendirilebilir. Ancak asprosin düzeylerinin hastalığın şiddetini doğrudan yansıtmadığı düşünülmektedir. Bu nedenle asprosin pankreas beta hücre hasarı ve inflamasyonuyla ilişkili yeni bir biyobelirteç adayı olarak düşünülebilir; ancak tanısal ve prognostik rolünü açıklamak için ek araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Asprosin; Endoskopik Retrograd Kolanjiyopankreatografi (ERCP); Pankreas Beta Hücreleri; Pankreatit

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Introduction

Acute pancreatitis is a serious inflammatory disease with local and systemic complications. Assessing severity and managing complications are crucial. Hospitalizations rise, but mortality decreases with experience [1,2].

Recent studies have observed an increasing occurrence of diabetes mellitus (DM) following acute pancreatitis. Exocrine pancreatic dysfunction leads to the development of DM, also known as Type 3c DM or secondary pancreatic DM [3]. Due to pancreatitis, all cells in the pancreas are affected, and beta cell damage is also observed. For this reason, there is a risk of impaired insulin secretion and the development of DM [4].

Asprosin was discovered in 2016 and is an adipokine secreted from fat tissue. It increases glucose release from the liver, and high levels of asprosin have been associated with DM [5, 6]. The literature has demonstrated a potential association between asprosin and pancreatic cancer [7]. It has also been observed that asprosin is associated with beta cell damage in mice. Recently, new ways were found to explain how asprosin causes inflammation, cell dysfunction, and apoptosis in human primary islet cells and pancreatic β-cells. Studies have shown that asprosin supports β-cell apoptosis in Mouse Insulinoma 6 (MIN6) cells [8, 9].

In pancreatic β cells, asprosin has been shown to induce inflammation and apoptosis through Toll-like receptor 4 (TLR4)-mediated pathways and reduce insulin secretion, suggesting a potential role in β-cell damage and metabolic disorders [10].

Pancreatitis is the most common complication of ERCP, occurring in 2–10% of cases, with mortality about 1 in 500 [11].

PEP may result from contrast reaction, obstruction, or ductal pressure. Papillary trauma during ERCP causes edema and enzyme activation [12,13].

We chose to investigate asprosin, a potential but unexplored marker in acute pancreatitis, specifically in post-ERCP cases due to the homogeneity and iatrogenic origin of this patient group. In contrast to other etiologies such as gallstones, alcohol use, or hyperlipidemia, which may involve additional pathophysiological mechanisms affecting pancreatic beta-cell function, post-ERCP pancreatitis results directly from an interventional procedure. This distinction helps minimize potential confounding factors. Consequently, it allows for a more accurate assessment of whether changes in serum asprosin levels are directly related to the pathophysiology of pancreatitis itself.

Material and Method

All procedures in this study were approved by the Ethics Committee and conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and its subsequent revisions (Date: 18/09/2024, Approval Number: TABED 2-24-387). A total of 25 patients diagnosed with PEP and 21 healthy control participants who presented to the Department of Internal Medicine and Clinic of Ankara Bilkent City Hospital were prospectively enrolled. Informed consent was obtained from all participants. The diagnosis of PEP was established based on the criteria defined by Cotton et al. According to these criteria, PEP diagnosis requires the presence of new or worsened epigastric abdominal pain within 24 hours following ERCP, serum amylase and/or lipase levels elevated to at least three times the upper limit of normal, and a clinical course necessitating hospitalization for a minimum of 2 days (14).

PEP diagnosis followed Cotton criteria, while severity was graded per the revised Atlanta definitions, which are widely used for acute pancreatitis severity stratification and facilitate comparability with prior AP literatüre. Due to the limited sample size of the study, patients were grouped into two categories—mild and moderate-severe—for analysis. This approach was chosen to enhance the reliability of statistical analyses and to make comparisons between groups more meaningful (15, 16).

The Bedside Index for Severity in Acute Pancreatitis (BISAP) score was assessed based on five criteria: blood urea nitrogen (BUN) level exceeding 25 mg/dL, Glasgow Coma Scale (GCS) score below 15, presence of SIRS with a score of 2 or more, age over 60 years, and detection of pleural effusion on imaging

studies. These assessments were performed upon hospital admission and, when necessary, completed within 48 hours (17).

Ranson criteria were also applied at admission and during the first 48 hours to assess severity, using standard clinical and laboratory parameters (18).

ERCP procedures were performed in accordance with the indications outlined by the American Society for Gastrointestinal Endoscopy (ASGE) and the European Society of Gastrointestinal Endoscopy (ESGE) guidelines. Patients referred for ERCP generally presented with signs of biliary obstruction that could not be explained by non-invasive imaging methods and exhibited bile duct dilatation. Additionally, to prevent potential alterations in asprosin levels, only patients without signs of cholangitis or pancreatitis prior to the procedure and who did not require emergency intervention—those scheduled for elective ERCP—were included in the study (19).

All procedures were performed under sedation by experienced gastroenterologists following the standard protocols of the Ankara Bilkent City Hospital Gastroenterology Clinic. Patient-related risk factors such as normal bilirubin levels, history of PEP, diabetes, and smoking were considered exclusion criteria, and patients with these characteristics were not included in the study.

In this study, no pharmacological treatments, except for non-steroidal anti-inflammatory drugs, were administered to prevent the development of PEP. The procedures were performed by experienced operators, and the patients had no history of smoking, with no procedure-related risk factors such as difficult cannulation, pancreatic duct injection, or pancreatic stent placement being applied to any of the patients. Although these procedure-related risk factors are important for PEP development, the aim of our study was to evaluate asprosin levels in patients who developed pancreatitis after ERCP; therefore, separate analyses of these risk factors were not conducted. Given the limited sample size and the inclusion of only patients who developed pancreatitis, the occurrence of pancreatitis itself was considered sufficient for the study regardless of the presence or absence of these interventions.

In this study, the focus was specifically on cases of PEP rather than general acute pancreatitis cases. The primary reason for this was that PEP represents an iatrogenic form of pancreatitis with a defined timing. This approach allowed for the exclusion of pre-existing inflammatory processes and minimized the influence of confounding factors that could affect asprosin levels . This controlled setting enabled a more isolated and reliable evaluation of the role of asprosin, a novel potential marker, in the pathophysiology of acute pancreatitis.

Serum asprosin levels were measured using a sandwich-type enzyme-linked immunosorbent assay (ELISA). Measurements were performed with the Human Asprosin ELISA Kit (Cat. No: EH4176) from FineTest® (Wuhan Fine Biotech Co., Ltd., China). According to the manufacturer, the kit's analytical performance characteristics include a detection range of 1.563–100 ng/mL and a sensitivity of 0.938 ng/mL. All measurements were conducted exclusively on serum samples following the manufacturer's protocol.

In the PEP group, serum samples were collected 4 hours after the procedure, following an 8-hour fasting period prior to ERCP. In the control group, serum samples were obtained at the end of a 12-hour fasting period. Serum samples and other blood tests from both groups were analyzed simultaneously at the Ankara Bilkent City Hospital Central Biochemistry Laboratory after a total fasting duration of 12 hours.

The reason for collecting blood samples 4 hours post-ERCP is that potential marker related to pancreatic injury—particularly pancreatic enzymes such as amylase and lipase—begin to rise rapidly during this timeframe, becoming clinically significant for evaluation. Therefore, obtaining serum samples at 4 hours post-procedure allowed monitoring of the early pancreatic response and provided an appropriate and methodologically justified time point for assessing the early-phase dynamics of potential novel potential marker like asprosin. Due to the unpredictable nature and low incidence of PEP, pre-procedure serum asprosin levels were not measured in this group (20).

The control group consisted of healthy individuals over the age of 18 with no prior history of ERCP,

Table 1: Demographics of control and post-ERCP pancreatitis groups

	Control group (n=21)	Post Ercp pancreatitis group (n=25)	p
Age (years), mean ±SD	53.2 ±23.1	61 ±19.8	0.223
Female gender, n (%)	12 (57.1%)	15 (60%)	0.845

Continuous variables are presented as mean ± standard deviation (SD) and compared using the independent samples t-test. Categorical variables are expressed as number (percentage) and compared using the Chi-square test. A p-value<0.05 was considered statistically significant.

pancreatitis, gastrointestinal interventions, or abdominal surgery. Additionally, participants had no systemic diseases and exhibited routine laboratory values within normal limits. This selection was made to minimize potential confounding factors that could arise from procedure-related or inflammatory variables.

Exclusion criteria included the presence of chronic kidney disease, known coronary artery disease, diabetes mellitus, alcohol dependence, hyperlipidemia, sepsis, hypertension, malignancy, acute cholangitis, smoking, acute pancreatitis due to causes other than ERCP, acute cholecystitis, pre-procedural infections, acute renal failure, chronic pancreatitis, pancreatic duct cannulation or stent placement, age under 18, and failure to provide written informed consent.

Statistical Analysis

The statistical analysis was performed using SPSS 26.0 (IBM Corp., Armonk, NY, USA). Categorical data were summarized as frequencies and percentages, and the chi-square test was used to compare differences between groups. To verify the normality of continuous variables, both histograms and the Shapiro-Wilk test were utilized. Normally distributed data were expressed as means with standard deviation (SD), and the Student's t-test was employed for group comparisons. For variables not following a normal distribution, medians with the interquartile range (25th and 75th percentiles) were reported, and the Mann-Whitney U test was used to assess differences between groups.

The relationship between asprosin levels and other laboratory and clinical parameters was assessed using Spearman's rank correlation coefficient. For normally distributed data, Pearson's correlation was used for correlation analysis. Correlation analyses were conducted to evaluate the relationships between asprosin levels and parameters such as amylase, lipase, bilirubin, i, and other relevant clinical and laboratory parameters.

The severity of PEP was categorized into mild and moderate-severe groups, and the asprosin levels between these groups were compared using the Mann-Whitney U test. The significance level for all statistical tests was set at $p<0.05$. The required sample size was estimated using G*Power 3.1 software for a two-tailed independent samples t-test, based on an assumed effect size due to the absence of prior similar studies. At a significance level of $\alpha = 0.05$, and following Cohen's classification of effect sizes, the comparison between patients with PEP (n = 25) and healthy controls (n = 21) yielded an estimated statistical power of approximately 80% for detecting a large effect (Cohen's d = 0.88).

In subgroup analyses, PEP patients were categorized as having mild (n = 13) or moderate-to-severe (n = 12) disease. The observed difference in serum asprosin levels between these subgroups corresponded to a large effect size (Cohen's $d \approx 1.38$). A post-hoc power analysis indicated that, with the available sample sizes, the achieved power was approximately 91%, exceeding the commonly accepted threshold of 80%. Therefore, the study is adequately powered to detect large effects in both the overall and subgroup comparisons.

However, it should be noted that the ability to detect medium or small effect sizes is limited, and this

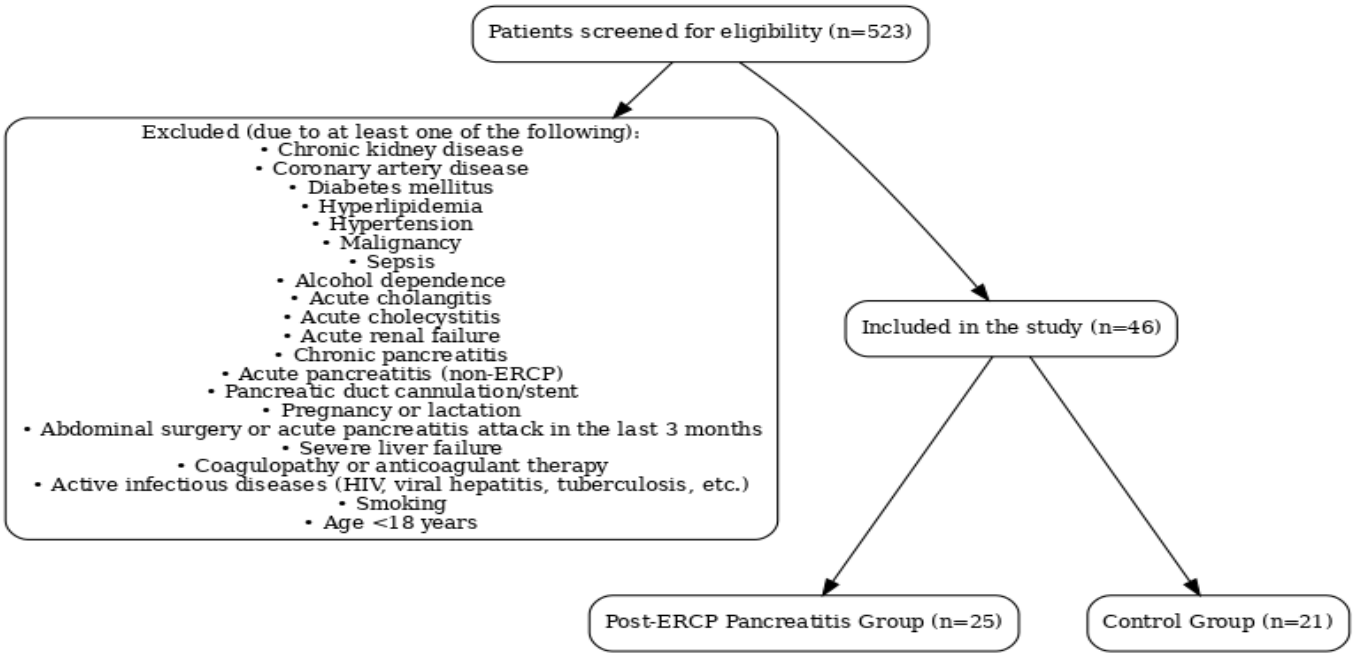

Figure 1. Flowchart of the study.

limitation has been clearly acknowledged in the discussion of study limitations.

Results

Table 1 shows demographics. Mean age was 53.2 in controls and 61 in PEP, with no significant difference (p=0.223). Female ratio was similar (57.1% vs. 60%, p=0.845).

Table 2 shows labs. Urea, creatinine, albumin were similar. AST was higher in PEP (p=0.029). ALT and ALP increased but not significantly. GGT was higher (p=0.008). LDH showed no difference.

As expected, amylase and lipase levels, key markers of pancreatitis, were markedly elevated in the PEP group, with statistically significant differences observed for both parameters (p<0.001). Both total and direct bilirubin levels were higher in the pancreatitis group (p=0.007 and p=0.004, respectively), which may be associated with biliary obstruction. While procalcitonin levels were higher in the pancreatitis group, the difference was not found to be statistically significant (p=0.317).

WBC and neutrophils were higher in PEP (p=0.016, p=0.001), lymphocytes lower (p=0.018). Hemoglobin and platelets were similar. CRP was higher but not significant. Asprosin was markedly higher in PEP (p<0.001).

Serum asprosin levels were higher in the PEP group compared to the control group (Figure 2), supporting the role of asprosin as a biochemical potential marker of beta cell damage in the context of pancreatic inflammation.

Asprosin was higher in PEP (p<0.001). No correlations were found in controls. In PEP, asprosin correlated negatively with leukocytes (r=-0.433) and lymphocytes (r=-0.44). No associations with other labs or severity scores (p>0.05) (Table 3).

Asprosin levels were slightly higher in mild vs. moderate-severe cases but not significant (p=0.347) (Figure 3).

Although asprosin levels were higher in the mild pancreatitis group, this difference was not statistically significant (p=0.347). Sample sizes for each group are indicated above the boxes.

To further illustrate the relationship between serum asprosin levels and immune cell counts, scatter plots were generated for leukocyte and lymphocyte counts in the PEP group. As shown in Figure 4A, an inverse linear trend was observed between asprosin levels and leukocyte count (r=–0.433; p=0.031), suggesting that higher asprosin levels are associated with a lower leukocyte/lymphocyte count; causality cannot be inferred. Similarly, as depicted in Fig-

Table 2: Comparison of Laboratory Parameters Between Control and Post-ERCP Pancreatitis Groups

Parameter	Control group (n=21)	Post ERCP pancreatitis group (n=25)	p
Body mass index (kg/m^2) (mean ± SD)	26.4 ± 3.2	26.8 ± 3.5	0.721
Fasting glucose (mg/dL) (median/IQR)	95 (88–104)	98 (90–110)	0.412
Total cholesterol (mg/dL) (mean ± SD)	188 ± 32	192 ± 35	0.683
LDL cholesterol (mg/dL) (mean ± SD)	112 ± 26	115 ± 28	0.644
HDL cholesterol (mg/dL) (median/IQR)	48 (42–55)	47 (41–54)	0.587
Triglycerides (mg/dL) (median/IQR)	135 (110–155)	140 (115–160)	0.497
Urea (mg/dL) (median/IQR)	33 (24-45)	28 (21-43)	0.279
Creatinine (mg/dL) (median/IQR)	0.76 (0.66-1)	0.75 (0.61-1.06)	0.627
Albumin (g/L) (median/IQR)	38 (32-39)	38 (35-39)	0.626
Aspartate Aminotransferase (U/L) (median/IQR)	19 (13-31)	32 (18-89)	**0.029**
Alanine Aminotransferase (U/L) (median/IQR)	23 (19-35)	28 (23-138)	0.122
Alkaline phosphatase (U/L) (median/IQR)	93 (74-109)	118 (74-181)	0.182
Gamma glutamyl transferase (U/L) (median/IQR)	26 (16-81)	104 (36-276)	**0.008**
Lactate dehydrogenase (U/L) (mean ± SD)	237,8 ± 62.3	231.5 ±54.9	0.718
Amylase (U/L) (median/IQR)	72 (55-93)	706 (361-1115)	**<0.001**
Lipase (U/L) (median/IQR)	43 (30-64)	837 (348-1420)	**<0.001**
Total bilirubin (mg/dL) (median/IQR)	0.5 (0.3-0.7)	1 (0.5-1.7)	**0.007**
Direct bilirubin (mg/dL) (median/IQR)	0.1 (0.1-0.2)	0.5 (0.2-1)	**0.004**
Procalcitonin (µg/L) (median/IQR)	0.04 (0.03-0.06)	0.08 (0.03-0.2)	0.317
Leukocyte (x10^9/L) (median/IQR)	6.67 (5.83-8.89)	10.37 (6.78-12.31)	**0.016**
Neutrophil (x10^9/L) (median/IQR)	4.41 (3.78-5.32)	8.5 (5.08-9.93)	**0.001**
Lymphocyte (x10^9/L) (median/IQR)	1.75 (1.13-2.18)	1.16 (0.83-1.43)	**0.018**
Hemoglobin (g/dL) (mean ± SD)	11.8 ±2.4	12.8 ±2	0.153
Platelet (x10^9/L) (median/IQR)	265 (196-310)	216 (188-285)	0.440
CRP (mg/dL) (median/IQR)	8 (4-16)	11 (5-28)	0.559
Asprosin (ng/mL) (median/IQR)	1.93 (1.6-2.34)	3.3 (2.78-3.84)	**<0.001**

Data are presented as median (interquartile range) for non-normally distributed variables and as mean ± standard deviation for normally distributed variables. Comparisons between groups were performed using the Mann–Whitney U test or independent samples t-test, as appropriate. A p-value<0.05 was considered statistically significant.

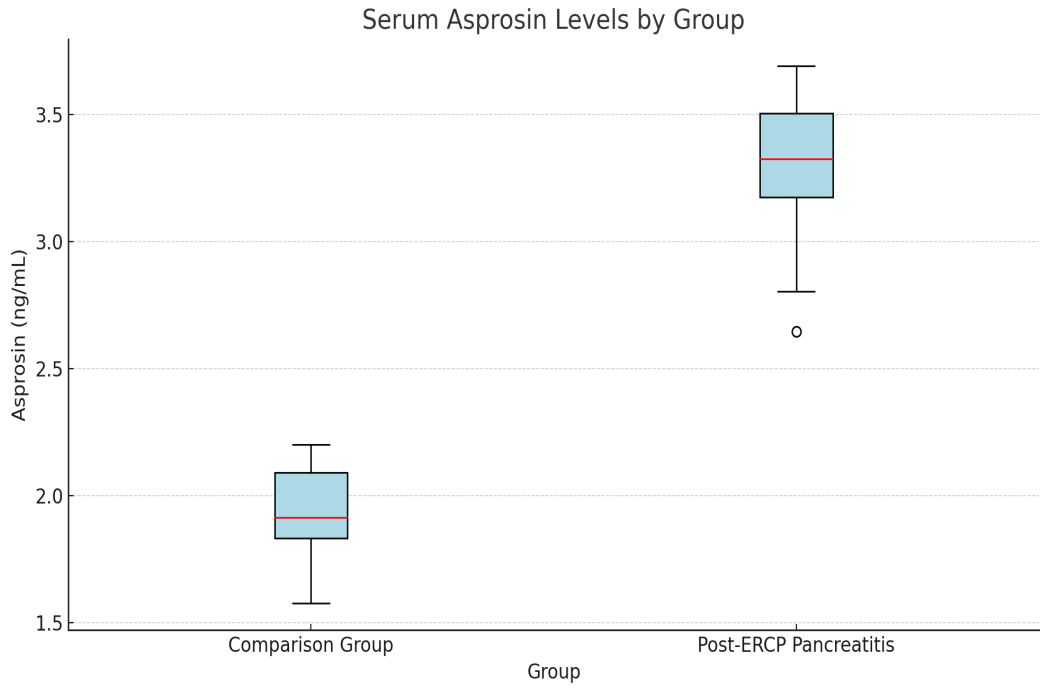

Figure 2. Box plot showing serum asprosin levels in the post-ERCP pancreatitis group (n=25) and the control group (n=21). The horizontal line within each box represents the median, the lower and upper edges represent the interquartile range (IQR), whiskers denote the minimum and maximum values within 1.5×IQR, and circles indicate outliers. Statistical comparison was performed using the Mann–Whitney U test.the interquartile range (IQR), whiskers denote the minimum and maximum values within 1.5×IQR, and circles indicate outliers. Statistical comparison was performed using the Mann–Whitney U test. **(p<0.001)**

ure 4B, a strong inverse relationship was observed between asprosin concentrations and lymphocyte count (r=–0.440; p=0.028), potentially reflecting an immunosuppressive effect or redistribution of lymphocytes in the inflammatory response. These visualizations reinforce the statistical findings and highlight a potential link between increased asprosin levels and systemic inflammation in PEP.

A significant inverse correlation was found between serum asprosin concentrations and leukocyte count (Spearman ρ = –0.521, p = 0.003). This finding may suggest that higher asprosin levels are associated with a lower systemic inflammatory response or reduced lymphocyte-mediated immune activity. However, as these data are based solely on correlation, no causal relationship can be inferred, and the potential influence of confounding factors should be considered in the interpretation of these results. A statistically significant negative correlation was observed between serum asprosin levels and lymphocyte count (Spearman's ρ=–0.553, p=0.002), indicating that increased asprosin concentrations might be linked to decreased lymphocyte-mediated immune activity.

Discussion

Asprosin is a peptide secreted from adipocytes that plays an important role in glucose metabolism and affects insulin secretion [21]. This study's results indicate an increase in asprosin levels in patients with PEP. This finding may be an indication that beta cell damage occurs during the course of pancreatitis. However, we found no relationship between the asprosin level and the Ranson and BISAP scores, which determine the severity of pancreatitis. This suggests that asprosin levels may not directly reflect the severity of pancreatitis but may indicate beta cell damage. However, since beta cell function could not be directly measured in patients with pancreatitis, the elevation in asprosin was interpreted as an indirect

Table 3: Correlations of asprosin levels with other parameters in control and post-ERCP pancreatitis groups

Parameter	Control group (n=21)		Acute pancreatitis group (n=25)	
	r	p	r	p
Body mass index (kg/m^2)	0.045	0.82	–0.061	0.77
Fasting glucose (mg/dL)	0.102	0.65	–0.085	0.69
Total cholesterol (mg/dL)	0.073	0.74	–0.042	0.81
LDL cholesterol (mg/dL)	0.088	0.70	–0.052	0.78
HDL cholesterol (mg/dL)	–0.066	0.76	0.071	0.72
Triglycerides (mg/dL)	0.089	0.70	–0.058	0.75
Age	0.312	0.156	0.039	0.852
Urea (mg/dL)	0.381	0.088	0.006	0.977
Creatinine (mg/dL)	0.305	0.179	-0.134	0.522
Aspartate Aminotransferase (U/L)	0.191	0.408	0.123	0.559
Alanine Aminotransferase (U/L)	0.091	0.695	0.076	0.719
Alkaline phosphatase (U/L)	-0.07	0.763	0.245	0.238
Gamma glutamyl transferase (U/L)	0.361	0.108	0.112	0.594
Lactate dehydrogenase (U/L)	0.165	0.475	-0.311	0.13
Amylase (U/L)	0.119	0.617	-0.162	0.44
Lipase (U/L)	-0.002	0.993	-0.035	0.869
Total bilirubin (mg/dL)	-0.079	0.734	-0.046	0.828
Direct bilirubin (mg/dL)	0.186	0.42	0.117	0.577
Procalcitonin (µg/L)	-0.009	0.969	-0.224	0.281
Leukocyte (x10^9/L)	-0.121	0.602	-0.433	**0.031**
Neutrophil (x10^9/L)	-0.044	0.849	-0.336	0.1
Lymphocyte (x10^9/L)	-0.283	0.215	-0.44	**0.028**
Hemoglobin (g/dL)	-0.395	0.076	0.008	0.97
Platelet (x10^9/L)	-0.236	0.303	-0.119	0.57
CRP (mg/dL)	-0.004	0.985	-0.163	0.436
Ranson's criteria	-	-	-0.059	0.779
BISAP score	-	-	-0.073	0.729

Spearman correlation analysis was used to evaluate the relationship between serum asprosin levels and clinical/laboratory parameters. Correlation coefficients (r) and corresponding p-values are presented for each group. A p-value<0.05 was considered statistically significant and is highlighted in bold.

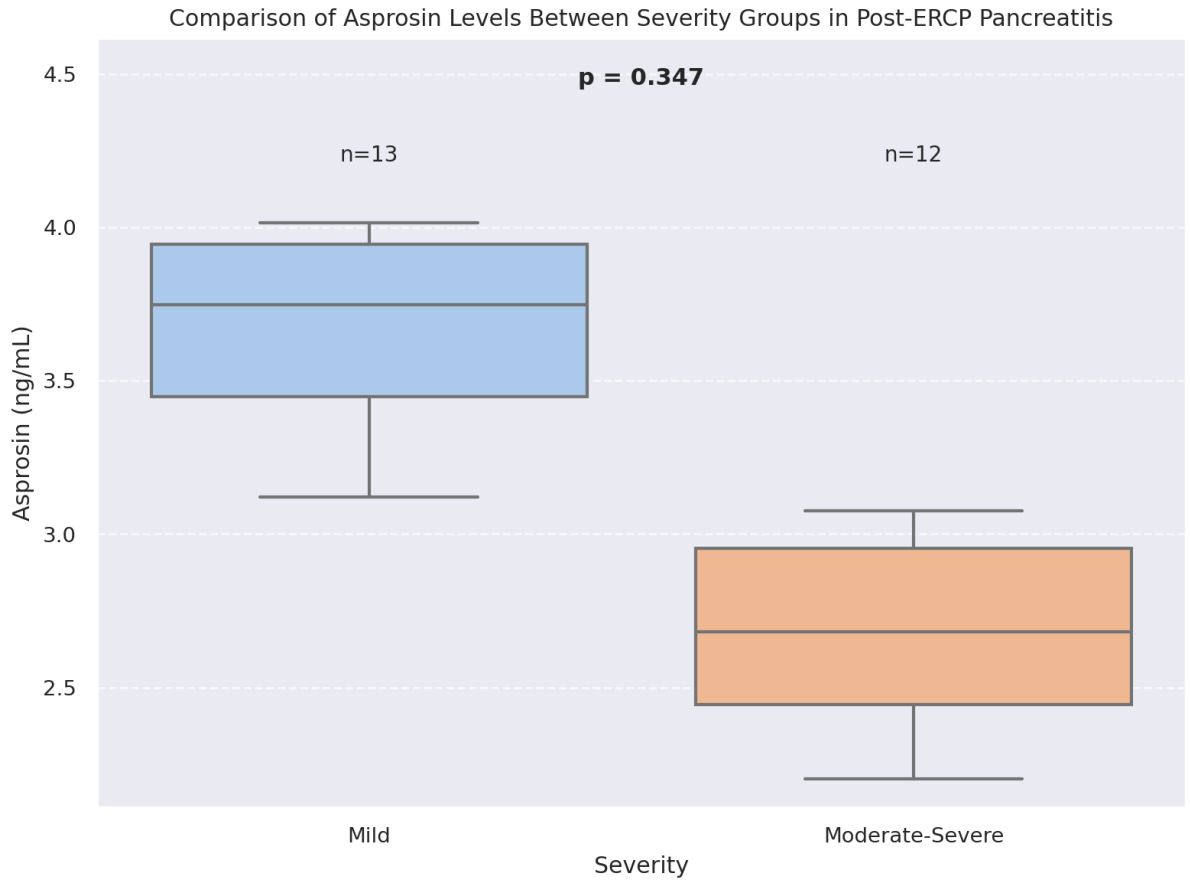

Figure 3. Comparison of serum asprosin levels between mild and moderate–severe post-ERCP pancreatitis groups. The horizontal line within the box represents the median value, the lower and upper edges of the box indicate the interquartile range (IQR). Whiskers represent the minimum and maximum values within 1.5×IQR, and circles denote outliers. Group sizes (n) are indicated above each box. Statistical comparison was performed using the Mann–Whitney U test.

marker of beta cell damage. Causality cannot be established in this study; thus, mechanistic investigations are warranted.

PEP is iatrogenic and usually resolves quickly, with rare complications. The effect on beta cells is unclear. Demonstrating beta cell injury would add to the literature. Lee et al. showed asprosin with palmitate increased cytokines, reduced insulin release, and harmed beta cells. Other studies found that reducing beta cell inflammation improved insulin secretion and glycemia. [9,22]. Earlier research has demonstrated that asprosin stimulates glucose production in the liver through the activation of cyclic AMP (cAMP) as a secondary messenger, a molecule that is also involved in regulating inflammatory processes [23–25]. Pancreatitis is a condition characterized by inflammation of the pancreas, which can disrupt the function of beta cells [26]. In our study, we found that asprosin levels increased in PEP patients. This suggests that asprosin levels increase in response to inflammatory processes of the pancreas. We found a link between PEP and asprosin, which is a sign of beta cell damage. We believe that measuring asprosin levels will help us predict the onset of diseases related to pancreatic beta cell dysfunction in the future.

The correlation analysis revealed a relationship between serum asprosin levels and amylase, lipase, systemic inflammatory index, AST, lymphocytes, and neutrophils across the entire population. We did not find a relationship between asprosin levels and pancreatitis severity scores. This suggests a potential relationship between asprosin levels and beta cell damage, rather than the severity of pancreatitis. The literature has demonstrated a correlation between oxidative stress and asprosin levels [27].

Several studies have shown a notable rise in the expression and secretion of pro-inflammatory cy-

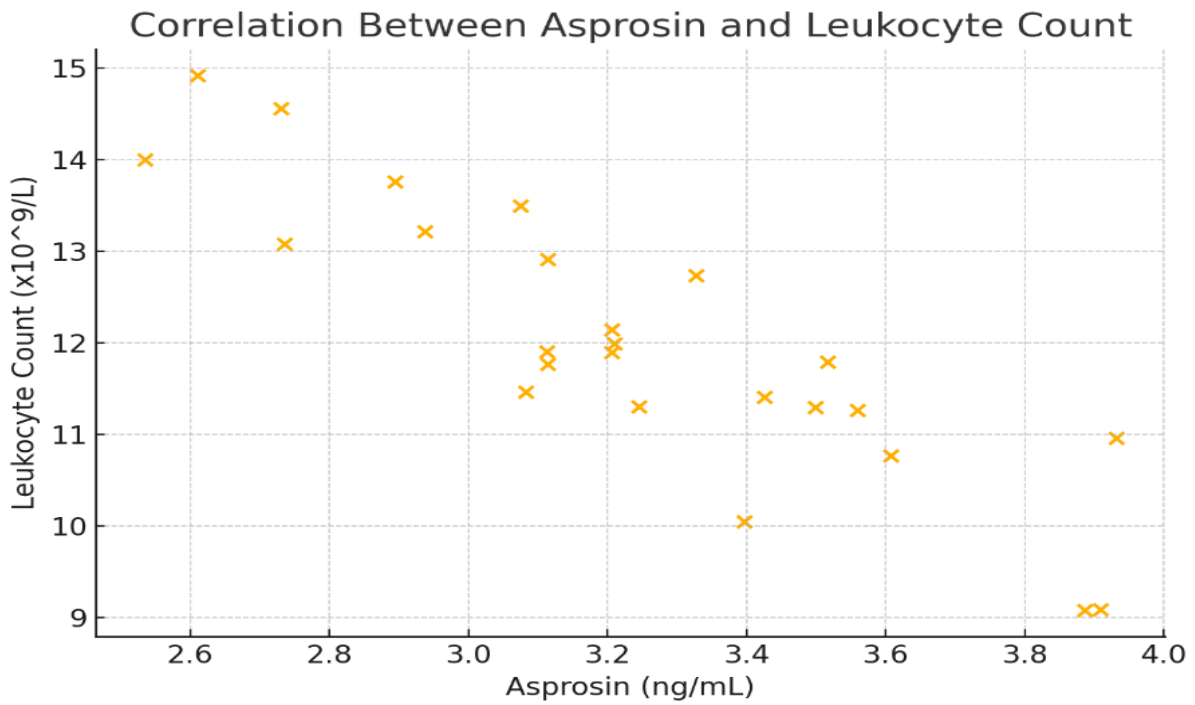

Figure 4a. Scatter plot showing the correlation between serum asprosin levels (ng/mL) and leukocyte count ($\times 10^9$/L) in patients with post-ERCP pancreatitis. Each dot represents an individual patient. A negative trend was observed between asprosin concentration and leukocyte count.

tokines upon asprosin stimulation, and our study established a relationship between the systemic inflammatory index and asprosin levels [28, 29]. In our study, we did not find a relationship between age and asprosin levels, similar to the study by Wang et al. [30].

Previous studies have shown that asprosin not only blocks the insulin signaling pathway but also stimulates the increase of triglyceride and total cholesterol concentrations in peripheral blood. Studies have also demonstrated its potential to suppress insulin secretion from pancreatic beta cells and disturb the balance of blood glucose. Studies have shown that injecting an asprosin antibody into mice improves insulin sensitivity [31]. Even though we don't fully understand how asprosin affects the beta cell, some studies have shown that it causes apoptosis by stopping the beta cell's autophagy through the AMPK-mTOR pathway [8]. Furthermore, studies have demonstrated an increase in asprosin levels in patients' Chronic renal disease, diabetic nephropathy, and terminal renal failure [32]. We excluded these patients from our study to avoid any limitations. Previous studies have found a relationship between asprosin levels and DM in patients diagnosed with the disease for more than 10 years [31]. In contrast to that study, our study included participants without a diagnosis of DM in both groups. In this way, the likelihood of asprosin levels being influenced by other conditions was minimized.

Since the c peptide level varies according to the fasting and satiety status and these patients had to fast for at least 8 hours before the procedure and this period varies from patient to patient, c peptide was not measured [33].

In our study, serum asprosin levels were found to be higher in patients with mild post- PEP compared to those with severe forms of the condition. This finding suggests that asprosin may play a compensatory role during the early or less intense phases of inflammation. Asprosin is an adipokine primarily secreted during fasting that contributes to glucose homeostasis; however, it has also been shown to participate in immune and inflammatory responses. It has been reported to enhance the secretion of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-8. In mild

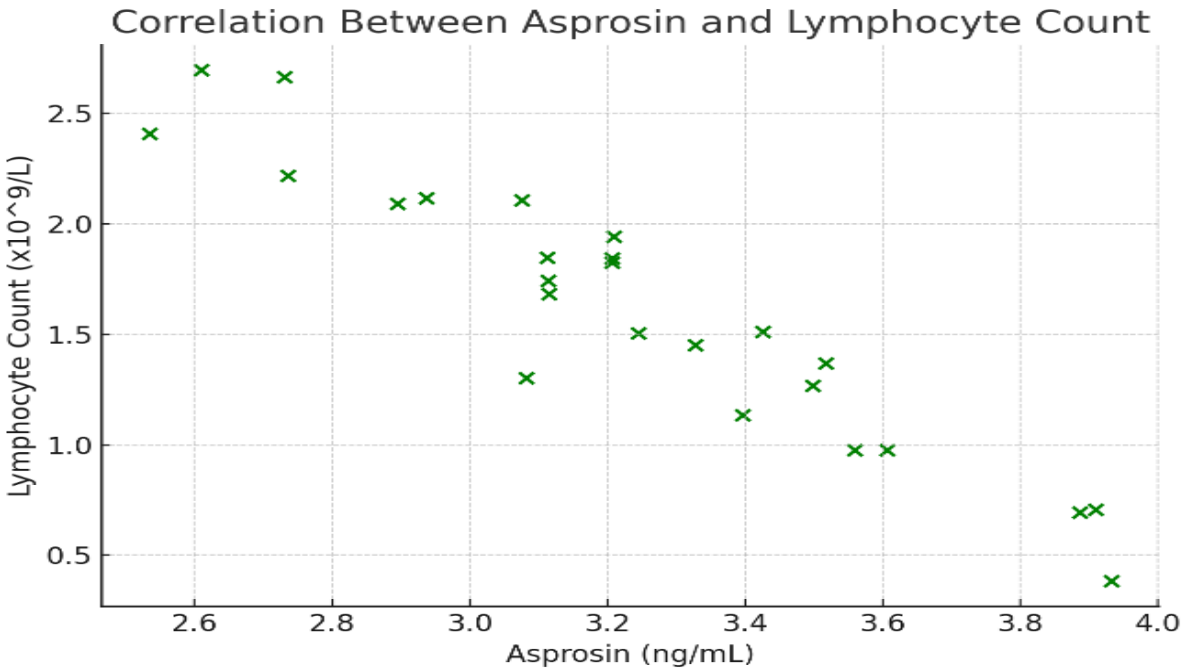

Figure 4b. Scatter plot showing the correlation between serum asprosin levels (ng/mL) and lymphocyte count (×10^9/L) in patients with post-ERCP pancreatitis. Each dot represents an individual patient. A negative correlation between asprosin concentration and lymphocyte count is observed.

PEP cases, the systemic inflammatory response may remain limited, allowing for the continued or even increased secretion of asprosin. In contrast, severe PEP is typically associated with widespread tissue damage, organ dysfunction, and metabolic disturbances, all of which may negatively affect asprosin production or its levels in circulation (34, 35).

One of the limitations of our study is the relatively small sample size, which may restrict the generalizability of the findings. Additionally, the lack of pre-ERCP asprosin measurements represents another significant limitation. Future prospective cohort studies involving serial measurements before and after ERCP would provide more robust and reliable data. Due to the cross-sectional design of our study, it is not possible to establish a causal relationship. Further molecular and experimental research is required to determine whether asprosin plays an active role in pancreatic beta-cell dysfunction.

Since PEP is an unpredictable complication, we did not measure asprosin levels prior to ERCP. However, the absence of baseline measurements limited our ability to assess intra-individual changes and interpret dynamic variations in asprosin levels over time.

Our study also lacked a control group consisting of individuals who underwent ERCP but did not develop pancreatitis. This limits our ability to determine whether the observed changes in asprosin levels are solely attributable to pancreatitis or potentially influenced by the ERCP procedure itself. However, to date, there is no evidence in the literature suggesting that ERCP, in the absence of secondary complications , directly alters serum asprosin levels. Therefore, based on the current body of evidence, it is more plausible that the observed increase in asprosin is associated with the pathophysiology of pancreatitis. Nonetheless, our findings should be interpreted in light of this limitation.

Conclusion

Serum asprosin levels were higher in PEP cases compared to the control group. Asprosin may represent a potential biomarker associated with pancreatitis-induced beta cell stress rather than clinical severity. To better evaluate this relationship, prospective studies incorporating serial pre/post-ERCP sampling and

direct assessments of β-cell function are needed. These findings may contribute to a better understanding of the complex interactions between pancreatitis and beta cell injury and may guide future research efforts.

List of Abbreviations

ALT – Alanine Aminotransferase
ALP – Alkaline Phosphatase
AST – Aspartate Aminotransferase
BISAP – Bedside Index for Severity in Acute Pancreatitis
BUN – Blood Urea Nitrogen
CRP – C-Reactive Protein
CT – Computed Tomography
DM – Diabetes Mellitus
ELISA – Enzyme-Linked Immunosorbent Assay
ERCP – Endoscopic Retrograde Cholangiopancreatography
GGT – Gamma-Glutamyl Transferase
GCS – Glasgow Coma Scale
LDH – Lactate Dehydrogenase
MRI – Magnetic Resonance Imaging
NSAID – Non-Steroidal Anti-Inflammatory Drug
PEP – Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis
RRT – Renal Replacement Therapy
SD – Standard Deviation
SIRS – Systemic Inflammatory Response Syndrome
TLR4 – Toll-like Receptor 4
ULR – Upper Limit of Reference

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Informed Consent: Written informed consent was obtained from all participants.

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Review / Derleme

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Decoding Autism: The Role of Synaptic Dysfunction in Neurodevelopment

Otizmin Şifresini Çözmek: Nörogelişimde Sinaptik Disfonksiyonun Rolü

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ABSTRACT

The human brain is comprised of intricate neuronal networks that comprise approximately 100 trillion synapses, which underpin functional connectivity. Disruptions in these synaptic connections play a fundamental role in several neurological disorders, such as autism spectrum disorder (ASD). During early development, genes related to ASD, coupled with environmental stressors, effect brain development and neural circuit formation. Genes associated with the structure and functioning of synapses demonstrate a significant prevalence of mutations within individuals diagnosed with ASD. In addition, environmental factors and mitochondrial dysfunction affecting synaptic metabolism also contribute to the pathophysiology of ASD. Understanding the link between synaptic dysfunction and autism symptoms may provide new perspectives for therapeutic approaches. The purpose of the present analysis is to furnish the reader with an overview of the findings pertaining to synaptic dysfunction in ASD.

Keywords: Autism spectrum disorder, Synaptic transmission, Synapse

ÖZET

İnsan beyni, yaklaşık 100 trilyon sinaps içeren karmaşık nöronal ağlardan oluşmakta ve bu yapılar, işlevsel bağlantısallığın temelini oluşturmaktadır. Bu sinaptik bağlantılardaki bozulmalar, otizm spektrum bozukluğu (OSB) da dahil olmak üzere çeşitli nörolojik hastalıklarda kritik bir rol oynamaktadır. Erken gelişim sürecinde, OSB ile ilişkili genler çevresel stres faktörleri ile birlikte beyin gelişimini ve nöral devre oluşumunu etkilemektedir. Sinaptik yapı ve işlevle ilişkili genler, OSB'de belirgin şekilde mutasyona uğramaktadır. Ayrıca, sinaptik metabolizmayı etkileyen çevresel faktörler ve mitokondriyal işlev bozukluğu da OSB'nin patofizyolojisine katkıda bulunmaktadır. Sinaptik disfonksiyon ile otizm semptomları arasındaki ilişkinin anlaşılması, terapötik yaklaşımlar açısından yeni bakış açıları sunabilir. Bu derleme, OSB'de sinaptik disfonksiyon ile ilgili bulguların genel bir değerlendirmesini sunmayı amaçlamaktadır.

Anahtar Kelimeler: Otizm spektrum bozukluğu, Sinaptik iletim, Sinaps

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that affects early intellectual development. It is characterized by impaired motor coordination, deficits in social communication, and repetitive, restricted behaviors. ASD was originally categorized into multiple diagnoses, including Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder-not otherwise specified, but these are now unified under the broader ASD classification (1).

The etiology of ASD involves a heterogeneous mix of genetic and environmental factors. Genome-wide association studies have been undertaken to identify rare variants, single nucleotide polymorphisms (SNPs), and de novo copy number variations (CNVs) contributing to ASD risk. These have led to the discovery of many ASD-associated genes, now included in major genomic databases (2).

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Recent findings suggest these genetic alterations converge on core neurodevelopmental pathways (3). Neurobiological studies show dysregulation in gene expression—via epigenetics and transcription—alongside disrupted synaptic communication and neurotransmission. Many ASD-related genes are expressed during critical stages of brain development, from prenatal to early postnatal periods (4), and their protein products are regulated by neural activity via transcription, splicing, translation, and degradation (5).

At the structural level, synapses represent the fundamental communication units between neurons, composed of a presynaptic terminal that releases neurotransmitters, a synaptic cleft, and a postsynaptic membrane equipped with specialized receptors (6). Genes such as *NRXN, NLGN, SHANK3,* and *DLG4*—frequently implicated in ASD—play essential roles in synapse formation, stabilization, and signal transduction (7). Animal and genetic studies consistently demonstrate that mutations in these and other ASD-related genes, including *FMR1, MECP2, UBE3A,* and *TSC1/2,* lead to disruptions in synaptic integrity and circuit-level connectivity, ultimately contributing to deficits in social interaction, sensory processing, and cognitive function (8-11). Together, these findings highlight synaptic dysfunction as a central mechanism in ASD pathophysiology (12).

The following sections examine core molecular mechanisms underlying synaptic dysfunction in ASD, with a particular focus on synaptic cell adhesion molecules, postsynaptic scaffold and signaling proteins, mitochondrial impairment, and the resulting excitation/inhibition (E/I) imbalance. While additional contributors—such as epigenetic modulation, impaired synaptic pruning, and environmental factors—are also implicated in ASD pathophysiology, they are only briefly addressed or remain beyond the scope of this review.

Synaptic Cell Adhesion Molecules

Synaptic cell adhesion molecules (CAMs) play a central role in establishing and maintaining synaptic connectivity. By spanning the synaptic cleft, these molecules mediate structural alignment between pre- and postsynaptic neurons and modulate key processes such as synaptogenesis, synaptic stabilization, and plasticity mechanisms including long-term potentiation (LTP) and long-term depression (LTD) (13). Among the CAMs, neurexins (NRXNs), neuroligins (NLGNs), and Contactin-Associated Protein-Like 2 (CASPR2) have been extensively linked to ASD through both genetic and functional studies.

Neurexins

NRXNs are presynaptic adhesion molecules that interact with postsynaptic neuroligins (NLGNs) to form trans-synaptic complexes essential for synaptic differentiation and communication. The three NRXN genes—*NRXN1, NRXN2,* and *NRXN3*—each produce multiple alpha and beta isoforms that regulate synapse specificity, plasticity, and neurotransmitter release (14).

Disruptions in NRXNs, particularly *NRXN1*, are among the most frequently reported mutations in ASD. Both gene deletions and duplications impair synaptic transmission, disturb E/I balance, and lead to altered neural circuitry associated with social deficits and repetitive behaviors (15).

Studies on *Nrxn1* knockout (KO) mice show impairments in synaptic organization and cognitive functions, underscoring its role in neurodevelopment (16). Given their central function in synaptic communication, *NRXNs* remain a major focus in ASD research, particularly with regard to understanding how their dysfunction impacts neural circuit formation and cognitive processes (17). The investigation of these molecules may offer valuable insights that could inform the development of therapeutic strategies aimed at restoring synaptic balance in individuals with ASD.

Neuroligins

NLGNs are postsynaptic adhesion molecules that play a crucial role in synapse identity and functional development (14). They interact with presynaptic NRXNs to facilitate synapse formation and stabilization. NLGNs exist in multiple isoforms, each with distinct localization and functional properties. Specifically, NLGN1 is primarily found in glutamatergic excitatory synapses, while NLGN2 is localized to γ-aminobutyric acid-ergic (GABAergic) inhibitory synapses, playing a crucial role in maintaining E/I balance (18).

Functional studies demonstrate that *NLGN1* overexpression increases excitatory signaling and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) to NMDA (N-methyl-D-aspartate) receptor ratios, while *NLGN2* overexpression strengthens inhibitory responses (19, 20). Notably, *Nlgn1* KO mice exhibit impaired hippocampal LTP, reduced dendritic spine density, and increased repetitive grooming behavior—core features of ASD. The phenotypes observed in these models, including spatial memory deficits and disrupted corticostriatal signaling, support a critical role for NLGNs in synapse morphology and ASD-like behavior (19).

Contactin-Associated Protein-Like 2

CASPR2, encoded by the *CNTNAP2* gene, is a member of the NRXN family. CASPR2 was initially identified as a synaptic protein involved in synapse formation and maintenance, but recent research has expanded its functional relevance to neurodevelopmental processes. CASPR2 plays significant roles in dendritic arborization, dendritic spine stabilization, and AMPAR trafficking, highlighting its importance in neuronal connectivity and signaling (21, 22).

The presence of recessive mutations and chromosomal inversions in the *CNTNAP2* gene has been identified in individuals diagnosed with both intellectual disabilities and ASD. *CNTNAP2,* alongside genes such as *KMT2C* and *SHANK3,* has been associated with ASD, speech, and language impairments. These mutations are known to disrupt critical protein domains involved in language-related neural functions (23, 24).

Cntnap2 KO mice exhibit ASD-like behaviors including repetitive actions, restricted social interactions, and impaired communication (21). These models also show disrupted cortical inhibitory interneuron migration, further supporting *CNTNAP2*'s contribution to the neural mechanisms underlying ASD (22). Additionally, CNTNAP2 dysfunction is associated with altered sensory processing, likely due to impaired Kv1 potassium channel function, which contributes to neuronal hyperexcitability and pain hypersensitivity (25).

SNP rs2710102 in the *CNTNAP2* gene has been associated with language development deficits in ASD. Individuals carrying the A-allele tend to exhibit lower language skills compared to G-allele homozygotes, despite no significant difference in overall communication abilities. Moreover, studies in *Cntnap2*-deficient mice showed that dysfunction of this gene influences affective processing beyond basic sensory and motor domains (26). These findings highlight the gene's broad involvement in both synaptic and circuit-level phenotypes relevant to ASD.

Postsynaptic Density and Scaffold Proteins

The postsynaptic density (PSD) is a protein-dense region located at the postsynaptic membrane of excitatory synapses, essential for regulating synaptic transmission and plasticity (6). This specialized structure hosts neurotransmitter receptors, kinases, structural proteins, and signaling molecules essential for synaptic function (27).

Scaffold proteins, as core components of the postsynaptic density (PSD), organize and stabilize multiprotein complexes at synaptic sites. Through interaction domains such as PDZ (PSD-95/Discs large/ZO-1) and SH3 (Src Homology 3), they orchestrate intracellular signaling cascades and modulate synaptic structure in an activity-dependent manner (28). Disruptions in these proteins lead to synaptic instability and are linked to ASD-related neurodevelopmental deficits (29).

PSD-95

PSD-95, encoded by *DLG4,* is a key scaffold protein at glutamatergic synapses, regulating receptor clustering, dendritic spine morphology, and NMDA receptor-mediated plasticity (27). It plays a central role in trafficking NMDA and AMPA receptors and in maintaining synaptic integrity during maturation (30).

Mutations in *DLG4* have been associated with increased ASD risk, and loss-of-function variants impair synaptic connectivity, leading to social and cognitive dysfunctions (27, 31). Proteomic analyses in individuals with idiopathic ASD indicate downregulation of PSD-95-associated complexes, contrib-

uting to delayed synaptic maturation and cognitive impairment (32).

Animal models revealed that environmental exposures can alter PSD-95 expression, disrupting plasticity and inducing ASD-like behaviors (33). *Dlg4* KO mice exhibit repetitive behaviors and social deficits, while pharmacological upregulation of PSD-95 restores synaptic function and ameliorates behavioral abnormalities in ASD models (34).

HOMER Protein Family

The HOMER family consists of three homologous genes—*HOMER1, HOMER2,* and *HOMER3*—encoding proteins that act as multimodal scaffolds. These proteins interact with type I metabotropic glutamate receptors (mGluR1–5), SH3 and multiple ankyrin repeat domains (SHANK) proteins, and inositol1,4,5-trisphosphate receptors (IP3Rs), anchoring them to cytoskeletal components and supporting synaptic signaling and plasticity. HOMER proteins are essential for cell signaling, neuronal excitability, synaptic neurotransmission, and neural plasticity (35).

Two experimental findings link HOMER dysfunction to ASD: First, *Homer1a* is upregulated in the basolateral amygdala (BLA) of valproic acid (VPA)-exposed rats, a widely used ASD model. Overexpression of *Homer1a* in the BLA of healthy mice induces ASD-like behaviors, such as impaired fear conditioning and reduced social interaction (36). Second, HOMER1A a interacts with SHANK3, and disruption of this interaction affects synaptic plasticity and connectivity, suggesting a mechanistic pathway contributing to ASD phenotypes (37).

SH3 and multiple ankyrin repeat domains protein 3

SHANK3 is a key postsynaptic scaffold protein highly enriched in the postsynaptic density (PSD) of excitatory synapses. It plays a central role in synaptic organization by linking neurotransmitter receptors, such as NMDA and AMPA receptors, to intracellular signaling complexes and the actin cytoskeleton. SHANK3 directly interacts with PSD-95, HOMER, and other Membrane-Associated Guanylate Kinase (MAGUK) proteins, thereby contributing to synaptic strength, plasticity, and spine morphology (28).

Genetic studies have identified *SHANK3* mutations and deletions as one of the most consistent and penetrant monogenic causes of ASD (38). Animal models with *Shank3* deficiency exhibit core ASD-like phenotypes, including impaired social behaviors, repetitive actions, and disrupted synaptic transmission (39). Given its structural and functional significance, SHANK3 represents a critical node in the molecular architecture of ASD-related synaptic dysfunction.

The Discs Large Protein Subfamily

DLG proteins, including DLG1, DLG2, DLG3, and DLG4, are members of the MAGUK family and regulate glutamatergic receptor positioning and postsynaptic signaling. These proteins contain three PDZ domains and serve as scaffolds for NMDA and AMPA receptor complexes, and link extracellular stimuli to postsynaptic signaling pathways (40, 41).

In addition to postsynaptic roles, DLG proteins influence ion channel trafficking, presynaptic excitability, and neurotransmitter release (42). Their differential expression across development suggests stage-specific functions (30). Genetic variants in the *DLG1* gene and 3q29 microdeletions have been associated with ASD and schizophrenia (43). In mice, *Dlg2* (PSD-93) deficiency results in reduced corticostriatal synaptic transmission and ASD-like behaviors, including social withdrawal and cognitive inflexibility (44).

Figure 1 highlights the NRXN–NLGN trans-synaptic complex, postsynaptic scaffold proteins (PSD-95, SHANK, HOMER), and their roles in anchoring AMPA and NMDA receptors at excitatory synapses.

Synaptic Signaling and Protein Synthesis Regulators

Synaptic signaling and protein synthesis are critical for neurodevelopment and cognitive function. In ASD, disruptions in these processes contribute to abnormal plasticity, impaired neuronal communication, and altered protein regulation. Key molecular regulators of these pathways include Fragile X Mental Retardation Protein (FMRP), Tuberous Sclerosis Complex (TSC), Phosphatase and Tensin Homolog

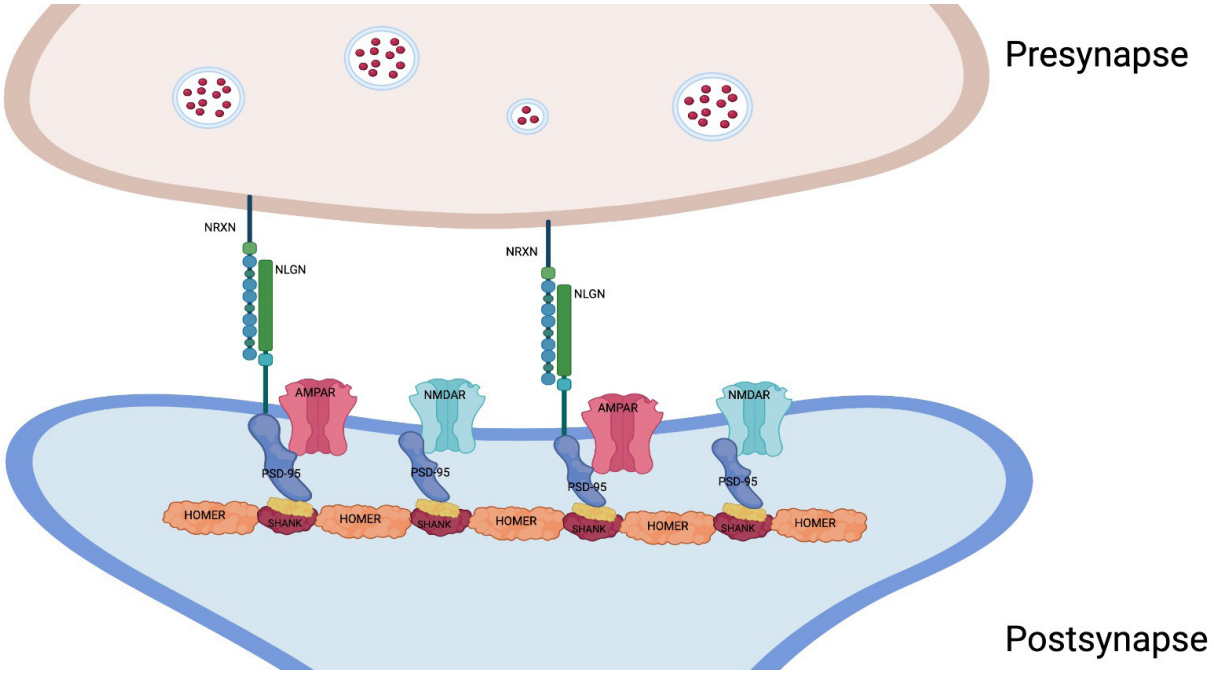

Figure 1. NRXN–NLGN trans-synaptic complex, postsynaptic scaffold proteins (PSD-95, SHANK, HOMER), and their roles in anchoring AMPA and NMDA receptors at excitatory synapses.NLGN: neuroligins, NRXN: Neurexins, PSD95: Post-synaptic Density Protein 95, SHANK: SH3 and multiple ankyrin repeat domains protein. This figure was created using a personally licensed BioRender account.

(PTEN), Methyl-CpG Binding Protein 2 (MECP2), and Ubiquitin-Protein Ligase E3A (UBE3A). Mutations or dysregulation of these proteins are strongly implicated in ASD pathophysiology and represent promising targets for therapeutic intervention (45).

Fragile X Mental Retardation Protein

FMRP is an RNA-binding protein encoded by the *FMR1* gene and is essential for local protein synthesis at synapses. Its loss leads to Fragile X Syndrome (FXS), the most common inherited cause of intellectual disability and a well-established monogenic contributor to ASD. Approximately 20–30% of males with FXS meet full ASD diagnostic criteria, and 2–4% of ASD cases are linked to FXS (46). FXS is caused by CGG trinucleotide repeat expansion (>200 repeats) in the promoter region of *FMR1,* leading to transcriptional silencing. In addition, point mutations have been observed to contribute to FXS pathogenesis, albeit less frequently.

FMRP regulates synaptic plasticity by controlling the translation of specific mRNAs, including those related to dendritic spine maturation. *Fmr1* KO models exhibit social deficits, seizures, anxiety, and synaptic abnormalities—features overlapping with ASD (47). While the exact mechanism linking FXS and ASD remains unclear, studies using human genetics and animal models suggest that FMRP interacts with key translation regulators such as CYFIP1 and eIF4E. Dysregulation of these interactions alters protein synthesis and contributes to ASD-related phenotypes (45, 48)

TSC1/TSC2 complex

The *TSC1* and *TSC2* genes encode hamartin and tuberin, which form a complex that negatively regulates the mammalian target of rapamycin (mTOR) pathway, a central mediator of cell growth and metabolism. Mutations in either gene cause TSC, a multisystem disorder often presenting with epilepsy, intellectual disability, and ASD. Up to 36% of individuals with TSC mutations develop ASD features (21).

Loss of TSC function results in hyperactive mTOR signaling, disrupting synaptic plasticity and neuronal morphology (49). Impaired synaptic plasticity, neuronal morphological abnormalities, and aberrant cell growth and proliferation are observed in TSC-related conditions (50). *TSC2* mutations are particularly enriched among individuals with ASD, further implicating the mTOR pathway in ASD pathophysiology (49).

Phosphatase and Tensin Homolog

PTEN is a tumor suppressor gene that antagonizes the PI3K/AKT/mTOR pathway. While best known for its role in cancer, germline *PTEN* mutations are also associated with neurodevelopmental abnormalities and ASD (51).

Approximately 10-20% of individuals with ASD and macrocephaly harbor PTEN mutations (52). These mutations have also been observed in Cowden syndrome, a condition characterized by macrocephaly and increased cancer risk. Some individuals with Cowden syndrome have been found to exhibit autistic traits (53). Genetic sequencing of the PTEN gene in ASD patients has identified three heterozygous germline mutations and one missense mutation (54).

Mouse models with *Pten* mutations (e.g. *Pten*$^{m3m4/m3m4}$) display ASD-like behaviors and molecular hallmarks of ASD (55). Studies have shown that ASD- and cancer-associated *PTEN* germline mutations predominantly occur in the ATP-binding motif type B and around the highly conserved P-loop in the active site, supporting their pathogenic significance (56). Furthermore, PTEN regulates alternative splicing of ASD-associated transcripts, affecting synaptic function and gene expression regulatory processes. This mis-splicing is developmentally regulated and involves other susceptibility genes, underscoring the multifactorial nature of PTEN-associated ASD (52).

Methyl-CpG Binding Protein 2

MECP2 encodes a transcriptional regulator that plays a crucial role in neuronal development, and its mutations are known to cause Rett Syndrome (RTT) and ASD (57). Loss of *MECP2* function leads to delayed language development, psychomotor regression, seizures, and impaired social interactions following an initial period of normal development (58). Studies suggest that MECP2 deficiency is linked to Brain-Derived Neurotrophic Factor (BDNF) dysregulation, cytokine production in astrocytes, and impaired neuronal dendritic branching (59). Additionally, MECP2-deficient astrocytes fail to support normal dendritic morphology in wild-type neurons (60). In *Mecp2*-null mice, astrocyte-specific restoration of *MECP2* expression was found to rescue normal neuronal dendritic morphology, improving locomotion, anxiety, and respiratory deficits (61). *MECP2* mutations and duplications have been identified in ASD cohorts and are linked to stereotypic behaviors, communication deficits, and sensory abnormalities (62, 63).

Ubiquitin-Protein Ligase E3A

UBE3A is a dual-function protein that acts as both a ubiquitin ligase and a transcriptional co-activator. Loss of maternal *UBE3A* expression leads to Angelman Syndrome (AS), characterized by severe intellectual disability, ataxia, epilepsy, and autistic features (64, 65).

Some experts classify AS as a syndromic form of ASD, as studies indicate that a subset of AS individuals meet the full diagnostic criteria for ASD (66). In ASD individuals, 15q11-q13 duplications are among the most common chromosomal abnormalities (67). This region includes the PWS/AS critical locus, which is subject to genomic imprinting. While maternally inherited duplications in this region are strongly associated with ASD, paternally inherited duplications tend to result in a normal phenotype (68). These findings underscore the critical role of UBE3A and 15q11-q13 genomic variations in ASD, highlighting their impact on neurodevelopment and potential therapeutic targets for intervention (69).

Synaptic Metabolic Stress and Excitatory/Inhibitory Imbalance in ASD

Emerging evidence suggests that disruptions in neuronal metabolism and synaptic energy homeostasis are closely linked to E/I imbalance, a core feature in the pathophysiology of ASD. Synaptic transmission is highly energy-dependent, requiring tightly regulated ATP production, calcium buffering, and redox homeostasis to maintain functional neurotransmitter signaling. Mitochondrial dysfunction, frequently reported in ASD, impairs these processes and disrupts the delicate balance between excitatory glutamatergic and inhibitory GABAergic signaling (70, 71).

Mitochondrial Dysfunction and Synaptic Vulnerability

Mitochondria are essential for sustaining synaptic plasticity and neuronal survival through ATP synthe-

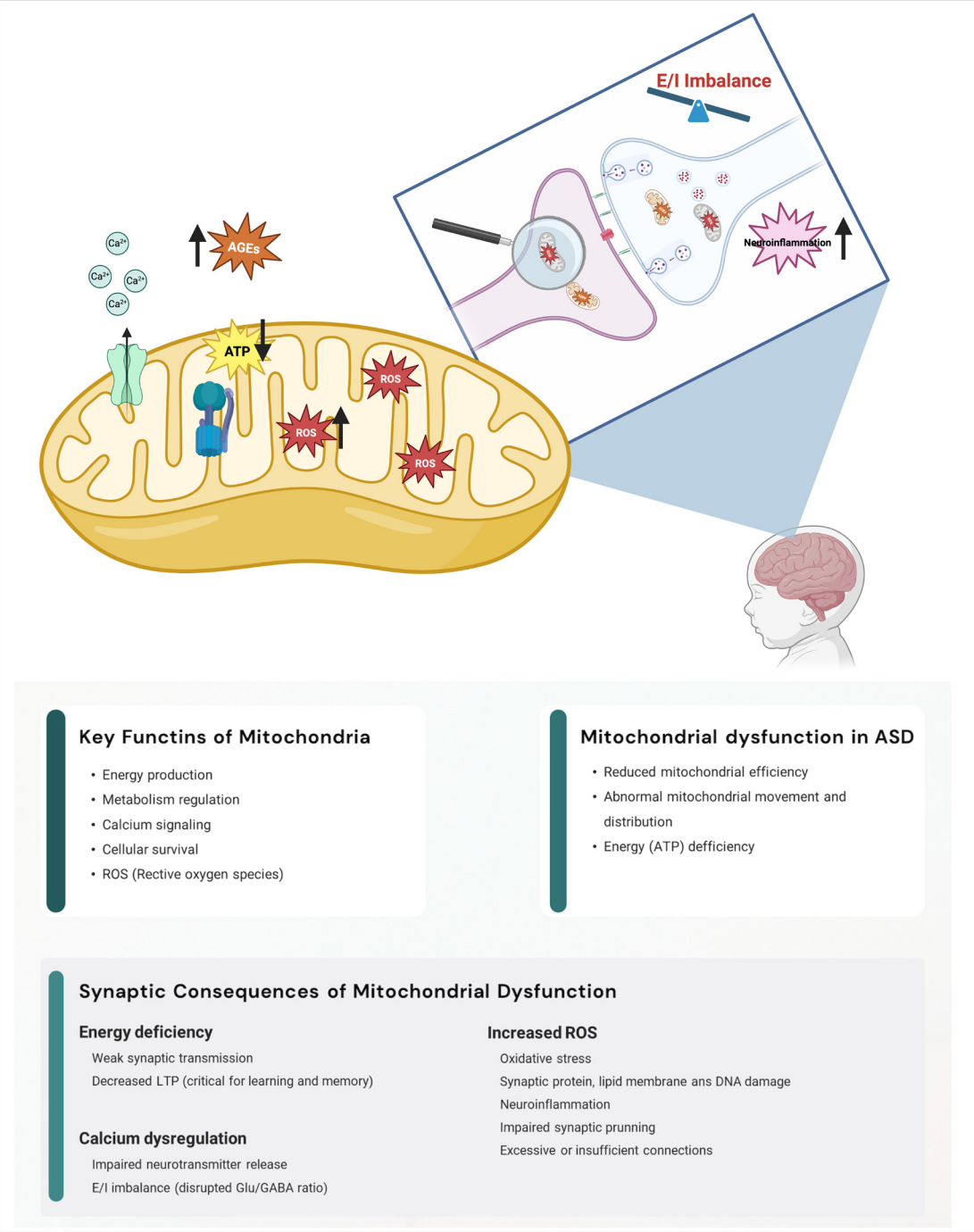

Figure 2. The schematic illustration of mitochondrial dysfunction in ASD. Arrows pointing upwards represent increases, whereas arrows pointing downwards represent decreases. AGEs: advanced glycation end-products, ROS: reactive oxygen species. This figure was created using a personally licensed BioRender account.

sis, calcium regulation, and reactive oxygen species (ROS) detoxification. Studies have shown that neurons from individuals with ASD exhibit impaired mitochondrial movement, abnormal distribution at synaptic terminals, and reduced respiratory efficiency (72). Such dysfunction compromises LTP-related processes and synaptic pruning, both vital for cognitive development and circuit refinement.

Elevated ROS levels can damage synaptic lipids and proteins, disrupt calcium-dependent vesicle release, and promote neuroinflammation. Postmortem studies confirm elevated oxidative stress in ASD brains, implicating mitochondrial redox imbalance in synaptic pathology (73). Furthermore, in syndromic ASD models such as FXS, mitochondrial impairment is closely linked to FMRP deficiency, affecting both energy metabolism and synaptic transmission (45, 73).

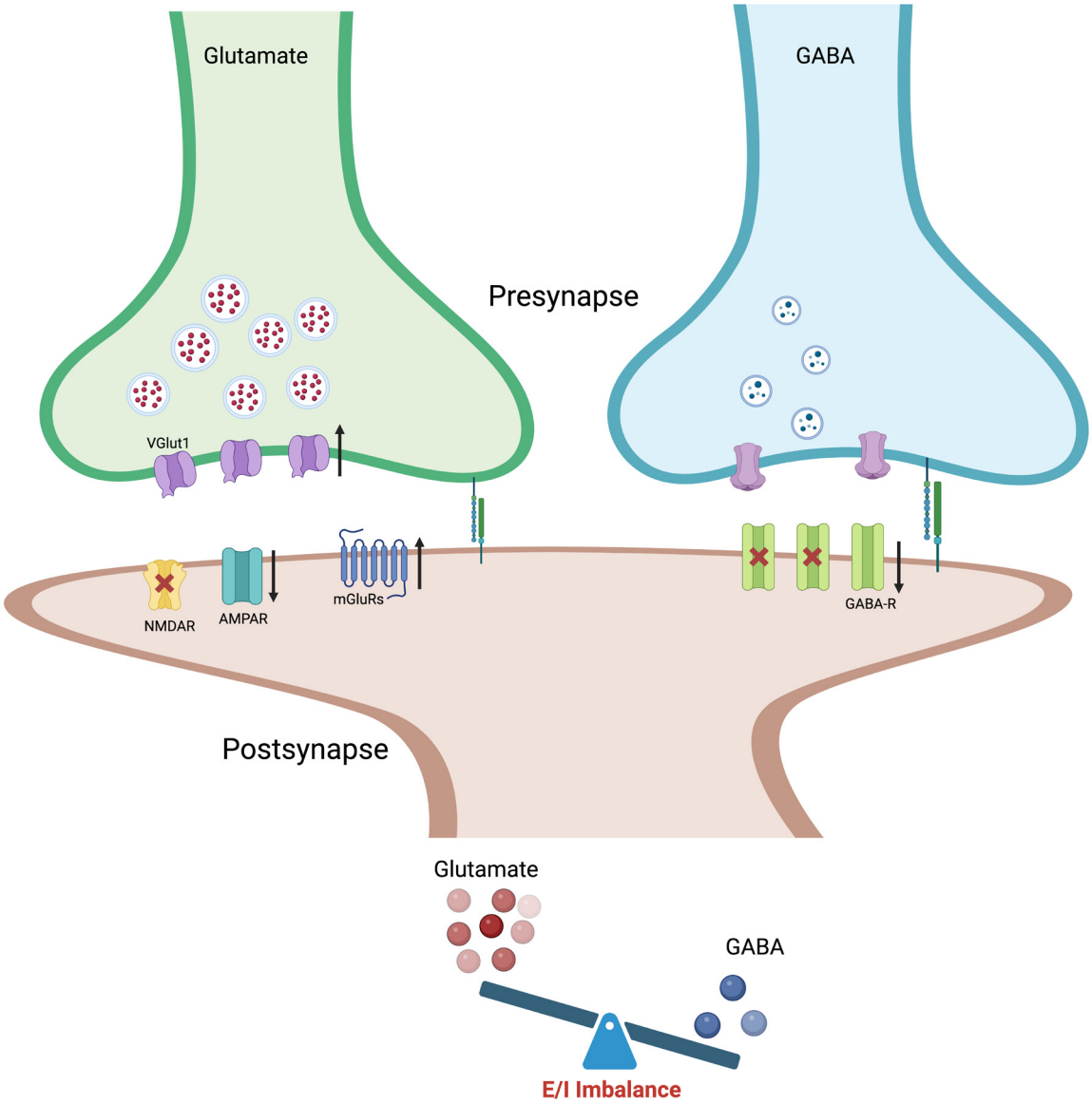

Figure 3. The schematic illustration of excitatory/inhibitory imbalance in ASD. Arrows pointing upwards represent increases, whereas arrows pointing downwards represent decreases. A cross (X) symbol denotes dysfunction in the corresponding component. AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, GABA: Gamma-aminobutyric acid, NMDAR: N-methyl-D-aspartate receptors, VGlut1: Vesicular glutamate transporter 1, mGluRs: metabotropic glutamate receptor. This figure was created using a personally licensed BioRender account.

Recent pharmacological studies targeting synaptic mitochondria have shown promise. For instance, activation of 5-HT1A receptors via eltoprazine improved mitochondrial performance and synaptic function in a Drosophila FXS model (74). Additionally, the accumulation of advanced glycation end-products (AGEs) and their precursor methylglyoxal (MG) has been linked to mitochondrial dysfunction and synaptic impairments, contributing to cognitive decline. Samantha et al. (2025) have examined the role of AGE and MG accumulation in synaptic mitochondrial stress and transmission in transgenic mice. The study found that overexpression of glyoxalase 1 (GLO1), an enzyme that detoxifies AGEs, reduced AGE accumulation, restored mitochondrial function, and improved cognitive performance in aging mice. Ex vivo and in vitro studies on hippocampal neurons confirmed that GLO1 rescued LTP and synaptic transmission, mitigating synaptic plasticity deficits (75).

Frye et al. (2024) further demonstrated that individuals with ASD and neurodevelopmental regression (NDR) exhibit hyperactive yet fragile mito-

chondrial respiration, showing reduced resilience to oxidative stress compared to typically developing siblings (76). Taken together, these findings underscore the vulnerability of synaptic mitochondria in ASD. Importantly, mitochondrial impairments not only disrupt local synaptic energy metabolism and calcium signaling but also converge on one of the core neurobiological mechanisms of ASD: E/I imbalance. Figure 2 illustrates the key functions of mitochondria at the synapse and the consequences of mitochondrial dysfunction, including ATP deficiency, oxidative stress, impaired calcium buffering, and downstream effects on E/I balance.

Excitation/Inhibition Imbalance and Synaptic Signaling Disruption

The fine-tuned balance between excitatory glutamatergic and inhibitory GABAergic neurotransmission is crucial for normal brain development, synaptic plasticity, and cognitive function. Disruption of this E/I balance represents a core pathophysiological mechanism in ASD, contributing to altered network dynamics and behavioral abnormalities (77). Figure 3 visually summarizes key alterations in excitatory and inhibitory synaptic components—including changes in receptor expression and presynaptic transporters—culminating in the disrupted E/I balance observed in ASD.

GABAergic Dysfunction in ASD

GABA is the major inhibitory neurotransmitter in the brain. Dysfunction of GABAergic signaling has been extensively documented in ASD. Ionotropic *GABAA* and metabotropic *GABAB* receptor genes—particularly *GABRB3* and *GABRA5*—are located within the 15q11–q13 chromosomal region, which is frequently duplicated in individuals with ASD (78, 79).

Mouse models of this duplication recapitulate ASD-like behaviors, including poor social interaction, behavioral inflexibility, and abnormal ultrasonic vocalizations (80). Postmortem studies have revealed reduced expression of GABAergic genes and related proteins in ASD brain tissue (81). Furthermore, the expression of GABAB receptors, particularly GABAB1 and GABAB2, has been found to be decreased in ASD brain samples, which may be critical for maintaining the E/I balance (82).

Glutamatergic Signaling Alterations

Glutamate serves as the primary excitatory neurotransmitter in the central nervous system. Its receptors include ionotropic types (AMPA, NMDA, and kainate receptors) and metabotropic types (mGluRs) (83). The paucity of postmortem tissue available for research has restricted the progress of studies on glutamate receptor expression in ASD. Nevertheless, postmortem cerebellar tissue samples have revealed a reduction in AMPAR density and an increase in mGluRs expression, suggesting altered glutamatergic signaling in ASD (84). Changes in AMPAR subunit expression influence LTP and LTD, fundamental mechanisms linked to learning and memory. Disruptions in these mechanisms could contribute to cognitive deficits in ASD (47).

NMDAR dysfunction has been observed in *Shank3, Nlgn1, Fmr1* KO mice, and *Mecp2* transgenic mouse models (85). Although *Nlgn1*-KO mice display increased repetitive behaviors, they do not exhibit the full spectrum of autism-associated behavioral impairments. Mutations in the genes encoding glutamate receptors, particularly *GRIN2B, GRIN2A,* and *GRIK2* have been associated with ASD in human genetic studies (86).

Among metabotropic receptors, mGluR1 and mGluR5 have been the most extensively studied in ASD and related disorders, including FXS, obsessive-compulsive disorder, and intellectual disability (87). Elevated mGluR5 protein levels have been reported in the vermis region of the cerebellum in children with ASD compared to controls (88). *mGluR5* KO mice exhibit cognitive deficits, highlighting its importance in synaptic regulation (89).

Epigenetic and Structural Contributors to E/I Imbalance

Emerging evidence suggests that E/I imbalance in ASD may also result from transcriptional and epigenetic dysregulation. Histone modifications such as H3K4me2/3, H3K9ac, H3K27ac, H3K9me2, and H3K27me3 have been shown to affect the expression of the genes related to excitatory and inhibitory

transmission in the prefrontal cortex of ASD patients (90). Additionally, synaptic structural studies using VGlut1 (presynaptic excitatory marker) and PSD-95 (postsynaptic marker) revealed increased excitatory and decreased inhibitory synapse numbers in the upper cortical layers of ASD brains, confirming functional synaptic imbalance (91). Conditional KO mice for *Arid1b*, an ASD risk gene, also demonstrate interneuron dysfunction and cortical hyperexcitability, further supporting the genetic basis of E/I dysregulation (92).

Environmental Influences on Synaptic Function

Beyond genetic and cellular mechanisms, growing evidence highlights the significant impact of environmental factors on synaptic dysfunction in ASD. Microglial cells, which support synaptic homeostasis and pruning, have been implicated in ASD pathophysiology. Dysfunction in these cells may lead to neuroinflammation and impaired synaptic refinement, both of which can disrupt neural circuit development (93).

Environmental insults during prenatal development may alter microglial activity, contributing to E/I imbalance and aberrant network formation. For instance, a recent mouse study examined the effects of valproic acid (VPA) and Poly I:C exposure during embryogenesis. Male offspring exhibited both stereotypical behaviors and impaired social interactions, while female mice showed only repetitive behaviors. Remarkably, restoration of GABA levels on postnatal day 10 prevented the onset of ASD-like behaviors, suggesting that environmental factors can perturb neurotransmitter balance and synaptic function (94).

Additionally, prenatal exposure to endocrine-disrupting chemicals such as Bisphenol A (BPA) and its analogs has emerged as a potential risk factor. 4-hydroxyphenyl 4-isoprooxyphenylsulfone (BPSIP), a BPA substitute, is frequently detected in breast milk and placental tissue. A 2024 study by Zhang et al. demonstrated that early-life exposure to BPSIP (0.02–0.5 mg/kg/day) in mice led to increased anxiety and impaired spatial memory in both sexes. Synaptic plasticity was altered via changes in Synapsin I (SYN1) and PSD-95 levels in the cerebellum and hippocampus (33).

It should be noted that these examples represent a subset of known environmental contributors to ASD. Due to space constraints, only select cases are highlighted here.

Therapeutic Approaches and Future Directions

Several studies have focused on targeting synaptic dysfunction in ASD, exploring various synaptic proteins, ion channels, and regulatory mechanisms. These therapeutic approaches can be classified into pharmacological and non-pharmacological interventions (78). Research has demonstrated that impairments in AMPAR-mediated neurotransmission are linked to autism, particularly in conditions involving SHANK3 or SYNGAP1 protein dysfunction. The use of AMPAR modulators, such as Perampanel, has been proposed as a potential treatment for these synaptic dysfunctions (95).

Another promising avenue is the development of genetic-based interventions, including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based gene editing, which aims to correct mutations affecting synaptic function. Such approaches are currently under investigation for their potential in restoring normal synaptic signaling in ASD (96, 97).

Conclusion

Taken together, these findings suggest that synaptic dysfunction in ASD arises from complex interactions among adhesion molecules, postsynaptic scaffold proteins, intracellular signaling pathways, mitochondrial impairments, and neurotransmitter imbalance. Figure 4 provides an integrative schematic representation of the excitatory and inhibitory synapses, highlighting how synaptic, signaling, and metabolic components converge to drive ASD-related neurodevelopmental disturbances. Therapeutic efforts focusing on restoring synaptic function through targeted pharmacological treatments and genetic interventions hold promise for future treatment strategies. Continued research is necessary to further elucidate the molecular underpinnings of ASD and develop effective, personalized therapies to improve outcomes for individuals affected by the disorder.

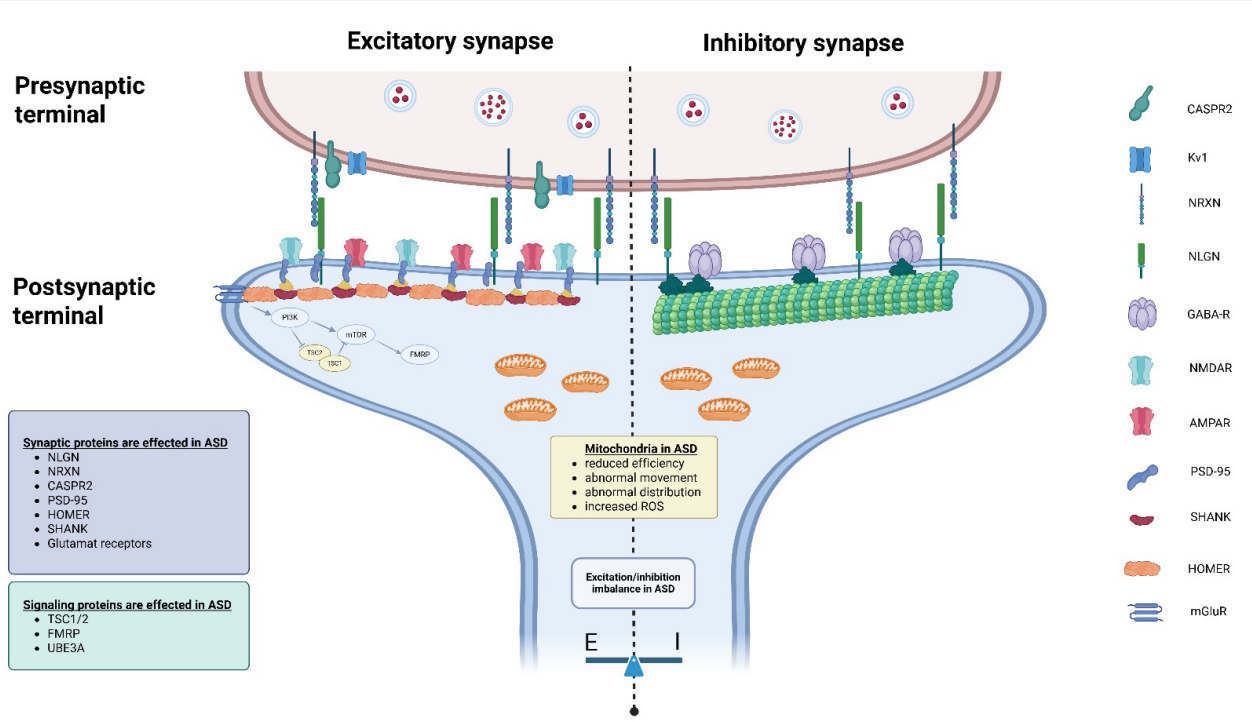

Figure 4. The schematic representation of the key synaptic and signalling proteins affected in autism spectrum disorder (ASD). The excitatory synapse illustrates the involvement of postsynaptic glutamate receptors (AMPA, NMDA, mGluR), scaffold proteins (PSD-95, SHANK, HOMER), and the neurexin–neuroligin adhesion complex, while the inhibitory synapse displays GABA receptors and associated presynaptic elements. The inclusion of proteins such as TSC1/2, mTOR, FMRP, and UBE3A is intended to represent altered intracellular signalling pathways. Mitochondria have been observed to reflect ASD-related changes, including reduced efficiency, abnormal distribution, and increased ROS. The imbalance between excitation and inhibition (E/I) is central to the pathophysiology of ASD, resulting from both synaptic structural and functional alterations. GABAR: Gamma-aminobutyric acid receptor. This figure was created using a personally licensed BioRender account.

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Bridging Statistical Rigor and Clinical Usability: The CORMeta App for Meta-Analysis of Correlated Outcomes

Klinik Uygulamalarda CORMeta Meta-Analiz Uygulaması

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ABSTRACT

Background: In clinical research, multiple outcomes are often measured within the same cohort, leading to statistical dependencies that violate assumptions of traditional meta-analytic methods. While advanced models can accommodate such correlations, they typically require programming expertise, limiting accessibility for many physician-researchers.

Objective: We present a user-friendly, interactive Shiny web application designed to perform meta-analyses of correlated outcomes, with particular relevance for cohort-based clinical datasets.

Methods: The application implements a modified multivariate meta-analytic framework that accounts for the correlation structure of outcomes within cohorts. Users can upload their data, define correlation matrices, and filter observations by any variable (e.g., age, domain, exposure) without writing code. The application provides graphical output (forest plots) along with estimates of overall effect size, heterogeneity (τ^2), and p-values.

Results: A demonstration dataset on prenatal alcohol exposure and neurodevelopmental outcomes is simulated to illustrate the application's functionality. The application automatically generates correlation matrices where needed, adjusts for intra-cohort dependencies, and produces interpretable results suitable for clinical research reports.

Conclusion: This open-access application bridges the gap between complex statistical modeling and clinical usability. It enables physicians to conduct robust meta-analyses of correlated outcomes with ease, supporting evidence-based practice and local research initiatives. The tool is particularly valuable in multi-domain or multi-cohort studies where outcome correlation is non-negligible.

Availability: The CORMeta app is freely available at https://fasdprojectapp.shinyapps.io/CORMetaApp/ . Users can upload their own summary datasets or explore the app using a preloaded sample dataset (example_data.csv), which is included with the source code. The complete app and codebase are available at https://github.com/takkaya/Meta-Analysis-of-Correlated-Outcomes-Made-Easy-Introducing-CORMeta-for-Clinicians.git

Keywords: meta-analysis, correlated outcomes, Shiny app, clinical research, multivariate models

ÖZET

Arka Plan: Klinik araştırmalarda, aynı kohort içinde birden fazla sonuç ölçütü değerlendirilmekte olup bu durum, geleneksel meta-analiz yöntemlerinin varsayımlarını ihlal eden istatistiksel bağımlılıklara yol açmaktadır. Bu tür korelasyonları dikkate alabilen gelişmiş modeller mevcut olmakla birlikte, bu modellerin kullanımı genellikle programlama bilgisi gerektirdiğinden, birçok hekim araştırmacı için erişilebilir değildir.

Amaç: Kohort tabanlı klinik veri setleriyle ilişkili korele sonuçlar üzerinde meta-analiz yapabilmeyi kolaylaştırmak amacıyla, kullanıcı dostu ve etkileşimli bir Shiny web uygulaması sunmaktayız.

Yöntemler: Bu uygulama, kohort içi sonuçların korelasyon yapısını dikkate alan, uyarlanmış bir çok değişkenli meta-analiz çerçevesini uygulamaktadır. Kullanıcılar veri yükleyebilir, korelasyon matrislerini tanımlayabilir ve herhangi bir değişkene (örneğin yaş, alan, maruziyet) göre gözlemleri filtreleyebilir; tüm bunlar kod yazmadan gerçekleştirilebilmektedir. Uygulama, grafiksel çıktılar (orman grafikleri) ile birlikte genel etki büyüklüğü, heterojenite (τ^2) ve p-değerleri gibi sonuçları sunmaktadır.

Bulgular: Uygulamanın işlevselliğini göstermek amacıyla, doğum öncesi alkol maruziyeti ve nörogelişimsel sonuçlara ilişkin simüle edilmiş bir veri seti kullanılmıştır. Uygulama, gerekli durumlarda korelasyon matrislerini otomatik olarak oluşturur, kohort içi bağımlılıkları dikkate alır ve klinik araştırma raporlarına uygun, yorumlanabilir sonuçlar üretir.

Sonuç: Bu açık erişimli uygulama, karmaşık istatistiksel modelleme ile klinik uygulanabilirlik arasındaki boşluğu kapatmaktadır. Hekimlerin, korele sonuçlar üzerinde sağlam meta-analizler yapabilmesini kolaylaştırarak kanıta dayalı uygulamaları ve yerel araştırma girişimlerini desteklemektedir. Araç, özellikle çok alanlı veya çok kohortlu çalışmalarda, sonuçlar arasındaki korelasyonun göz ardı edilemeyecek düzeyde olduğu durumlarda büyük değer taşımaktadır.

Anahtar kelimeler: Monosemptomatik noktürnal enürezis, Desmopressin, Enürezis alarmı

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1. Introduction

Meta-analysis plays an important role in synthesizing evidence across studies, particularly in clinical research where multiple outcomes are often reported within the same cohort. In such settings, traditional univariate meta-analytic approaches may be inappropriate [1], as they typically assume that outcomes are statistically independent. This assumption is frequently violated when correlated outcomes—such as neurodevelopmental domains or imaging markers—are measured within the same study population. Ignoring these dependencies can lead to biased estimates and underestimated uncertainty [2].

To address this issue, advanced multivariate meta-analytic methods have been developed. These methods explicitly account for the correlation structure among outcomes. One such method is multivariate meta-analysis, which allows for the simultaneous synthesis of multiple outcomes across studies, estimating multiple pooled effects while preserving their interrelationships. Another technique, the three-level meta-analytic model [3], accommodates dependencies by modeling the nested structure of effect sizes, where non-independence arises from clustering rather than repeated measurements on the same individuals.

An additional strategy is the two-stage meta-analysis using summary measures, which incorporates robust variance estimation (RVE) [4]. RVE adjusts the standard errors of pooled effects or meta-regression coefficients without needing to model the dependence structure explicitly. Instead, it approximates the correlation between outcomes to estimate between-study variance and derive near-optimal weights.

Building upon these advances, Akkaya Hocagil et al. (2022) proposed a novel hierarchical meta-analysis tailored for cases where each cohort provides multiple, potentially correlated endpoints [5]. They applied this method to data from six longitudinal cohorts that used various cognitive assessments to measure child neurodevelopment. Their model assumes that all endpoints reflect effects of exposure on a shared underlying construct. A major advantage of this approach is its ability to integrate multiple outcomes within cohorts and evaluate whether consistent patterns emerge across cohorts.

Despite these methodological developments, adoption of these methods in clinical setting has been limited due to the requirement of statistical and programming expertise to run such models. Consequently, many clinician-researchers rely on simpler, less suitable methods that may compromise the validity of their results.

To bridge this gap between methodological rigor and clinical usability, we developed a user-friendly, interactive Shiny web application, CORMeta. The application is designed specifically for conducting meta-analyses of correlated outcomes and tailored for researchers working with cohort-based datasets where multiple outcomes are measured per study. The application enables users to upload their own data, define correlation structures, and customize the analysis through an intuitive graphical interface without requiring any programming skills. It provides comprehensive outputs, including forest plots, pooled effect estimates, heterogeneity statistics, and confidence intervals.

In this paper, we introduce the methodological framework underpinning the application, demonstrate its use with a simulated dataset that mimics a real-world dataset on prenatal alcohol exposure and neurodevelopment, and discuss its potential to improve evidence-based practice in clinical settings where accounting for outcome correlation is essential.

2. Statistical Framework

2.1. Multivariate Meta-Analysis of Correlated Outcomes

In many clinical studies, multiple outcomes are reported within the same patient cohort. When these outcomes are synthesized across studies, conventional univariate meta-analytic methods may not be appropriate, as they assume independence among effect sizes. This assumption is violated when outcomes are measured on the same participants, leading to correlated effect estimates and underestimation of standard errors.

To address this issue, we implement a multivariate meta-analytic (MVMA) framework that explicitly

models the correlation between multiple outcomes measured within the same study. This approach allows for the simultaneous estimation of multiple effect sizes while accounting for their interdependencies, improving the accuracy and interpretability of pooled estimates.

While traditional multivariate meta-analysis estimates separate pooled effects for each outcome, our framework simplifies to a univariate structure when only one outcome is available per study but retains valid heterogeneity and standard error estimation by incorporating user-specified outcome correlations. This enables flexible modeling of both single and multiple correlated endpoints within cohorts.

The general form of the multivariate model [6] is:

$\hat{\theta}_i \sim N(\theta_i, V_i)$, $\theta_i \sim N(\mu, \Sigma)$

Where:

- $\hat{\theta}_i$ is the vector of observed effect sizes from study i
- V_i is the corresponding within-study variance-covariance matrix
- μ is the overall vector of true effect sizes
- Σ is the between-study variance-covariance matrix capturing heterogeneity and outcome correlations

2.2. Modeling Within-Cohort Correlation Structure

To correctly estimate V_i, the within-study variance-covariance matrix must reflect the correlations between outcomes within each cohort. In our application, users can either:

- Upload a custom correlation matrix that reflects outcome dependencies (e.g., based on domain knowledge, pilot data, or previous validation studies), or
- Allow the application to automatically generate a pooled or cohort-aware dummy correlation matrix from cohorts reporting more than one outcome, under the assumption of a shared correlation structure.

If users do not have access to an empirically derived correlation matrix, they may choose to generate a dummy matrix using a predefined or manually selected value of ρ (residual correlation). This approach is particularly useful when exact correlations are unknown but can be reasonably approximated based on outcome type and measurement context. Suggested default values, informed by prior literature [7,8], include:

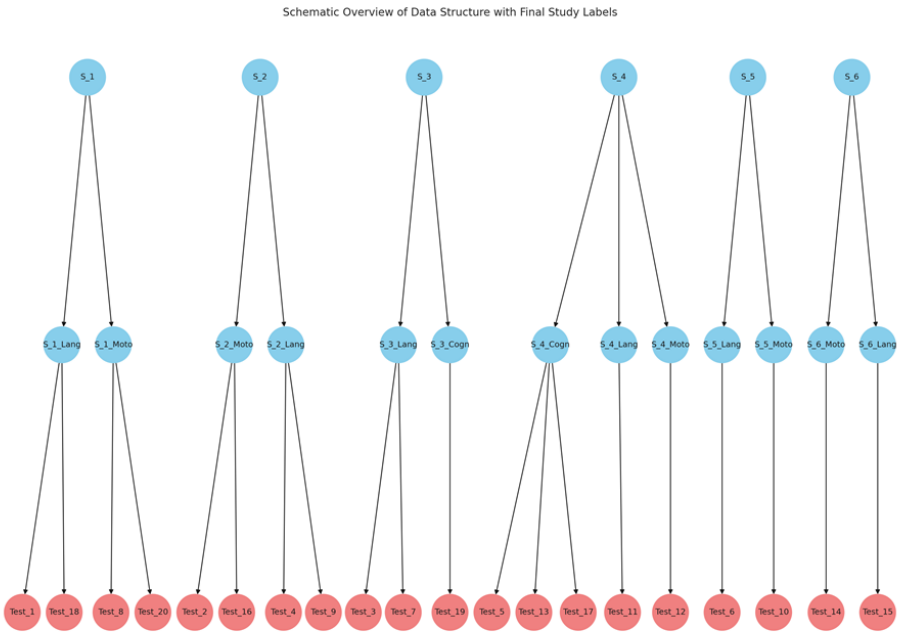
- Surrogate endpoints: ρ=0.1users must ensure that stratification does not lead to overfitting
- Psychological test scores: ρ=0.5
- Longitudinal repeated measures: ρ=0.8
- Independent outcomes: ρ=0

This flexibility ensures that outcome dependency is explicitly incorporated into the meta-analytic model, thereby avoiding biased estimates and inflated precision that arise from ignoring within-cohort correlations. However, when only one outcome is provided per study and users select the “Independent” correlation option (ρ = 0), the residual correlation matrix becomes the identity matrix, and the analysis simplifies to a standard univariate meta-analysis [9]. In this scenario, the model behavior is equivalent to conventional random-effects meta-analysis methods (e.g., **rma.uni()** from the **metafor** package [10]), and results (effect size, standard error, τ^2) are interpreted as in traditional meta-analyses.

2.3. Estimation of Effect Sizes and Heterogeneity

Parameter estimation is performed using Restricted Maximum Likelihood (REML), which is robust for estimating between-study variance components in multivariate models. REML jointly estimates the pooled effect sizes (μ) and the between-study covariance matrix (Σ), which includes both heterogeneity (e.g., τ^2) and cross-outcome correlations. To derive study-specific and overall estimates, we employ Best Linear Unbiased Predictions (BLUPs), which improve precision by borrowing strength across correlated outcomes and cohorts. The primary heterogeneity measure reported is τ^2, representing the variance of the true effect sizes across studies, adjusted for outcome correlation.

Figure 1. Schematic representation of the multilevel data structure used in CORMeta



3. Application Architecture and Design

3.1. Overview of the Shiny App Framework

The CORMeta application was developed using the Shiny web framework for R, which facilitates the creation of interactive, browser-based tools. Shiny allows reactive programming and dynamic UI rendering, enabling users to upload data, adjust parameters, and view real-time outputs without requiring any programming expertise. This architecture was chosen to maximize accessibility for clinician-researchers who may not have experience with statistical software or coding. The app runs entirely in the browser, either via a hosted web server or locally, and does not require users to install R or other dependencies.

3.2. Backend Libraries Used

The core analytical engine of the application builds upon the widely used metafor package in R, which offers flexible modeling of complex random-effects structures, REML estimation, and forest plot visualization. To accommodate correlated outcomes within cohorts, we extended metafor's functionality by incorporating methodology described in Akkaya Hocagil et al. (2022), enabling hierarchical multivariate meta-analysis with user-specified correlation structures [5].

Additional libraries such as 'shinyWidgets', 'DT', and 'ggplot2' are used for enhancing user interface components, data tables, and graphics respectively. These packages enable seamless interaction and customization, even for users unfamiliar with R.

3.3 User Workflow and Application Guidance

Clinical studies frequently assess multiple outcomes within the same group of participants. For example, in studies of child neurodevelopment, researchers may evaluate several domains such as language, motor skills, and cognitive function each assessed using multiple standardized tests. These outcomes are inherently correlated, as they are measured on the same individuals and often reflect overlapping constructs.

CORMeta is specifically designed to handle this type of complex, multilevel data structure. The tool assumes and accommodates the following hierarchy:

1. Study Level (e.g., S_1 to S_6): Each study or cohort includes participants assessed under a common study protocol.
2. Domain Level (e.g., Language, Motor, Cognitive): Within each study, outcomes are organized into broader conceptual domains.
3. Test Level (e.g., $Test_1$ to $Test_{20}$): Each doma-

Table 1. Structure of the Summary Dataset Used in the CORMeta application

cohort_new	domain	dependent	age	beta_alcohol	se_beta
Study_1	Language	Test_1	7	-0.011	0.163
Study_1	Language	Test_18	5	0.747	0.144
Study_1	Motor	Test_20	6	0.121	0.271
Study_1	Motor	Test_8	7	0.892	0.284
Study_2	Language	Test_4	5	-1.507	0.199
Study_2	Language	Test_9	5	-0.204	0.299
Study_2	Motor	Test_16	5	-0.318	0.202
Study_2	Motor	Test_2	6	-0.991	0.278
.	.	.	.	.	.
.	.	.	.	.	.
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.	.	.	.	.	.
.	.	.	.	.	.

in contains specific outcome measures or tests (e.g., vocabulary comprehension for language, reaction time for motor skills).

4. Correlation Structure: Outcome measures within the same domain and study are typically correlated, as they are administered to the same participants and assess related aspects of development. CORMeta explicitly models this correlation structure, leading to more accurate effect estimates and confidence intervals.

To operationalize this hierarchical data structure within the CORMeta application (Figure 1), users follow a structured workflow that mirrors the organization of their dataset. The interface is designed to guide users through each step of the analysis process—beginning with uploading properly formatted data and continuing through model specification, correlation handling, and result interpretation. Below, we outline the key steps for preparing and analyzing your data using the application.

Step 1: Upload Summary Data

Users begin by uploading a .csv file containing study-level summary data (Table 1). The dataset must include the following columns:

- Effect Size Estimates: A column containing regression coefficients or other effect size estimates (e.g., beta_alcohol).
- Standard Errors: A corresponding column with the standard errors of the effect sizes (e.g., se_beta).
- Study Identifier: A column indicating the study or cohort from which each estimate originates (e.g., cohort_new).
- Outcome Label: A column specifying the outcome measure or domain being assessed (e.g., domain or dependent_label_new).

Each row represents a single outcome measured within a study cohort. The dataset includes the study identifier (cohort_new), outcome domain (e.g., language, motor, cognitive), specific test name (de-

pendent), participant age at assessment, estimated effect size (beta_alcohol) for prenatal alcohol exposure, and its associated standard error (se_beta). Multiple outcomes from different domains are often reported within the same cohort, reflecting the hierarchical structure of the data. Once the file is uploaded, the application automatically detects all column names for use in subsequent steps.

Step 2: Specify Column Names

Using the dropdown menus, users select the appropriate column names corresponding to:

- Effect size (Select Effect Size Column)
- Standard error (Select Standard Error Column)
- Study/Cohort ID (Select Study ID Column)

These menus are dynamically populated based on the uploaded dataset, reducing the risk of typos and promoting flexibility across datasets with varying column names.

Step 3: Upload or Generate Correlation Matrix

If available, users can upload a pre-computed residual correlation matrix (e.g., derived from model residuals). Alternatively, the user can generate a study-aware dummy correlation matrix using a predefined or manually selected value of ρ (residual correlation), based on common scenarios [7,8] such as:

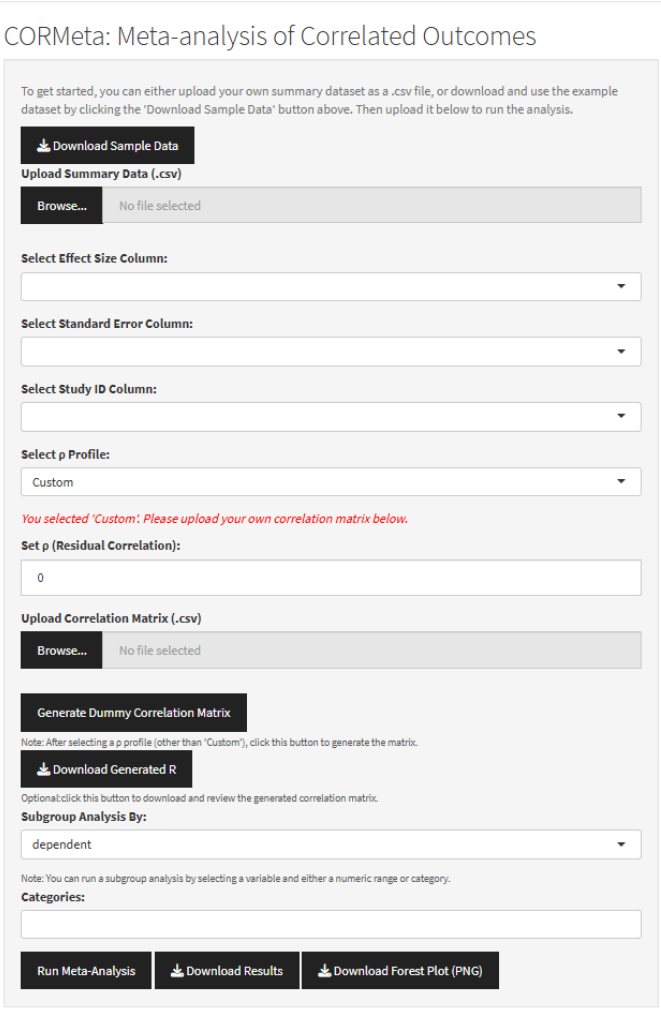
- Surrogate endpoints (ρ = 0.1)
- Psychological test scores (ρ = 0.5)
- Longitudinal repeated measures (ρ = 0.8)
- Independent outcomes (ρ = 0)

After selecting an appropriate correlation profile, clicking the “Generate Dummy Correlation Matrix” button creates a block-diagonal matrix that assumes constant residual correlations within each cohort and independence between cohorts. This matrix can be previewed and downloaded for verification or further refinement. The flexibility to specify or simulate within-study correlations ensures that the meta-analytic model appropriately accounts for outcome dependency, avoiding biases and overconfident inferences that arise when correlations are ignored.

Step 4: Optional Subgroup Analysis

Users may optionally perform subgroup analyses by selecting a variable from the *Subgroup Analysis By* dropdown menu. Based on the chosen variable type, the application dynamically displays appropriate filtering controls—such as a range slider for continuous variables or a checkbox selector for categorical variables—allowing users to restrict the analysis to a specific subset of the data.

Figure 2. Screenshot of the CORMeta user interface during Step 2.



Users assign column roles using dropdown menus that are automatically populated based on the uploaded dataset. This design minimizes user error and allows flexibility in handling datasets with diverse naming conventions.

Step 5: Run Meta-Analysis

After defining the inputs, the user initiates the meta-analysis by clicking Run Meta-Analysis. The app executes the modified multivariate meta-analytic model, accounting for the correlation structure and study identifiers as described in Section 3.

Step 6: Review and Download Results

Upon completion, the application displays:

- A forest plot summarizing the pooled effect
- A data table of the included studies and parameters
- A printed summary of key statistics, including:
- Pooled estimate ($\hat{\mu}$)
- Standard error (SE)
- Between-study variance (τ^2)
- P-value

Results can be exported as an Excel workbook containing both the input data and the statistical summary using the Download Results button. Users can also download the forest plot as png file.

4. Demonstration: Case Example

4.1. Description of Demonstration Dataset

To demonstrate the functionality of the application, we generated a simulated dataset that mimics real-world cohort studies examining the association between prenatal alcohol exposure and neurodevelopmental outcomes in children. The simulated data reflect the typical structure of such studies, including:

Multiple correlated neurodevelopmental outcomes (e.g., cognitive, motor, language),

Binary exposure variable (prenatal alcohol exposure: exposed vs. unexposed),

Several cohorts contributing data, each with unique sample sizes and measurement variability,

A shared latent structure where all outcomes reflect an underlying neurodevelopmental construct influenced by exposure.

4.2. Analytical Setup

Users selected three neurodevelopmental outcomes—cognitive, motor, language domains—for analysis. For each cohort, effect estimates and associated standard errors were derived from regression models examining the association between prenatal alcohol exposure and each outcome given all potential confounders. These estimates reflect adjusted regression coefficients (e.g., from linear models), accounting for relevant covariates within each study. Because multiple outcomes were measured on the same individuals within each cohort, the estimates are likely correlated. To account for this within-study correlation structure, users can specify or upload a correlation matrix, enabling multivariate meta-analysis that appropriately pools effects across outcomes and cohorts.

4.3. Interpretation of Output

The application generated pooled effect size estimates for each outcome, along with 95% confidence intervals and τ^2 heterogeneity statistics. The forest plot provided a clear visual summary of the effect sizes, illustrating both individual study estimates and the overall pooled estimate for each outcome. The results revealed moderate negative associations between prenatal alcohol exposure and all three outcomes, with the largest effect observed in expressive language. Heterogeneity was moderate across outcomes, indicating variation in effect sizes that was partially explained by cohort-level differences.

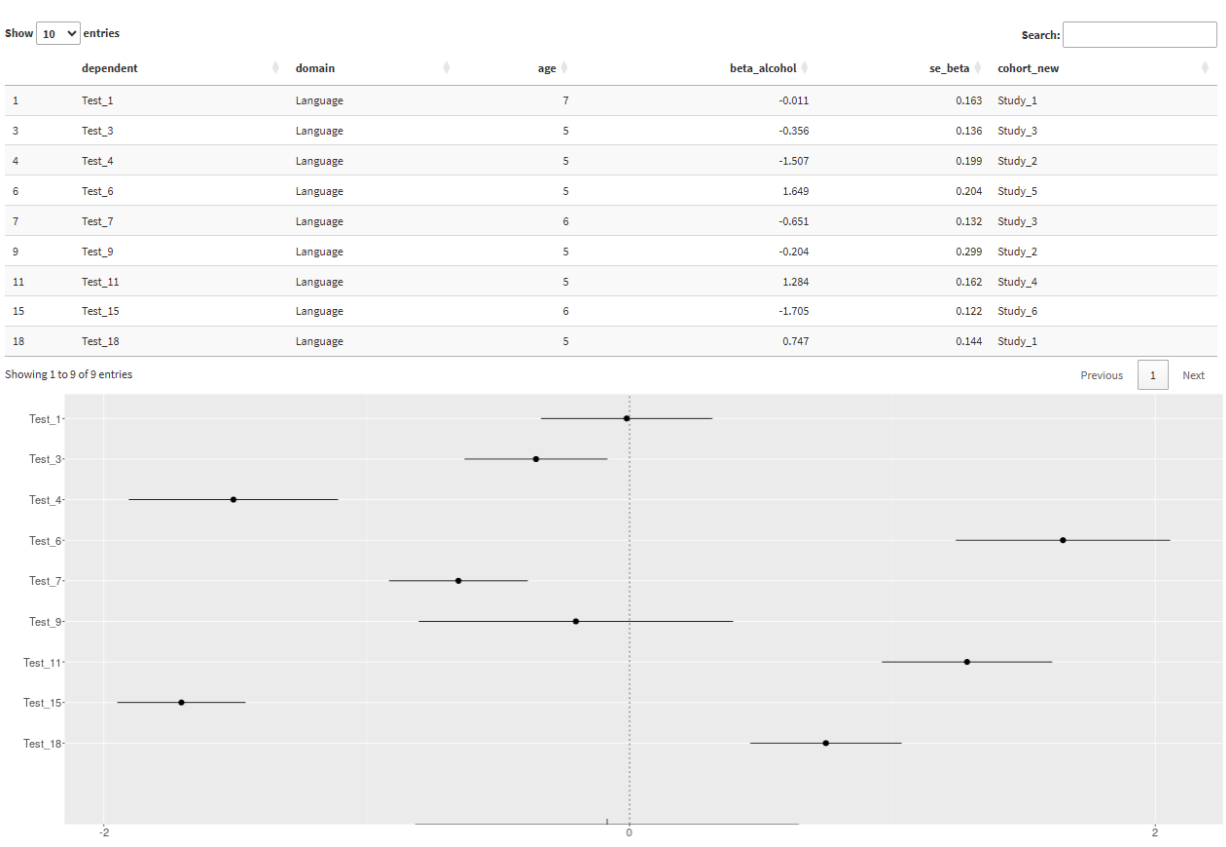
4.3. Example Forest Plot and Heterogeneity Results

Each dot represents the estimated regression coefficient for the association between prenatal alcohol exposure and a specific neurodevelopmental test (e.g., Test_1, Test_3, Test_4, etc.). Horizontal lines denote the 95% confidence intervals, while the vertical dashed line at zero represents the **null** hypothesis of no effect.

Most of the CIs span the null value, suggesting that the estimated associations are not statistically significant at the 0.05 level. Notably, no test shows a confidence interval that is clearly separated from zero, indicating limited evidence of a consistent effect of prenatal alcohol exposure across the evaluated outcomes (Figure 3).

The pooled estimate of the effect was:

Figure 3. Forest plot and summary statistics generated by the CORMeta application.



Show 10 entries Search:

	dependent	domain	age	beta_alcohol	se_beta	cohort_new
1	Test_1	Language	7	-0.011	0.163	Study_1
3	Test_3	Language	5	-0.356	0.136	Study_3
4	Test_4	Language	5	-1.507	0.199	Study_2
6	Test_6	Language	5	1.649	0.204	Study_5
7	Test_7	Language	6	-0.651	0.132	Study_3
9	Test_9	Language	5	-0.204	0.299	Study_2
11	Test_11	Language	5	1.284	0.162	Study_4
15	Test_15	Language	6	-1.705	0.122	Study_6
18	Test_18	Language	5	0.747	0.144	Study_1

Showing 1 to 9 of 9 entries Previous 1 Next


```
$mu_hat
            [,1]
[1,] -0.08580883

$se
          [,1]
[1,] 0.3648488

$tau2
[1] 1.154688

$pvalue
          [,1]
[1,] 0.8199697
```

In Figure 3, the upper panel shows the study-level data table and resulting forest plot for 10 language domain outcomes across multiple cohorts. Each point represents the estimated effect of prenatal alcohol exposure on the respective test, with horizontal lines denoting 95% confidence intervals. The lower panel presents the pooled effect size estimate ($\hat{\mu}$), standard error (SE), between-study variance (τ^2), and p-value derived from the multivariate meta-analytic model.

- Mean estimate **($\hat{\mu}$):** −0.086
- Standard error (SE): 0.364
- Between-study variance **(τ^2):** 1.15
- P-value: 0.820

These findings indicate substantial heterogeneity across outcomes, reflected in the large τ^2 value and variable CI widths. The results highlight the value of using a multivariate meta-analytic approach that accounts for correlations among outcomes and between-study variability. Despite the nonsignificant overall effect, this approach provides more efficient and nuanced insight than a series of univariate models.

5. Discussion

This paper presents a web-based application, CORMeta, designed to perform multivariate meta-analyses of correlated outcomes, addressing a critical gap in usability for clinician-researchers. While multivariate models are well established in statistical literature, their adoption in clinical research has been limited by accessibility barriers, particularly the requirement for programming expertise and manual data formatting. By integrating these models into a Shiny interface, we allow users to use advanced meta-analytic methods and we promote default values, informed by prior literature methodological rigor in evidence synthesis.

The primary innovation lies in the application's ability to handle outcome correlations that arise when multiple endpoints are measured within the same study population. Failure to account for these correlations can lead to underestimated standard errors, inflated precision, and potentially misleading inferences [11, 12]. Our framework addresses this by allowing users to input custom correlation matrices or apply empirically derived default structures, enabling flexibility while preserving statistical validity.

The demonstration using neurodevelopmental outcomes following prenatal alcohol exposure illustrates how outcome domains with known correlation (e.g., language and cognition) can be simultaneously modeled, yielding pooled estimates that better reflect underlying study heterogeneity. This is especially relevant in pediatrics, neurology, and psychiatry, where multidimensional assessments are standard practice.

Despite its strengths, the application has limitations. It currently supports only continuous outcomes and assumes homogeneity of correlation structures across studies when automatic estimation is used. Additionally, while subgroup analysis is supported, users should be cautious of overfitting or sparsity when performing subgroup analyses. Future updates may incorporate Bayesian estimation, imputation of missing data, and support for binary or time-to-event outcomes to expand functionality.

6. Conclusion

We developed and validated an open-access, clinician-friendly Shiny application that enables multivariate meta-analyses of correlated outcomes. By removing the need for programming knowledge and providing real-time visualization and customization, the app supports clinician-researchers in conducting more accurate and transparent evidence syntheses. This tool is particularly valuable in multi-domain or multi-cohort studies where statistical dependencies among outcomes are non-negligible. We encourage the research community to adopt, adapt, and contribute to the tool as part of broader efforts to enhance methodological accessibility in clinical research.

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Author Contributions: Concept: T.A.H., Design: T.A.H., Data Collection or Processing: T.A.H., Analysis or Interpretation: T.A.H., Literature Search: T.A.H., Writing: T.A.H.

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