Acta Medica Academica

Journal of Department of Medical Sciences
of Academy of Sciences and Arts of Bosnia and Herzegovina

Mariia, a Ukrainian Refugee, in Front of the Peristyle at Diocletian's Palace, Split, Croatia

ISSN 1840-1848 (Print) Volume 53 Number 3 December 2024 ISSN 1840-2879 (Online)

Online First www.ama.ba

Acta Medica Academica

Contents
2024 Vol. 53, No. 3

Acta Medica Academica

www.ama.ba

AIMS AND SCOPE

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SUBSCRIPTION

Acta Medica Academica is published triannually. The annual subscription fee is € 50 outside of Bosnia and Herzegovina.

PUBLISHER CONTACT INFORMATION

Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina. Contact person: Husref Tahirović, E-mail: htahirovic@anubih.ba

COVER PHOTO PICTURE

Mariia, a Ukraina Refugee, in Front of Peristyle at Diocletian's Palace, Split, Croatia. The illustration is done according to the paper by Shmatkova, et al., "Resilience in the Face of War: a Collaborative Autoethnography of a Ukrainian Refugee Student's Journey through Europe Striving to Find Oneself". Photo: Saša Burić, Split, Croatia.

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CIRCULATION

200 copies.

THE JOURNAL IS INDEXED IN

Medline/PubMed; PMC; Scopus; Embase; EBSCOhost; CAB Abstract/Global Health Databases; DOAJ; CrossRef.

Acta Medica Academica (ISSN 1840-1848) is an international peer-reviewed triannual journal which is printed as a continuation of the journal Works of the Academy of Sciences and Arts of Bosnia and Herzegovina, Department of Medical Sciences, founded in 1953. *Acta Medica Academica* Online (ISSN 1840-2879) offers free access to all articles at www.ama.ba.

Review Article
Acta Medica Academica 2024;53(3):237-273
DOI: 10.5644/ama2006-124.458

HPV-Related Cancers in Bosnia and Herzegovina: A Comprehensive Review

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Received: 12 September 2024; **Accepted**: 17 October 2024

Abstract
This review assesses the burden of human papillomavirus (HPV)-related cancers in Bosnia and Herzegovina (BH), aiming to inform strategies for prevention and early detection. Despite the availability of highly effective HPV vaccines and screening programs, HPV-related cancers remain a significant public health burden worldwide. We conducted a comprehensive search of PubMed and GLOBOCAN to identify all available data on HPV prevalence/genotype and HPV-related malignancies in BH, including information on HPV vaccination and cervical cancer screening. A comprehensive literature search revealed limited data on HPV prevalence and HPV-related cancers, as well as the absence of a national HPV vaccination or cervical cancer screening program in BH. In the largest study with available data from BH, HPV prevalence was 43% among women undergoing routine gynecologic exams. HPV-16 was identified as the most common cause of cervical cancer. The HPV prevalence was 50% in head and neck cancer, with HPV-18 being the most prevalent subtype. HPV was detected in 80% of patients with colorectal cancer, and HPV-16 was the most common subtype. **Conclusions.** HPV-related cancers, particularly cervical cancer, represent a significant public health problem in BH. Implementation of a national HPV vaccination program, along with organized cervical cancer screening is essential to reduce HPV-related morbidity and mortality. Addressing systemic challenges, such as establishing a comprehensive cancer registry, is essential for effective HPV prevention and control. Raising public awareness about HPV infection, its consequences, and the importance of prevention is essential for vaccine acceptance and promoting healthy behaviors. By investing in HPV prevention, BH can significantly improve the health and well-being of its population, particularly women.

Key Words: Human Papillomavirus ▪ Bosnia and Herzegovina ▪ Cancers ▪ Vaccination ▪ Screening.

Introduction

Overview of Human Papillomavirus (HPV) and Its Association with Various Cancers

The HPV virus is a small deoxyribonucleotide acid (DNA) virus that is the most common cause of sexually transmitted diseases worldwide. HPV is primarily transmitted through sexual contact, including vaginal, anal, and oral sex. While most HPV infections are asymptomatic and resolve spontaneously, persistent infections with certain high-risk HPV subtypes can lead to precancerous changes and ultimately cancer. There are over 200 identified HPV subtypes, but only 12 are considered high-risk and linked to cancer development (1). Cervical cancer is the most recognized HPV-related malignancy. HPV-16 and 18 are primarily responsible for its development. However, HPV's oncogenic potential extends beyond the cervix. It is a significant causative factor in other anogenital cancers, including vaginal, vulvar, anal, and penile cancers. It is a primary cause of vaginal and vulvar cancers, contributing to approximately 75% and 69% of cases, respectively (2). Globally, there has been a substantial increase in HPV-related oropharyngeal cancers, linked to rising rates of sexually transmitted HPV infection. HPV-related oropharyngeal cancers generally have a more favorable prognosis compared

to those not associated with HPV (3). While less prevalent, HPV is also linked to cancers of the oral cavity and larynx, although the prevalence of HPV in these cancers is lower compared to oropharyngeal cancer (4).

Prevention and early detection are crucial in reducing the burden of HPV-related diseases. HPV infection can be effectively prevented through vaccination with one of three available HPV vaccines: bivalent, quadrivalent, and 9-valent (5, 6). These vaccines target both low-risk and high-risk HPV subtypes, with all three protecting against high-risk HPV subtypes 16 and 18, which are responsible for most HPV-related cancers. Early detection of asymptomatic precancerous lesions caused by HPV infection through screening tests is crucial for preventing invasive cancer.

Importance of Studying HPV-related Cancers in BH

Cancer represents a major public health concern globally, including in BH. According to GLOBOCAN data, an estimated 14,265 new cancer cases are diagnosed annually in BH, resulting in approximately 8590 cancer-related deaths each year (7). HPV is responsible for approximately 790,000 cancers worldwide each year, accounting for about 5% of all cancers (8). Despite being a globally recognized preventable disease, BH faces substantial gaps in HPV prevention and control. The absence of a unified national cervical cancer screening program and HPV vaccination rates contributes to a high risk of HPV-related cancers among the population. Cervical cancer is the most common cancer among these cases and a leading cause of cancer-related deaths in women in developing countries (Figure 1). The situation in BH is further complicated by high rates of high-risk HPV infections among women of reproductive age and the absence of a national cervical cancer screening or vaccination program (9). The prevalence of high-risk HPV subtypes 16 and 18 among women of reproductive age further underscores the urgent need for effective prevention and control measures.

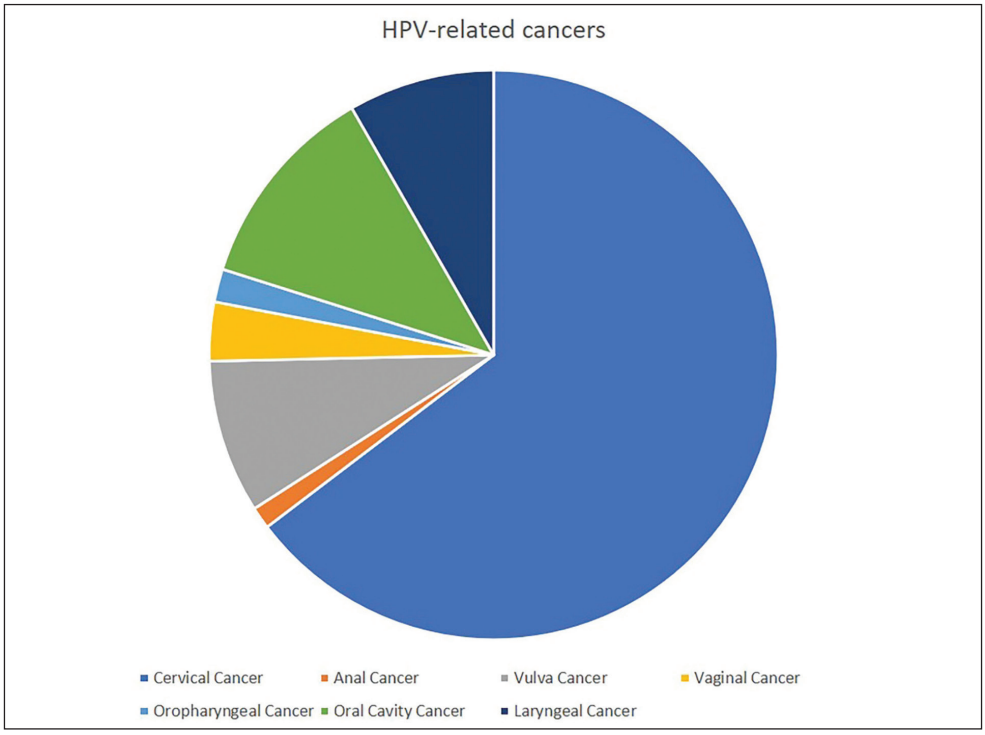

Figure 1. The frequency and distribution of HPV-related cancers in Bosnia and Herzegovina (adapted from: https://hpvcentre.net/statistics/reports/BIH_FS.pdf).

According to available data, cervical screening through the Pap test is widely accessible in 98% of surveyed institutions in BH (10). However, only 26% of these institutions have documented written protocols for conducting Pap tests. HPV testing is currently limited to specific regions of BH, including Tuzla, Sarajevo, and Banja Luka. Despite the HPV vaccine registration in BH since 2007, its role in cervical cancer prevention was not formally recognized until the 2011 Federation of Bosnia and Herzegovina (FBiH) health strategy (11). Despite this acknowledgment, a nationwide HPV vaccination program remains absent. Furthermore, comprehensive data on HPV prevalence within the general population in BH is currently unavailable (12). Beyond cervical cancer, a limited number of studies from BH explored and identified high-risk HPV subtypes in other cancers, such as head and neck, and colorectal cancers (13, 14).

We present a comprehensive review of the published literature and the current state of HPV-related cancers in BH. A thorough literature search revealed 23 studies on HPV prevalence and associated cancers in BH (Table 1) (9, 11, 13-33).

Table 1. Overview of Key Findings from HPV-Relevant Studies in Bosnia and Herzegovina

Authors	Cancer subtype	Participants (N)*	Age	HPV subtype	HPV (%)†	Most common HPV subtype (%)§	Detection method
Davies et al. 2023‡	Cervix	NA	NA	NA	NA	NA	NA
Davies et al. 2023‡	Cervix	NA	NA	NA	NA	NA	NA
Muhovic-Pasic et al. 2022	Cervix	375	NA	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 Low-risk HPVs: 6, 11	2018: (54.43); 2019: (42.33); 2020: (39.08); 2021: (31.81)	HPV-16: 2018: (13.92); 2019: (11.04); 2020: (9.19); 2021: (13.03)	Multiplex PCR reaction
Gavrankapetanovic et al. 2022	NA	1517	Mean 33 (range 18 to 61)	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	(43)	HPV-16: (22.5)	Real-time PCR
Sadiković et al. 2020	Cervix	800	18 to 40	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	(33.5)	High-risk HPV: (33.5)	Hybrid capture II HPV test
Božić et al. 2020	Head and neck	50	NA	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68)	(22)	NA	Single PCR Nested PCR Real-time PCR
Jahic et al. 2020	Cervix	11051	NA	HPV 16,18,31, 35,39,45,51, 52,56,58,59, 66,68	NA	HPV-16: (50.5)	HPV in situ hybridization HPV genotypes 14 Real-TM Quant
Al-Thawadi et al. 2020	Head and neck	98 of 123 had interpretable results	Mean 62.8	High-risk HPVs: 16, 18, 31, 33, 35, 45, 51, 52, 58	(50)	HPV-18: (56)	PCR IHC
Gupta et al. 2020	Colorectal (96% rectal cancer)	106	Mean 65 (range 41 to 86)	High-risk HPVs: 16, 31, 18, 51, 52, 45	(80)	HPV-16: (53)	PCR IHC
Salimović-Bešić et al. 2019	Cervix	105	Average: Younger group 26.2; Range 19-30 Older group 40.9; Range 31-62	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	HPV DNA test: Younger group (83.9); Older group (67.6) DNA and mRNA test: Younger group (75.8) Older group (83.9)	HPV-16 in women aged ≤30 years: DNA test (32.1); mRNA test (26.4) HPV-16 in women aged >30 years: DNA test (33.0); mRNA test (29.8)	Real-time PCR amplification (HPV High Risk Typing Real-TM test) RNA-based assay: Real-time NASBA reactions

Continuation of Table 1

Authors	Cancer subtype	Participants (N)*	Age	HPV subtype	HPV (%)†	Most common HPV subtype (%)§	Detection method
Jahić et al. 2017	Cervix	3244	Average 41	High-risk HPV	NA	High-risk HPV in women with ASCUS (51); LSIL (71);	NA
Radić et al. 2017	Cervix	101	NA	HPV types: 11, 16, 18, 31, 35	(17.7)	HPV-16: (35.3)	Multiplex PCR
Jahić et al. 2016	Cervix	1784	Average 37.6	High-risk HPV	NA	High-risk HPV in women with ASCUS (51); CIN 1 (88)	In situ by hybridization
Salimović-Bešić et al. 2015	Cervix	105	Average 36.6	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 Probable high- risk HPVs: 53, 66 Low-risk HPV: 70	(50.4)	HPV-16: (32.6)	Multiplex real-time PCR test
Iljazović et al. 2014	Cervix	283	Mean 51.7	High-risk and low-risk HPVs: 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74	(94.7)	HPV-16: (95.5)	SPF-10 broad spectrum primers followed by deoxyribonucleic acid enzyme immunoassay and genotyping by reverse line probe assay (LiPA25, version 1)
Asotic et al. 2014	Cervix	6376	NA	NA	NA	HPV positive: CIN I (43.10); CIN II (27.93); CIN III (25.69); CIS (0.52); Normal findings (2.76)	PCR
Salimović-Bešić et al. 2013	Cervix	105	Average 31.6	High-risk HPVs: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59	(72.4%	HPV-16 in ASCUS (20.8); LSIL (30.6); HSIL (48.9)	HPV typing: multiplex real-time PCR mRNA typing: real-time NASBA assay
Poljak et al. 2013‡	Cervix	NA	NA	NA	NA	NA	NA
Bray et al. 2013‡	Anogenital, Head and neck	NA	NA	NA	NA	NA	NA
Seme et al. 2013‡	Cervix	NA	NA	NA	NA	NA	NA
Jahic et al. 2013	NA	100	35.7	High-risk and low-risk HPVs	NA	HPV positive in: LSIL (46); HSIL (48.9); normal Pap test (14)	Digene HPV test, Hybrid capture
Salimović-Besic et al. 2007	Cervix	148	NA	HPVs: 6, 44, 53, 66, 68, 72, 73	NA	NA	Hybrid Capture 2 HPV DNA PCR-PGMY11/ PGMY09 PCR-CPI/CPIIG
Iljazović et al. 2006	Cervix	55	NA	NA	NA	High-risk HPV after three months of therapy: (71.4)	Digene HPV Test-Hybrid Capture II

*Tested; †Prevalence; ‡Positive; §Prevalence. These studies address HPV-related cancer in Bosnia and Herzegovina but do not provide specific data regarding HPV positivity and HPV prevalence; HPV=Human papillomavirus; NA=Not applicable; PCR=Polymerase chain reaction; DNA=Deoxyribonucleotide acid; IHC=Immunohistochemistry; NASBA=Nucleic acid sequence-based amplification assay; ASCUS=Atypical squamous cells of undetermined significance; LSIL=Low-grade squamous intraepithelial lesion; HSIL=High-grade squamous intraepithelial lesion; CIN=Cervical intraepithelial neoplasia; CIS=Carcinoma in situ.

Cervical Cancer

Pathogenesis, Progression, and Types of Cervical Cancer

Cervical cancer develops due to persistent infection with high-risk HPV types, particularly HPV-16 and HPV-18 (34). HPV evades the host immune system by downregulating immune responses, allowing the infected cells to persist and proliferate (35). The mechanism by which is this happening is the virus entry in the basal cells of the cervical epithelium through micro-abrasions, following genome integration into the host DNA (36). This integration leads to the overexpression of viral oncogenes E6 and E7, which are central to the pathogenesis of cervical cancer. The E6 protein binds to and degrades the tumor suppressor protein p53, preventing the normal process of apoptosis and allowing cells with damaged DNA to survive and proliferate (37). Simultaneously, the E7 protein inactivates the retinoblastoma protein (pRb), leading to uncontrolled cell cycle progression and increased cellular proliferation (38). Persistent chronic inflammation due to the infection creates a microenvironment conducive to cancer development, with inflammatory cytokines and reactive oxygen species inducing DNA damage (37, 38). In advanced stages, it can metastasize to distant organs such as the lungs, liver, and bones (39). Clinically, early-stage disease may be asymptomatic or present with nonspecific symptoms like abnormal vaginal bleeding or discharge (37-39). The two primary types of cervical cancer are squamous cell carcinoma (SCC) and adenocarcinoma, accounting for 69% and 25% of all cases. The remaining ~6% include small cell (neuroendocrine) carcinoma (SCNC), primary cervical lymphomas, and soft tissue tumors, such as rhabdomyosarcoma (40-42). SCC typically progresses through a series of precancerous stages, known as cervical intraepithelial neoplasia (CIN). Screening methods such as the Pap test and HPV DNA testing play a critical role in detecting these precancerous changes, allowing for early intervention and reducing the risk of progression to invasive cancer (43). The treatment for SCC often involves surgical intervention, especially in the early stages, which may include conization, hysterectomy, or trachelectomy. In more advanced cases, radiation therapy, chemotherapy, or a combination of both is commonly employed. A literature search identified a single study on the treatment of cervical cancer in BH.

This study reported promising outcomes for inoperable locally advanced cervical cancer treated with chemobrachyradiotherapy. The treatment included external radiotherapy, concurrent low-dose brachytherapy with cisplatin and ifosfamide, and consolidation treatment with the same chemotherapy. The five-year local control rate was 94%, disease-free survival was 72.8%, overall survival was 76.6%, disease-specific survival was 88% and toxicity was acceptable (44). The outcomes of this study were similar to those reported in the original study by Vrdoljak et al., which used an identical protocol (45). Recent advances in targeted therapies and immunotherapies are also being explored, particularly in recurrent or metastatic SCC, showing potential in improving patient outcomes (46, 47). Adenocarcinoma constitutes about 25% of all cervical cancer cases and originates from the glandular epithelial cells lining the endocervix. Unlike SCC, adenocarcinoma arises from the mucus-producing cells and has distinct pathological and clinical features. This subtype is also strongly linked to HPV infection, particularly HPV 18, but it tends to be more challenging to detect through conventional Pap smears because the glandular cells are located higher up in the cervix, often beyond the reach of standard sampling techniques (48, 49). Histologically, adenocarcinoma can be further subclassified into several subtypes among which endocervical adenocarcinoma is the most common (49, 50).

The clinical management of adenocarcinoma mirrors that of SCC, with early-stage disease often treated surgically and advanced disease requiring chemoradiation. However, due to its unique biology and the challenges associated with its detection, adenocarcinoma often necessitates more specialized diagnostic and therapeutic approaches (51). SCNC is a rare but highly aggressive form of cervical cancer, characterized by small, round cells

that resemble those seen in small-cell lung cancer (52). It is associated with a poor prognosis due to its rapid growth and high likelihood of metastasis. SCNC is also linked to HPV infection, particularly HPV 18. Treatment typically involves a combination of surgery, chemotherapy, and radiotherapy, but outcomes remain poor (53). Recent molecular studies provide some opportunities for targeted treatments, given that a subset of SCNCs may harbor PIK3CA/PTEN/AKT and programmed cell death protein 1/PD-L1 alterations (52).

Epidemiology of Cervical Cancer in BH

Cervical cancer represents a significant public health problem in BH, as in many other countries. The incidence is generally lower than in other regions, but it remains a concern. According to the data reported by the Institute of Public Health of the FBiH, which represents approximately 70% of the total population of BH, in 2020 cervical cancer was the fifth most diagnosed cancer in women with a crude incidence of 10.0/100,000 women (54). With 67 registered deaths in 2020, cervical cancer was the 10th leading cause of cancer-related death in women. In BH, much of the available data relies on estimates from neighboring countries and reports from the World Health Organization (WHO) and the Catalan Institute of Oncology (ICO)/International Agency for Research on Cancer (IARC) (7, 55).

Current estimates by the HPV Information Centre indicate that there are about 312 new cases of cervical cancer diagnosed in BH annually, which ranks cervical cancer as the 6th most frequent female cancer in this country. According to these estimations, 153 women die from cervical cancer every year, which places cervical cancer as the ninth leading cause of cancer deaths in BH (56). As reported by GLOBOCAN 2022, the estimated age-standardized rates (ASR) for cervical cancer are 14.1 per 100,000 for incidence and 7.1 per 100,000 for mortality (57), which is less than reported in 2018, when the age-standardized incidence rate was estimated at 23.9/100,000, with an age-standardized mortality rate of 7.9/100,000 (58).

There is much inconsistency in the available data regarding the epidemiology of cervical cancer in BH, which is mainly the result of irregular reporting of newly diagnosed cases, but also due to the high burden of the health care system, deficiency of health care workers and the lack of infrastructure for efficient, reliable and timely health information system (59). The latest study investigating the epidemiology of cervical cancer in BH was published ten years ago, where the reported crude incidence in Tuzla Canton varied from 18.5 in 2005 to 4.8/100,000 in 2000 (55).

Comparison with Global and Regional Statistics

Cervical cancer is the most common HPV-related malignancy in the female population, as shown in Figure 1. Women at risk for cervical cancer (Female population aged >=15 yrs) is roughly estimated to be 1.41 million. According to the estimates, the overall cancer incidence rate is 218.6 cases per 100,000 persons per year. According to GLOBOCAN, in 2022 cervical cancer was the fourth most common cancer in terms of both incidence and mortality in women worldwide, with an estimated 660,000 new cases and 350,000 deaths. It is the most diagnosed cancer in 25 countries and the leading cause of cancer death in 37 countries (57). Survival rates for cervical cancer often vary according to a country's level of development. Around 84% of cervical cancer cases and 88% of related deaths occur in developing countries (60). In contrast, among developed nations, the five-year survival rates are much higher. In the United States, the five-year survival rate is 91% for localized disease, 60% for regional disease, and 19% for metastatic disease. Similar data are reported in the United Kingdom, with a five-year survival rate of 95% for stage I, 70% for stage II, 40% for stage III, and 15% for stage IV (61, 62). There are large disparities in incidence and mortality between different countries, with about a 10-fold variation, the highest rates being recorded in Eastern Africa (incidence 40.4/100,000 and mortality 28.9/100,000) and the lowest rates found in Western Asia (incidence 4.1/100,000 and mortality 2.2/100,000)

(57). Variance is also present between different European regions, with an age-standardized incidence rate of 15.7, mortality of 6.3/100,000 in Eastern Europe, and an incidence of 6.4 and mortality of 2.2/100,000 in Southern Europe. This is probably due to the different prevalence of chronic HPV infections and limited access to screening and vaccination in developing countries.

In the Summary Report published by the ICO/IARC Information Centre on HPV and Cancer, there were 58,169 new cervical cancer cases annually in Europe (estimations for 2020), which ranks cervical cancer as the 9th leading cause of female cancer and the 3rd most common female cancer in women aged 15 to 44 years in Europe. There are huge variations between different European regions and countries, with the highest age-standardized incidence rate of cervical cancer cases attributable to HPV recorded in Montenegro (26.2/100.000 women), Romania (22.6/100,000), Serbia and Lithuania (18.7/100,000) and the lowest in Switzerland (3.4/100,000), Malta (3.7/100,000), Luxembourg and Finland (5.2/100,000). Compared to other European countries, BH is ranked 11th with 14.3 per 100,000 women (56). In Croatia, where organized screening has been present since 2012, the age-standardized incidence rate is estimated at 10.1 per 100,000 women. In Croatia, the incidence is still relatively high, with 276 cases annually (ASR 11.0/100,000), as is the mortality (ASR 4.2/100,000) (63). The age-standardized mortality rates per 100,000 women per year in BH are 5.2, while in Serbia, Croatia, and Montenegro, the rates are estimated at 7.9, 3.2, and 10.5, respectively (56).

Local Risk Factors for Cervical Cancer

Cervical cancer represents 1.8% of all cancer cases, with a corresponding mortality rate of 1.8% (7). In the 2014 analysis, most cases (92.2%) were histologically classified as SCC, and 95% tested positive for HPV. Infections were predominantly single, accounting for 95.5% of cases, with HPV-16 and 18 being the most prevalent, responsible for 77.8% of the positive cases. Other notable HPV types included HPV-45 (4.4%), HPV-33 (3.1%), HPV-51 (2.3%), and HPV-31 (2.2%). The mean age of individuals infected with the seven most common HPV types globally HPV-16, HPV-18, HPV-45, HPV-31, HPV-33, HPV-52, and HPV-58—was 51.1 years (with a standard deviation of 11.6 years). This is notably younger by six years compared to individuals infected with other HPV types, whose mean age was 56.3 years (with a standard deviation of 12.9 years) (11). Various reports from BH indicate a prevalence of cervical HPV infection ranging from 17% to 72% (Table 1) (9, 17, 20-23, 25, 26, 30-33). The five studies listed in Table 1 explored HPV-related cancer in BH but lacked specific information on HPV positivity and prevalence specific to the country (15, 16, 27-29). Furthermore, the data from these studies primarily reflected HPV status across Central and Eastern Europe.

In BH, various risk factors contribute to the prevalence of HPV infections and related cancers, particularly cervical cancer. These factors are shaped by socioeconomic challenges, cultural norms, and a fragmented healthcare system. One significant issue is the limited access to healthcare services, including regular cervical cancer screenings and HPV vaccinations. Socioeconomic disparities further compound this issue, as poverty and economic instability limit access to healthcare. Many individuals, particularly from low-income backgrounds, may not prioritize or afford preventive measures, such as screenings and vaccinations. Additionally, educational barriers and a lack of awareness about HPV contribute to high-risk behaviors and delayed diagnosis (64, 65). Cultural and social norms also influence HPV risk factors. Sexual behaviors, such as early initiation of sexual activity, multiple sexual partners, and inconsistent condom use, are associated with increased HPV transmission. In BH, cultural stigmas surrounding sexual health may limit open discussions and reduce awareness about protective measures. Traditional gender roles may further restrict women's access to preventive care and hinder their ability to discuss HPV-related concerns with healthcare providers. The fragmented healthcare

infrastructure in the country presents another challenge. Under-resourced and disjointed, the healthcare system struggles to implement comprehensive HPV prevention and treatment programs. This fragmentation hampers efforts to accurately assess the burden of HPV-related diseases and target interventions effectively (55). Additional risk factors include smoking and co-infections. Smoking is a known co-factor that can exacerbate the risk of HPV-related cancers, particularly cervical cancer, and its prevalence in BH may contribute to higher cancer rates among HPV-infected individuals (66). Up to 41% of adults in BH consume cigarettes (67). Other factors, such as co-infections with other sexually transmitted infections, such as chlamydia, also increase the risk of HPV persistence and progression to cancer, further compounded by limited sexual health education and resources (68).

Other HPV-Related Cancers

Vulvar Cancer: Epidemiology, Risk Factors, and Treatment

Vulvar cancer is the twenty-ninth most prevalent cancer in women worldwide, with around 47,342 new cases reported in 2022 (57). In Europe, it represents the nineteenth most common cause of cancer incidence in women with approximately 16,506 new cases in 2020 (69). According to data from the SEER database, five-year survival rates vary by stage: 85.6% for localized disease (stages I/II), 47.5% for regional or locally advanced disease (stages III/IVA), and 23.3% for stage IVB, which encompasses patients with pelvic nodal involvement (70). In geographic terms, there is around a 30-fold variation in the recorded incidence rates of vulvar cancer with the higher incidence in seven countries and three continents, amongst them Bahrain, Germany, the Netherlands, Canada, France, Australia, and the United Kingdom (71, 72). The incidence rates of vulvar cancer are approximately 2-fold higher in high-income countries (ASR = 1.56 per 100,000) than in low- and middle-income countries (ASR = 0.6 per 100,000), while the difference in mortality rates is less pronounced (ASR = 0.35 vs ASR = 0.27) (73). In Central and Eastern Europe and Central Asia, vulvar cancer incidence and mortality rates are 2-3 times higher than other anogenital cancer sites (28). The estimated age-standardized incidence in Europe is 1.68/100,000 women with a mortality rate of 0.51/100,000. The highest incidence in Europe is recorded in Germany, with an ASR of 3.61/100.000 and a mortality rate of 0.71. According to National Cancer Registry data, around 350 new cases of vulvar cancer are diagnosed in Poland each year, with 200 women dying from the disease (74). Croatia ranked tenth among EU-27 nations for age-standardized vulvar cancer incidence in 2022 and second in terms of vulvar cancer fatality estimates. Eastern Europe (Slovakia, Romania, Hungary, and Poland) had the highest fatality rates for vulvar cancer, with Germany ranking fourth (75). According to the Croatian National Cancer Registry data, 1451 women were diagnosed with invasive vulvar cancer and 814 women died due a vulvar cancer in the period 2011-2019. In BH, an estimated number of new cases of vulvar cancer for 2020 was 42 (76). The incidence is estimated at 1.06 and mortality at 0.57/100,000, which is lower than in Croatia and Serbia, where the estimated incidence is 1,67 for both countries and mortality at 0.54 and 0.69, respectively. Montenegro has the highest estimated mortality rate (0,90/100,000) in Europe, while the incidence rate of 1.36/100,000 is similar to other countries in the region (56).

The epidemiology disparities between different countries and regions are most likely a result of the availability of screening programs since the detection of vulvar cancer is linked to screening for cervical cancer. Another factor could also be the lack of public awareness, social, religious, and cultural differences, but also the lack of national cancer registries and adequate reporting of new cases (72).

Vulvar cancer primarily affects elderly women. More than 60% are keratinizing vulvar SCC (VSCC), followed by the basaloid type which is more common in young women and linked mostly to HPV-16 (72, 77, 78). Age, the presence of HPV, tobacco use, HIV infection, vulvar

intraepithelial neoplasia, and lichen sclerosus are the most common risk factors for vulvar cancer (77). As previously mentioned, basaloid carcinomas are more likely to be HPV-positive than keratinizing carcinomas. They share HPV-related factors with cervical cancer, such as lifetime sexual partners, age at first intercourse, and cigarette smoking. However, their etiologies differ (71). 30-60% of VSCCs are HPV related with significant variation across studies (78, 79). According to WHO 2020 classification, ESGO, and NCCN guidelines, it is mandatory to stratify VSCC into HPV-associated and HPV-independent using p16 immunohistochemistry (77, 80, 81). In addition, HPV-independent VSCC is divided into two categories: p53 mutant (p53mut) and p53 wild-type (p53wt), and therefore it is recommended to assess p53 status according to NCCN and ESGO guidelines for the proper management of patients with VSCC (77, 80). There is growing evidence that HPV-associated and p53wt cancers may have a better prognosis than those p53mut. Among retrospectively analyzed 413 samples of VSCC, the 5-year overall survival was 83% for HPVpos VSCC, 64% for HPVneg/p53wt VSCC, and 48% for HPVneg/p53mut VSCC. Women with HPVpos VSCC were younger at surgery (59 years) than those with HPVneg/p53wt VSCC or HPVneg/p53mut VSCC (73 and 75 years, respectively). The majority of patients with HPVpos VSCC (79%) or HPVneg/p53wt VSCC (81%) tumors had stage I/II disease, contrary to (57%) HPVneg/p53mut VSCC (Table 2) (82). In addition, a meta-analysis evaluating 18 studies, including 475 women with VSCC, reported that HPV-associated VSCC showed a significant correlation between p16pos/p16neg and overall survival (ranging from 62% to 81% vs 22% to 47% in 5-year OS).

Among them, four studies in this meta-analysis reported an overall survival according to p53, including 310 women with VSCC of which 166 (53.5%) were p53 positive and 144 were p53 negative. Women with p53 positive VSCC had a significantly worse 5-year OS (ranging from 35-63%) compared to p53 negative (ranging from 68-70%) (Table 2) (83). In the era of personalized medicine, a potential strategy to tackle high operation morbidity and pre-operative risk assessment based on the molecular subtype of VSCC is valuable in tailoring surgery, patient counseling, and planning adjuvant treatment for the patient's risk profile. A single study's findings revealed that the concordance of preoperative and postoperative molecular subtypes in a relatively small number (N=57) of samples was 91.2%. These findings could assist in therapy tailoring, particularly given the less aggressive behavior of HPV-associated VSCC and the fact that these cancers occur in younger

Table 2. Overview of the Relevant Studies Examining the Survival of Patients with HPV-Related Cancers According to p16, p53, and HPV Status

Author	Tumor type	Tested patients (N)	HPV Subtype(s)	Molecular subtype	OS (%)	Detection method
Kortekas et al.	Vulva	413 75 275 63	HPV-16, 18, 33	HPVpos HPV neg/p53mut HPV neg/p53wt	5y OS (83) (48) (64)	IHC
Sand et al.	Vulva	475 181 294 310 166 144	NA	p16pos p16neg p53pos p53neg	5y OS range (62-81) (22-47) 5y OS range (35-63) (68-70)	IHC
Feldbaum et al.	Vagina	43	NA	p16pos p16neg	Mean 49.5 months 25.3 months	IHC

OS=Overall survival; IHC=Immunohistochemistry; ISH=In-situ hybridization; HPV=Human papillomavirus; RNA=Ribonucleic acid; DNA=Deoxyribonucleotide acid; PCR=Polymerase chain reaction; NA=Not applicable.

women. On the contrary, in older and frail patients unfit for upfront surgery, in the light of recently published studies, definitive or neoadjuvant chemoradiotherapy or chemotherapy, which have shown durable responses, could also be an option (84-86). Comprehensive genomic profiling among HPV-associated and HPV-independent VSCC showed two distinct entities (87). HPV-positive VSCC exhibited PI3K/mTOR pathway mutations and was enriched in *FGFR3* and *PTEN* mutations (87, 88). In a cohort of HPVpos VSCC, 61% of tumors had genetic mutations in the PI3K/mTOR pathway. HPV-positive cancers sequenced from metastases had a significantly greater rate of *STK11* mutations, a negative regulator of mTOR signaling, than HPV-positive tumors sequenced from primary cancer (88). Depending on the stromal invasion, local treatment is recommended, or a wide local excision (T1a ≤1 mm of stromal invasion) or a radical partial vulvectomy (T1b >1 mm of stromal invasion), especially in cases with multifocal involvement, to obtain surgically negative margins (according to recent guidelines, a pathological minimal margin of >2-3 mm seems adequate). Groin treatment should be performed for tumors greater than T1a and could be performed in various ways depending on the tumor size and distance from midline; radical partial vulvectomy and ipsilateral inguinofemoral lymphadenectomy with or without sentinel lymph node biopsy or radical partial vulvectomy and bilateral inguinofemoral lymphadenectomy or a sentinel lymph node biopsy in selected cases. Adjuvant treatment (radiotherapy or chemoradiotherapy) is advised for patients with a positive margin and lymph node involvement. In patients with locally advanced inoperable VSCC, primary chemoradiotherapy or neoadjuvant platinum-based chemotherapy is recommended after a thorough multidisciplinary assessment in selected cases (77, 80). Systemic therapy, platinum-based chemotherapy, is recommended in a metastatic setting or recurrent, inoperable disease. In the post-progression setting, there are no standard treatments, although chemotherapy, VEGF inhibitors, immune checkpoint inhibitors, EGFR inhibitors, or, in the case of a *NTRK1-3*-positive tumor, larotrectinib or entrectinib, could be considered (77, 80, 89, 90). New promising approaches to the treatment of HPV-related cancers include adaptive T cell therapy (clinical trial NCT01585428, in which two of nine patients with metastatic cervical cancer had complete responses), cancer vaccines (ISA101, a peptide vaccine developed for HPV-related cancers), and intra-tumoral oncolytic viral therapy (79). A clinical trial using a T cell receptor that targets the E7 antigen is currently enrolling patients (NCT02858310) (91).

Vaginal Cancer: Epidemiology, Risk Factors, and Treatment

Primary vaginal cancer accounts for only 2% of female genital tract cancers in adulthood, with an estimated incidence of 18,800 new cases diagnosed worldwide in 2022 (57, 92). A report using population-based cancer registries found a threefold variance in recorded incidence rates for vaginal cancer. However, vaginal cancer incidence was lower and more stable than vulvar cancer, despite the larger HPV-attributable fraction among cohorts born 1940-50 and afterward. The Dominican Republic has the highest risk of vaginal cancer (ASR=2.7 per 100,000), followed by Malawi and Zambia, with rates of 1.4 and 1.3 per 100,000, respectively (71). According to GLOBOCAN, vaginal cancer ranks 33rd worldwide in terms of both incidence and mortality. The age-standardized rate (ASR) for incidence is 0.36 per 100,000, while the ASR for mortality is 0.15 per 100,000 (57). In Europe, the ASR of vaginal cancer incidence for 2020 is estimated to be 0.33 per 100,000, with the highest rates observed in Northern Europe at 0.38 per 100,000. An estimated number of new cases of vaginal cancer for 2020 in BH was 14 (93). The incidence is estimated at 0.41 per 100,000, which is higher than the rates in Croatia and Serbia, both of which have an incidence rate of 0.33 per 100,000. Conversely, Montenegro has the highest estimated incidence rates in Europe, with an ASR of 0.74 per 100,000 women (56). These data are comparable to those observed for cervical cancer, likely

attributable to the prevalence of chronic HPV infection, which is a major risk factor for both cervical and vaginal carcinoma. In adults, only 10% are vaginal-originating cancers, whereas the rest are spread from other locations such as the cervix, endometrial, vulva, and rectum (94). The majority of primary vaginal SCC cases are HPV-associated (Table 3); thus, the risk factors for vaginal SCC are the same as those for cervical cancer: multiple lifetime sexual partners, early age at first intercourse, and smoking. A history of vaginal adenosis [related or not to diethylstilbestrol (DES)] is another risk factor for some kinds of adenocarcinoma, as is past DES exposure and endometriosis (92). Individuals with AFAB (hysterectomized individuals assigned female at birth) and pre-existing cervical intraepithelial neoplasia are more than twice as likely to develop vaginal cancer (95). SCC is the most frequent histologic type accounting for ~90% of cases (92, 95). Persistent infection with high-risk HPVs has been found in vaginal malignancies, as well as 85-90% of vaginal intraepithelial neoplasia grades 2 and 3 (VaIN2). The most frequent form, HPV-16, is seen in 46-77% of vaginal malignancies. HPV-18 has been detected in lower percentages (78, 96).

Primary vaginal adenocarcinomas are very uncommon, while other morphologic subtypes are rarer (92, 95). In 2020, the WHO modified the categorization of female genital tumors and recommended a distinction between HPV-associated and HPV-independent vaginal SCC (81). A retrospective assessment of 43 vaginal cancer patients found that those with p16- positive diffuse staining had a significantly higher survival rate (~50 months) compared to those with p16-negative disease (~25 months) (Table 2) (97). The most important prognostic factors for vaginal cancer are the stage at diagnosis, tumor size greater than 4 cm, age, and tumor position outside of the upper region of the vagina. Adenocarcinoma has a worse prognosis than squamous cell carcinoma (98, 99). Patients with vaginal cancer are often treated with radiation, surgery, chemoradiation, or a combination of these treatments, regardless of their cancer subtype and HPV infection status. Treatment options differ by stage (92, 95). There are two options for early curative management: surgical excision (with microscopically clear margins without unnecessary morbidity) or chemotherapy and radiation therapy. Primary chemoradiotherapy involves external beam radiation (EBRT), brachytherapy, and cisplatin-based chemotherapy as the recommended protocol for stages II-IVA disease. Cisplatin-based chemotherapy should be administered concurrently. When cisplatin is not an option for vaginal cancer treatment, carboplatin or radiotherapy may be used instead. Premenopausal women should be informed about ovarian transposition early on (92, 98). For patients with limited distant (oligo-) metastatic disease at presentation, curative treatment options include stereotactic radiation, surgery, and radiofrequency ablation. Because vaginal cancer is a rare entity and similar to cervical cancer, treatment decisions are often based on cervical cancer guidelines. According to current guidelines for metastatic disease next-generation sequencing (NGS) and comprehensive molecular profiling are recommended. The following biomarkers should be tested: PD-L1, tumor mutational burden (TMB), p53, *RET* fusion, MSI-H, *NTRK1-3* fusions, and HER2 (95). The current standard of care for PD-L1 positive metastatic disease is a combination of cisplatin-based chemotherapy and pembrolizumab with or without bevacizumab, based on the results of KEYNOTE-826 study that revealed a statistically significant improvement in PFS, OS, and ORR (100). The addition of pembrolizumab to chemotherapy with or without bevacizumab continued to show significant survival benefits in PD-L1-positive tumors at a median follow-up of 39.1 months, with a median OS and PFS of 28.6 and 10.5 months versus 16.5 and 8.2 months in the pembrolizumab plus chemotherapy arm versus the placebo plus chemotherapy arm (101). Otherwise, based on the GOG-240 study, adding bevacizumab to cisplatin-based chemotherapy in the first-line setting of metastatic, persistent, or recurrent cervical cancer resulted in a substantial improvement in OS among patients receiving bevacizumab, especially in patients who were not treated with prior pelvic radiotherapy (102). Additional therapy options in

Table 3. Overview of the Representative Studies Reporting HPV Prevalence in HPV-Related Cancers

Author	Tumor type	Tested participants (N)	Age	HPV subtype	HPV positive (%)	Most common HPV subtype (%)*	Detection method
Komloš et al. 2011	Anal (invasive and in situ)	21	NA	High-risk and low-risk HPV; 6, 16, 52, 61	95.8	HPV-16 (90.5)	GP5+/6+ PCR / Inno-LiPA
Tachezy et al. 2007	Anal squamous cell carcinoma	22	Mean = 64.2; Range 47-86	High-risk HPV 16	81.8	HPV-16 (81.8)	GP5+/6+ PCR / RLB and sequencing
De Vuyst et al. 2009	Anal (29 studies)	955	NA	HPV-18 HPV-33	84.3	HPV-16 (73.4)	PCR
	Vaginal (14 studies)	136	NA	HPV-18 HPV-31	69.9	HPV-16 (53.7)	
	Vulvar (63 studies)	1,873	NA	HPV-33 HPV-18 HPV-6 HPV-11	40.4	HPV-16 (32.3)	
Frisch et al. 1997	Anal (invasive and in situ)	388	Median = 63; Range 26-94	High-risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 Low-risk HPV, 6, 11, 40, 42, 43, 44	84	HPV-16 (73.4)	PCR
Škamperle et al. 2013	Anal (3 studies)	43	NA	HPV-16, HPV-6, HPV-52, HPV-61	Overall HPV DNA prevalence = 90.7	HPV-16 (94.9)	PCR (MY09/11 / DBH and sequencing GP5+/6+ PCR / RLB and sequencing HPV-16/18 TS PCR and Linear Array® MY09/11 / HPV-16 TS PCR and sequencing HPV-16/18 TS PCR MY09/11 PCR / RFLP MY09/11 PCR / pU-1M/2R PCR GP5+/6+ PCR / IA and RLB Inno-LiPA® MY09/11 PCR / HPV-16/18 TS PCR HPV-16, -18, -33 TS PCR E6-E7 consensus PCR / HPV-16, -18, -31, -33, -45, -52, -59, -68 TS PCR)
	Cervical (24 studies)	2,531	Mean 50.2	HPV-16, HPV-18, HPV-31, HPV-33, HPV-45, HPV-35, HPV-39, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-68	Overall HPV DNA prevalence= 86.6	(59.6)	
	Vulvar (3 studies)	164	NA	HPV-16, HPV-33, HPV-45, HPV-58, HPV-6, HPV-42	Overall HPV DNA prevalence= 32.9	(22)	
Šimić et al. 2023	Oral cavity, oropharynx	76	Median= 61	High-risk HPV 16, 18	23.7	HPV-16 (77.7)	PCR

*Prevalence. Inno-LiPA®=INNO-LiPA HPV Genotyping Extra test (Innogenetics NV, Ghent, Belgium) or INNO-LiPA HPV genotyping test (Labo Biomedical Products, Rijswijk, the Netherlands); Linear Array®=Linear Array® HPV genotyping test (Roche Molecular Systems Inc., Alameda, CA, USA); PCR=Polymerase chain reaction; TS PCR=Type-specific PCR; DBH=Dot-blot hybridization, RLB=Reverse line-blot hybridization; IA=Immuno-assay–enzyme-linked oligosorbent assay; NA=Not applicable; HPV=Human papillomavirus; DNA=Deoxyribonucleotide acid.

subsequent lines include chemotherapy, immune checkpoint inhibitors (pembrolizumab, cemiplimab, nivolumab), biomarker-specific therapies based on agnostic tumor approvals (trastuzumab deruxtecan for HER2 positive tumors, selpercatinib for *RET* gene fusion-positive tumors, and TRK inhibitors for, *NTRK1-3* fusion-positive tumors) (103-109). Tisotumab vedotin (TV) is an

ADC (antibody-drug conjugate) that consists of an anti-TF (tissue factor) monoclonal antibody covalently linked to the microtubule-disrupting agent MMAE via a protease-cleavable linker. It is FDA-approved for the treatment of recurrent or metastatic cervical cancer with disease progression on or after chemotherapy based on phase 3, randomized, innovaTV-301/ENGOT-cx12/GOG-3057 where patients receiving TV had a 30% reduction in risk of death versus chemotherapy (110). Ongoing developments in the management of locally advanced cervical cancer include the use of immune-checkpoint inhibitors with chemoradiotherapy (KEYNOTE-A18 study (NCT04221945), and in recurrent/metastatic cervical cancer, TV might be established as a first-line treatment (111, 112).

Anal Cancer: Epidemiology, Risk Factors, and Treatment

Anal cancer is a rare disease that accounts for <3% of all gastrointestinal cancers with an annual incidence of 0.5-2.0 in 100,000 (113). Five-year overall survival (OS) rate improved from a mean estimate of 64% in 1980 to 75% in 2010 (114). Based on GLOBOCAN 2020, anal cancer ranks 30th globally in terms of both incidence and mortality (69). In 2020, there were 54,194 new cases (23,999 males and 30,195 females) of anal cancer worldwide, involving the anal canal, or anorectum (57). However, there are varying reports indicating an increase in incidence among both men and women over the past 20 years, particularly in high-income countries (113, 115). According to IARC, the estimated age-standardized incidence rate for anal cancer in Europe for 2020 was 0.66/100,000 for men and 1.05/100,000 for women, with the highest incidence for men recorded in Germany (1.20/100,000) and for women in France (2.53/100,000). The incidence rate estimated for BH was 0.19 for women and 0.20 for men, which is lower in comparison with Croatia, Serbia, and Montenegro, where the rates were estimated at 0.27, 0.36, and 0.38 for women and 0.34, 0.55, and 0.77 for men, respectively. The estimated mortality rate in Europe was 0.24 for women and 0.22 for men, with the highest recorded in the Czech Republic for both women (0.42/100,000) and men (0.55/100,000). The mortality rate for BH is estimated at 0.12/100,000 for men and 0.08 for women, which is lower than in Serbia, where it was estimated at 0.29 for men and 0.14 for women (56). As reported by GLOBOCAN 2022, BH is one of the countries with the lowest ASR of incidence (0.16) and mortality (0.04) in Europe (57). However, these estimates have a high degree of uncertainty because they are not derived from population-based cancer registries.

SCC constitutes 80–85% of all anal cancers. Adenocarcinomas are the second most common type, accounting for 5–18% of all cases, and in that case, should be treated as low rectal cancers (116). Anal carcinoma has been associated with HPV infection (anogenital warts), a history of receptive anal intercourse or sexually transmitted diseases, a history of genital tract cancer, immunosuppression from solid organ transplantation, or HIV infection. In addition, other risk factors include smoking, autoimmune disorders, and hematologic malignancies (116, 117). However, persistent infection with high-risk HPV variants (e.g., HPV-16, HPV-18) is strongly linked to anal cancer (118, 119). The prevalence and distribution of HPV in anogenital cancers in 16 Central and Eastern European countries including BH ranged from 81.8% to 100% (120). Overall, 37 (94.9%) of 39 HPV DNA-positive anal malignancies from the Slovenian and Czech cohorts were positive for HPV-16 (120-123). Meta-analysis of anogenital cancerous and precancerous lesions found the highest HPV prevalence in anal cancer (84.3%), predominately HPV-16 (73.4%) (78). A large study of tumor specimens of anal cancer discovered a high prevalence of high-risk HPV DNA in 84% of anal cancer specimens, particularly HPV-16, which was detected in 73% of them (Table 3) (124). Contrary to that, high-risk HPV was not found in any of the rectal cancer tissues tested, although various reports lately have stated the potential link between HPV and EBV co-presence as a possible contributing element to colorectal cancer development (124, 125). Among them, a report on the Qatari population found the presence of high-risk HPV in 52%

of colorectal cancer samples, whereas coinfection with more HPV subtypes was strongly correlated with advanced-stage colorectal cancer (125, 126). In addition to that finding, the high prevalence of high-risk HPV types (HPV-16 and HPV-18) among colorectal cancer samples in the Bosnian population was ~50% (Table 1) (14). Further research is needed to more thoroughly evaluate the potential role of the presence of high-risk HPV in colorectal carcinogenesis. An early-stage perianal disease that does not affect the anal sphincter and superficially invasive SCC of the anus can be treated with local excision, where negative margin excision can be accomplished without compromise of the adjacent sphincter muscles.

Combined chemoradiotherapy is the primary therapeutic preference for locoregional anal cancer (127). Over the past four decades, the current standard of care has been the combined modality of 5-FU (capecitabine) and mitomycin with radiotherapy where intensity-modulated radiotherapy is the preferred modality over 3D-conformal radiotherapy, according to the results of the phase 2 RTOG trial, which showed significant reductions in hematological, dermatological, and gastrointestinal toxicity (127–130). A retrospective National Cancer Database review of 10,524 patients with nonmetastatic disease from 2004 to 2015 revealed no benefit to OS with a higher dose of radiation of 54-60 Gy compared to 54 Gy in locally advanced anal cancer (HR 1.08, P=0.166) (131). Current guidelines propose the suggested dose according to the RTOG-0529 trial (127, 130). Ongoing research in locally advanced anal cancer focuses on strategies to reduce radiation-associated toxicities, such as bone marrow-sparing IMRT (VMAT) and proton beam radiotherapy (132, 133). Immunotherapy is currently recommended as a second-line treatment for metastatic cancer; preferably with nivolumab or pembrolizumab based on the NCI9673 and Keynote-158 studies, regardless of PD-L1 status (127, 134, 135). A post-hoc analysis and retrospective analysis within the NCT02919969 and NCI9673 studies showed that patients with durable responses to immune checkpoint inhibitors had higher levels of tumor-infiltrating CD8+PD-1+T cells, PD-L1-positive tumors, and HPV positivity, based on p16 IHC (134, 136). Overall survival, locoregional recurrence, and disease-free survival were improved in HPV-positive SCC compared to HPV-negative SCC (137). Immunotherapy is also being investigated in locoregional settings, particularly in combination with radiation therapy, because of its potential role as a sensitizer for immune checkpoint inhibitors by increasing antigen presentation by dendritic cells (138) and tumor-infiltrating lymphocytes, especially in patients with a high HPV16 viral load (139). When patients are diagnosed, approximately 10% have metastatic disease, and those with localized disease treated with CRT have a ~10% likelihood of metastatic recurrence (140). According to the InterAACT trial, a combination of carboplatin and paclitaxel is currently the optimal frontline therapy option for metastatic squamous cell anal carcinoma and is listed as a preferred first-line option in current guidelines (127, 140). Immune checkpoint inhibitors are currently being explored in combination with chemotherapy in treatment-naïve metastatic anal cancer, triplet therapy with the HPV-16 vaccine, NHS-IL12 tumor-targeted immunocytokine, and M7824 bifunctional fusion protein targeting PD-L1 and TGFβ in metastatic or refractory/recurrent HPV-associated malignancies, or with the EGFR/TGFβ fusion monoclonal antibody in locally advanced/unresectable or metastatic, immune-checkpoint-naïve EGFR-driven advanced solid tumors (NCT04444921, NCT04287868, NCT04429542) (141-143). Mutations or amplifications of the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) are another possible target in HPV-positive SCC. Alterations in other cancer drivers, like FBXW7 and KMT2D, occur at a low frequency, typically ~10-20% within most cohorts (144).

Oropharyngeal Cancer: Epidemiology, Risk Factors, and Treatment

Head and neck cancers (HNC) involve the upper aerodigestive tract, including the oral cavity,

nasopharynx, oropharynx, hypopharynx, and larynx. Squamous cell carcinoma is the most prevalent histology (145). Head and neck squamous cell carcinoma as a combined entity (HNSCC) is the sixth most prevalent malignancy worldwide, with an incidence in both sexes >890,000 new cases in 2022 (57, 146, 147). HNC often gets diagnosed in the advanced stage with a 5-year survival rate of only 40-50% (148, 149). The primary risk factors for HNSCC development include smoking and heavy alcohol consumption. Recently, HPV has been linked to oropharyngeal cancers. Tobacco and alcohol-induced HNSCC is decreasing in Western countries, whereas HPV-driven HNSCC, particularly oropharyngeal, is increasing in young people, especially non-drinkers and non-smokers (146, 150, 151). According to GLOBOCAN 2022, oropharyngeal cancer is ranked 24th in terms of incidence and 23rd in terms of mortality globally (57). The age-standardized rates (ASR) for oropharyngeal cancer are estimated to be 1.1 per 100,000 for incidence and 0.53 per 100,000 for mortality. In the United States, the overall incidence of HPV-positive oropharyngeal cancers is rising, especially among men (152), whereas the incidence of HPV-negative oropharyngeal cancers, which are primarily associated with tobacco and alcohol use, is declining (153). In the United States and certain regions of the European Union, the attributable fraction of HPV in newly diagnosed oropharyngeal cancers is estimated to be 60-70% (154). Globally, oropharyngeal cancer occurs two to three times more often in men than in women. However, women worldwide have a higher rate of HNC associated with HPV than men for cancers of the oropharynx and larynx. Women were more likely to have oropharyngeal cancer associated with HPV than men in Central-Eastern Europe (61.5% vs. 45.5%), Southern Europe (22.6% vs. 8.4%), and Western Europe (38.9% vs. 13%), but not in Northern Europe (50% vs. 50%) (150). The ASR for oropharyngeal cancer incidence in women across Europe is estimated at 0.92 per 100,000, with the highest rate observed in Western Europe at 1.53 per 100,000. Denmark has the highest incidence in Europe, with an ASR of 2.58 per 100,000. In contrast, BH has a lower estimated ASR of 0.27 per 100,000 compared to Serbia (0.42), Croatia (0.36), Slovenia (1.0), and Montenegro (0.64). The incidence of oropharyngeal cancer is considerably higher in men, with an ASR estimated at 3.74 per 100,000 in Europe. Eastern Europe reports the highest rate, with an incidence of 4.36 per 100,000. Romania has the highest ASR for incidence in Europe at 8.0 per 100,000. In contrast, BH has lower rates, with an ASR of 1.42 per 100,000, similar to Montenegro (1.18 per 100,000). The incidence rates in other regional countries are somewhat higher, with ASRs estimated at 2.72 for Serbia, 2.62 for Croatia, and 6.54 per 100,000 for Slovenia. The estimated ASR for mortality from oropharyngeal cancer in women across Europe is 0.28 per 100,000, with the highest mortality observed in Western Europe at 0.40 per 100,000. Denmark and Hungary have the highest mortality rates in Europe, with an estimated ASR of 0.56 per 100,000. Montenegro also exhibits a high estimated mortality rate of 0.53 per 100,000. In contrast, BH has a relatively low mortality rate of 0.07 per 100,000, which is lower compared to regional countries such as Serbia (0.17), Croatia (0.21), and Slovenia (0.24). The estimated ASR for oropharyngeal cancer mortality in men across Europe is 1.70 per 100,000, with the highest rate recorded in Eastern Europe at 2.31 per 100,000. Moldova reports the highest mortality rate in Europe, with an ASR of 5.03 per 100,000. Among other regional countries, Slovenia and Croatia also exhibit higher mortality rates, with ASRs of 3.06 and 2.22 per 100,000, respectively. In contrast, BH has a lower mortality rate of 0.80 per 100,000, which is similar to the rate in Montenegro at 0.66 per 100,000 (56).

Most cases of HPV-associated HNSCC contain HPV-16, which can induce carcinogenesis through the expression of oncoproteins E6 and E7. These oncoproteins promote angiogenesis, genomic instability, telomere shortening inhibition, apoptosis suppression, and contribute to invasion and metastasis through interaction with tumor suppressor proteins p53 and pRb (3,155-157). While HPV-16 is the most frequent type, genotyping differs based on gender and geography; the global prevalence

ranges from 0 to 60% (11). In a small Croatian cohort, the results were consistent with previous studies, but in Bosnian HNSCC samples, the most commonly expressed high-risk HPVs were HPV-18, with HPV-16 ranking fourth (Tables 1 and 3) (3, 13, 158). A retrospective analysis of 50 patients from the University Clinical Center of Banja Luka found that HPV was present in 27.3% of oropharyngeal malignancies. High-risk HPVs were found in 22% of head and neck cancer samples (Table 1) (19). Oropharyngeal cancer is classified as either HPV positive with a better prognosis or HPV negative with a worse prognosis, although multicenter, multinational individual patient data analysis suggests that double testing with p16 and HPV should be performed because their findings provide robust evidence of discordance in HPV and p16 prevalence in these patients, which translates to overall survival. The median overall survival for p16+/HPV+ cases was 15 years, while p16-/HPV- cases had a median of 3.5 years, p16-/HPV+ cases had a median of 5.3 years, and p16+/HPV- cases had a median of 6.7 years. Overall 5-year survival rates were 81.1% for p16+/HPV+, 40.4% for p16-/HPV-, 53.2% for p16-/HPV+, and 54.7%for p16+/HPV- cases (Table 2) (151, 159). Although these two groups (some will "argue" four) have distinct etiologies, the treatment is the same depending on the cancer stage. It includes (surgery, radiotherapy, radiotherapy and chemotherapy, radiotherapy and cetuximab +/- induction chemotherapy, radiotherapy and cisplatin +/- induction chemotherapy), and in recurrent, unresectable, or metastatic disease chemotherapy, immune checkpoint inhibitors, cetuximab, trastuzumab deruxtecan for HER2+ (score 3+) as a tumor-agnostic approach (3, 105, 160-169).

However, it could be an option to personalize treatment for specific patient groups, especially those who are eligible for oropharyngeal HPV-associated de-escalation, but there are still various obstacles and unanswered questions, although cisplatin-based chemoradiotherapy remains the standard of care for locoregionally advanced oropharyngeal cancer (170, 171). Besides HPV-positive oropharyngeal tumors, tumor-infiltrating lymphocytes (TILs) can play an important role in de-escalation treatment strategies. HPV-positive oropharyngeal cancer patients with high TILs exhibited a significantly better overall survival rate compared to those with low TILs (172-174). However, further research is needed to understand the total impact on survival and tailor treatment. Efficient strategies to tackle HPV-positive oropharyngeal cancer are still the subject of many trials and treatment de-escalation can take numerous forms: should we substitute cisplatin for a potentially less toxic agent, for example, cetuximab, although cetuximab showed underpowered regarding cisplatin in overall survival; should we use concomitant chemoradiotherapy with or without induction chemotherapy; should we use a single modality (surgery or radiotherapy) and eliminate chemotherapy; or at least reduce the dose of cisplatin; and lastly which dose of radiotherapy is appropriate without compromising local and distant control and overall survival (159, 175-178)?

Phase 2 clinical studies are ongoing to assess the efficacy and safety of the anti-HPV vaccine in combination with immune checkpoint inhibitors. TheOpcemISA phase 2 study examines the efficacy of the combination of ISA101b (a peltopepimut-S vaccine targeting E6/E7 HPV oncoprotein) with cemiplimab compared to cemiplimab alone in recurrent or metastatic HPV-16-positive oropharyngeal cancer. 198 patients with recurrent or metastatic squamous cell HPV-positive cancer of the oropharynx were included. There was no difference in the overall response rate between the two groups. However, in the sub-analysis, patients who had CPS ≥20 and added ISA101b to cemiplimab significantly increased ORR (28.1% vs. 23.3%) and OS (11.9 vs. 30.1 months) without a significant increase in toxicity (179). On a similar track, other studies were also ongoing, including NCT03978689 with CUE-101 (the first vaccine using the Immuno-STAT platform) in combination with pembrolizumab and NCT04180215 with HB-200 (the arenavirus vaccine) in combination with pembrolizumab in recurrent or metastatic HPV-16-positive head and neck cancers (180-182).

Cervical Screening

Importance of Cervical Screening in Preventing Cervical Cancer

The WHO has recently launched a global initiative aimed at advancing preventive strategies, screening, and treatment for cervical cancer. This initiative prioritizes the expansion of HPV vaccination programs, enhancement of screening and management protocols for both pre-invasive and invasive cervical lesions, and the provision of optimal therapeutic care for women diagnosed with invasive cervical cancer (183). The main goal of cervical screening is the prevention of cervical cancer by detection and treatment of precancerous intraepithelial lesions and early invasive cancers, to decrease mortality rates. There are two types of screenings: (i) organized population-based screening and (ii) opportunistic non-population-based screening. An organized population-based screening program is defined as a program that involves a defined target population, including specific age categories, methods, and intervals of screening. Also, there are mechanisms to identify the eligible individuals and send personal invitations to attend the screening (184). On the contrary, in opportunistic screening, the exams are performed randomly by a healthcare professional, the target population is not systematically invited, and the screening coverage depends on the frequency of visits to a doctor. Numerous studies in the past showed that organized population-based screening programs are more efficient, more cost-effective, and more equally distributed than opportunistic screening (185, 186). They also provide enhanced protection against the negative consequences that can arise from low-quality screenings or screenings conducted too frequently (187).

Overview of Cervical Screening Methods

Currently, the screening tests used in ongoing programs worldwide include cervical cytology, known as Pap test, HPV testing alone, or a combination of HPV testing and cytology. The technique of cervical cytology was developed by Papanicolaou and Babes in the 1920s and later improved by Papanicolaou (188). The conventional cytology technique involves collecting exfoliated cells from the transformation zone and endocervical canal. Cells collected for microscopic examination are applied to a glass slide for conventional cytology and commonly fixed using 95% ethyl alcohol covering the whole cellular area of the slide. According to general recommendations, cytological examinations are best scheduled approximately two weeks after the start of the previous menstrual period. To ensure accurate results, it is important to avoid sexual intercourse within 24 hours before the exam and refrain from using intravaginal estrogen products. Additionally, after childbirth, obtaining sufficient cervical samples for accurate interpretation is challenging until at least 8 weeks postpartum (184, 189). In the 1960s, cervical cytology was implemented for cervical cancer screening in several high-income countries. Over time, the focus of the Pap test has evolved from detecting invasive cancer to identifying precancerous lesions. After the implementation of cervical screening, there was a substantial decrease in the incidence of cervical cancer. It has been shown that in countries with a well-organized cytological screening, performed every three to five years in the age range from 35–64 years, the incidence of cervical cancer is reduced by 80% or more among screened women (190). It has been well-established that persistent infection with specific HPV types is closely linked to the development of cervical precancerous lesions and cancer. This understanding has prompted the consideration of detecting HPV genetic sequences as a potential alternative to traditional screening methods that rely on the microscopic examination of cervical cells (184). For the past two decades, HPV testing has emerged as a pivotal tool in cervical cancer prevention, offering a more precise approach to detecting high-risk HPV types associated with cervical malignancies. Today, there is an abundance of commercially available HPV tests. Most of these tests target multiple alpha-papillomaviruses types, including those with significant clinical relevance due to their carcinogenic potential. Specifically, 12

types, known as the IARC-2009 high-risk HPV types, are classified as carcinogenic (Group 1) by the IARC. These include HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (191). HPV-based screening offers 60-70% greater efficacy in protecting against invasive cervical cancer compared to cytology. Evidence from extensive randomized trials supports the initiation of HPV-based screening starting at age 30 and recommends extending screening intervals to a minimum of 5 years (192). Many trials compared co-testing (HVP and cytology) to HPV primary testing alone. Evidence suggests that co-testing is associated with higher costs, increased referral rates to colposcopy, and reduced positive predictive value for CIN2+ detection among referred women (193, 194).

Therefore, according to the WHO strategy for the elimination of cervical cancer, it is recommended that the screening should be performed with a high-performance test equivalent to or better than the HPV test (183). Over the past decade, numerous studies have examined the efficacy of collecting cervical material for HPV testing via vaginal self-sampling, to increase participation in cervical screening programs, particularly among women who are less likely to attend screening. The sensitivity of HPV testing for identifying cervical precancerous lesions and cancer using self-collected cervicovaginal samples was proved to be equivalent to that observed with conventional cytology or liquid-based cytology conducted with clinician-collected samples. However, the specificity of HPV testing with self-collected samples tends to be lower (195). With the development of more precise diagnostic tests, the use of self-collected samples for HPV testing could be considered a viable alternative in organized, population-based screening programs, particularly for women who have not participated in screening despite receiving a personal invitation (196). Aside from Pap and HPV tests, there is another affordable and straightforward technique, known as visual inspection with acetic acid (VIA). This screening method is widely utilized in mass screening programs in low-income regions. By applying a 3-5% acetic acid solution to the cervix, nuclear-dense lesions become visible as acetowhite areas. This test has a specificity of 82% (ranging from 64-98%) and a sensitivity of 84% (ranging from 66-96%), although it has a high rate of false positives (197).

Current Status and Challenges of Cervical Screening in BH

Although BH adopted screening protocols in alignment with recommendations from international health organizations, the implementation has progressed very slowly. According to the "Strategy for Prevention, treatment and Control of malignant diseases 2012-2020", in 2011 the government of the FBiH set a goal to implement and improve the organized population screening for cervical cancer, by developing individual population screening programs according to expert's consensus criteria and European recommendations on age and frequency, including the screening for cervical cancer for women based on cytological examination (198). To date, the screening program has been conducted on an opportunistic basis and includes mostly cytological examination and, in some parts of the country, HPV testing (199).

Comparisons with Cervical Screening Programs in Other Countries

More data regarding the screening program for cervical cancer are available from neighboring countries. In Serbia, there was notable progress in advancing preventive healthcare services for women's reproductive health, by initiating organized cervical cancer screening in 2012. To date, four screening cycles, each spanning three years, have been conducted among women aged 25 to 64. The current cervical cancer screening coverage across Serbia ranges from 35% to 68%, with evident regional disparities (200). According to the Institute for Public Health of Croatia, the Government adopted the National Program for Early Detection of Cervical Cancer in 2010, with its implementation commencing in December 2012 (201). During the initial implementation cycle (2013-2016), of the 414,018 women invited for screening, only 10%

responded to the test invitation. According to the European Health Interview Survey in 2019, the results of the second screening cycle in Croatia showed that a significant proportion of women aged 20-64 (76%) underwent a Pap test in the previous three years, while only 5% reported never having had the test (202).

In Montenegro, an organized and centralized cervical cancer screening program was initially launched as a pilot project in July 2016. Since February 2018, this program has been implemented nationally, targeting women aged 30 to 50 years. The primary screening method employed is HPV genotyping, with a screening cycle scheduled every five years (203). A review published in 2022 reported that the screening coverage in BH over five years was 30%, which is lower compared to neighboring upper-middle-income countries. Specifically, Serbia, North Macedonia, and Albania reported coverage rates of 66%, 67%, and 58%, respectively. In Montenegro, the coverage rate was 39%. For comparison, the average coverage of five-year screening programs in high-income countries is 77%, with coverage rates ranging from 66% to 88% (204).

HPV Vaccination

Overview of HPV Vaccines (Types, Efficacy, and Recommendations)

Persistent infection with high-risk human papillomavirus (HPV) types is the leading cause of cervical cancer, and HPV vaccines are a critical tool in preventing this and other HPV-related cancers. To effectively combat these HPV-related cancers, significant efforts are required to develop and implement efficient vaccination programs and strategies (205). Three main HPV vaccines that have been licensed and widely used are Cervarix, Gardasil, and Gardasil 9. Cervarix (GSK, Rixensart, Belgium) is a bivalent vaccine that targets HPV-16 and 18, which cause about 70% of cervical cancer cases. It is particularly effective in preventing cervical cancer but does not cover types that cause genital warts. Next, in line is Gardasil (Merck & Co, Whitehouse Station, NJ, USA), a quadrivalent vaccine with broader protection, covering HPV-6, 11, 16, and 18. Application has been widespread for both cervical cancer as well as genital warts. The most comprehensive option is Gardasil 9 (Merck &Co, Whitehouse Station, NJ, USA), a nine-valent vaccine showing protection against nine HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58. This vaccine offers expanded protection by targeting additional HPV strains, covering approximately 90% of cervical cancer cases and most genital warts. As a result, it is often the preferred choice in various vaccination programs due to its comprehensive coverage (206). All three HPV vaccines—Cervarix, Gardasil, and Gardasil 9—are developed using virus-like particles (VLPs), which are made from the L1 protein of the human papillomavirus (HPV). These L1 proteins self-assemble into VLPs that mimic the structure of the actual virus, but without containing any viral DNA. As a result, VLPs are non-infectious and cannot cause disease, making them a safe and effective foundation for vaccines. The resemblance of these VLPs to the natural virus plays a crucial role in their effectiveness. When introduced into the body, they trigger a strong immune response, allowing the immune system to recognize and produce antibodies against HPV. This response equips the immune system to quickly identify and neutralize the virus if the individual is exposed to it in the future. The high immunogenicity of the VLPs ensures that even without the use of strong adjuvants, the vaccines provide long-lasting protection against the targeted HPV types.

Clinical trials and long-term studies have shown that these vaccines have been effective in preventing infection for many years, significantly reducing the risk of HPV-related cancers and conditions. Moreover, because VLPs closely mimic the virus's outer shell, they generate an immune response that is both robust and specific to the HPV types they represent. This mechanism helps establish a strong memory response in the immune system, providing durable protection and reducing the incidence of HPV-related diseases in vaccinated populations (207). The current HPV vaccine

recommendations by the Advisory Committee on Immunization Practices (ACIP) recommend routine HPV vaccination for all preteens aged 11-12 years, though it can be started as early as age 9. The WHO endorses HPV vaccination for girls starting at age 9. For those who receive their first dose before the age of 15, a two-dose schedule is recommended, with the second dose given six to 12 months after the first. For those vaccinated after age 15 or those with certain immunocompromising conditions, a three-dose schedule is advised. Additionally, ACIP extended recommendations to include catch-up vaccination for everyone through age 26. Additionally, individuals aged 27-45 who were not adequately vaccinated earlier and are at risk of new HPV infections may also benefit from vaccination, though this is typically decided on a case-by-case basis with healthcare providers (208). When it comes to safety, HPV vaccines are well-studied and considered safe. Common side effects include mild pain, swelling, or redness at the injection site.

A systematic review by the WHO found no significant difference in serious adverse events between those vaccinated and those who received a placebo. The risk of severe reactions, such as anaphylaxis, is very low, estimated at 0.3-3 cases per million doses administered. Furthermore, extensive data analysis has shown no causal link between the vaccine and conditions like Guillain-Barré syndrome, complex regional pain syndrome (CRPS), or primary ovarian failure. WHO advises against the use of the HPV vaccine during pregnancy as a precautionary measure. However, research indicates that inadvertent administration of the vaccine during pregnancy does not elevate the risk of adverse outcomes for either the mother or the infant (208, 209). The results of the meta-analysis have confirmed that HPV vaccines do not result in increased risks of obstetric or birth complications (210).

Status of HPV Vaccination Programs in BH

BH is at the bottom of the ranking (immediately after Azerbaijan) in its HPV prevention efforts. BH faces challenges due to the absence of robust primary and secondary prevention strategies, including comprehensive vaccination programs and HPV screening services. Furthermore, it lacks reliable, evidence-based information on HPV prevention, which impedes public awareness and access to necessary preventive measures (211). What adds to the complexity of the issue is that healthcare responsibilities are divided among the FBiH, Republika Srpska (RS), and Brčko District (BD), each managing their healthcare initiatives, including HPV vaccination programs. As of mid-2021, FBiH had not integrated HPV vaccination into its health plans, and no vaccination programs were in place.

However, progress had been made in Canton Sarajevo where a free, voluntary HPV vaccination program for girls aged 11-12 using the 4-valent Gardasil vaccine began in November 2022, and by December 2023, the program was extended to include females aged 11-26. Additionally, pilot programs for girls aged 13-14 started in January 2023 in three other cantons, with plans to expand to the remaining six cantons by September 2023. In Republika Srpska, while HPV vaccination was recognized in health policies by mid-2021, no programs had been implemented. By June 2023, HPV vaccination was added to the Immunization Calendar, offering free, voluntary shots for girls and boys aged 11-14 through primary health clinics using the 9-valent Gardasil vaccine. The vaccine is also available for those aged 15 and older through regional public health units, although it's not free. Details on HPV vaccination in Brčko District were not provided, suggesting that further information might be needed to understand the status there (15). Given the estimated effectiveness of the current HPV vaccines, which could prevent up to 77.8% of cervical cancer cases in BH associated with HPV-16 and 18, there is significant potential for reducing the incidence of this disease. Additionally, if the cross-protection offered by these vaccines against non-vaccine HPV types proves to be long-lasting, an additional 6-10% of cases could be prevented (11).

Comparison with HPV Vaccination Coverage in Other Countries

Leading countries in HPV prevention include Denmark, Sweden, Finland, the United Kingdom, and Ireland. These nations have set a high standard by implementing comprehensive, best-practice policies. Their approach features gender-neutral vaccination programs that are freely available to all eligible individuals, resulting in notably high vaccination coverage. Additionally, they offer free HPV screening for adults, ensuring early detection and prevention of HPV-related conditions. These countries also excel in providing accessible and reliable information through government-supported websites, which help educate the public about HPV and its prevention (211). Coverage was highest in Australia, New Zealand (77%), and Latin America (61%), while Europe and North America reached 35%. In contrast, Northern Africa, Oceania (excluding Australia and New Zealand), and Asia had low coverage rates. Despite limited introduction in sub-Saharan Africa, nearly 20% coverage was achieved due to effective programs (212).

Barriers to Vaccine Access and Availability

As of June 2020, 107 out of 194 WHO Member States (55%) have introduced HPV vaccination nationwide or partially. However, the distribution is uneven: 85% of countries in the Americas and 77% in Europe have introduced the vaccine, compared to only 41% of low- and middle-income countries (LMICs) by the end of 2019. In 2019, 87% of new introductions occurred in LMICs, with six countries in sub-Saharan Africa, five in Latin America and the Caribbean, and three in Asia and the Pacific joining the program. GAVI has supported 19 LMICs, representing 35% of these countries. Thirty-three out of 107 programs (31%) were "gender neutral," vaccinating both boys and girls. Most programs (47%) targeted 12-year-olds, but LMICs generally targeted younger girls (9-10 years). In 2019, at least 35 million girls aged 9-14 were targeted, with 25 million in LMICs and 10 million in high-income countries (HICs). School-based delivery was the primary method in LMICs (90%), while HICs used both school-based (39%) and facility-based (48%) approaches. Globally, only 15% of girls and 4% of boys completed the full HPV vaccination course by 2019, with 20% and 5% receiving at least one dose, respectively, as shown in Figure 2 (212, 213). Seven of the ten most populous countries, including China, India, and Nigeria, have not fully introduced HPV vaccination, which affects global coverage, limiting it to 15%. Among the girls living in countries with HPV programs, only 53% received the final dose. Program performance averaged 67% for the first dose and 53% for the final dose. LMICs had higher first-dose coverage (80% vs. 72% in HICs) but also higher dropout rates (18% vs. 11%). Only five countries (6%) achieved over 90% coverage for the final dose, the target for global cervical cancer elimination by 2030. Twenty-two countries (21%) exceeded 75% coverage, while 35 countries (40%) had 50% or lower coverage, with 14 countries (16%) below 20%. By comparison, only 3% of countries globally have DTP3 vaccine coverage below 50% (212).

Public Health Implications and Recommendations

Impact of Inadequate Screening and Vaccination on Public Health

High-risk HPVs spread through sexual contact and are linked to anogenital and oropharyngeal malignancies (214, 215). On May 19, 2018, the Director General of WHO issued a global call to action aimed at eliminating cervical cancer, which has the greatest HPV-related disease burden (>90%). The main strategies that all countries should achieve by 2030 include 90% of girls fully vaccinated with the HPV vaccine by age 15, 70% of women screened with a high-performance HPV test by age 35 and again by age 45, and 90% of women with the cervical disease treated (15).

According to the Federal Ministry of Health's 2020 public health report, the second most common cause of death in the FBiH in 2020 was

malignant neoplasms (C00-C97). Women had a considerably greater incidence of cancer than men in the age range of 25 to 54 (54). Cervical cancer is the second most frequent female cancer in BH, as well as the third major cause of cancer death in women aged 15 to 44 years (76). Although the 2020 report does not include information on the prevalence of HPV infections and HPV-related malignancies, we could anticipate that this incidence may be linked to the fact that, besides breast cancer, HPV-related cancers such as cervical cancer are more common in this age group. Because of inadequate and ineffective screening, several countries in Central and Eastern Europe have a high cervical cancer burden; the estimated 14,300 new cases and 7200 deaths in 2008 are expected to rise 5% and 15%, respectively, to 15,000 cases and 8300 deaths by 2030 (28). The prevalence of HPV infections in women with normal cervical cytology in Central and Eastern Europe (based on samples from Croatia, the Czech Republic, Hungary, Lithuania, Poland, and Slovenia) revealed an overall prevalence of infection with high-risk HPV types of ~11% (28). However, reports from BH indicate a significantly greater prevalence. In a 10-year cross-sectional study of a Bosnian cohort of 1517 routinely screened women, 653 (43%) tested positive for HPV. Out of all the HPV-positive patients, 386 (59%) were infected with only one type of virus. HPV-16 was the most prevalent type (22.5%); however, the majority of patients were infected with HPV-16 or HPV-18 in combination with other HPVs. The average age of HPV-positive patients was 33.38±7.85, with a range from 18 to 61 years (Table 1) (18). In another study of women with positive cervical cytology (N=105), 16 different HPV strains were identified, with the majority being high-risk HPV types. HPV-16 was the most commonly found genotype in ~33% of women, while HPV-18 was detected in 7.5% of women (Table 1) (24). A report from nine Central and Eastern European countries (Bulgaria, Croatia, the Czech Republic, Hungary, Poland, Romania, Serbia, Slovakia, and Slovenia) provided quantitative evidence on the impact of early death due to HPV-related cancers in 2019. The societal and economic impact was calculated by analyzing the *"productivity loss of early death owing to HPV-related cancer, years of life lost (YLL), years of productive life lost (YPLL), and the present value of future lost productivity (PVFLP)"*. In 2019, HPV-related cancers caused 6,832 deaths, 107,846 YLL, 28,330 YPLL, and a PVFLP of approximately €151 million. Cervical cancer had the highest mortality burden, accounting for 72% of deaths, as well as the biggest PVFLP, at €114 million, accounting for 76% of total PVFLP (215). Although this report does not include data from Bosnia and Herzegovina, it demonstrates the socioeconomic burden of HPV-related malignancies, which result in significant productivity losses. If we consider that the life expectancy at birth for the FBiH population in 2019 was 77.13 years, slightly higher for women (79.25) than for men (74.93) according to the 2020 report, we can only anticipate negative effects of HPV-related carcinomas on socioeconomic aspects, regardless of the high unemployment rate (54). The prospective benefit of high-coverage HPV vaccination is expected to help reduce this increasing burden. For example, in the United States, the percentage of preventable cancers based on HPV-positive cancers would be nearly 80% through uptake of the 16/18 vaccine, with an additional 13% of cancers avoidable through the 9-valent vaccine, indicating a more than 90% decrease in HPV-positive cancers (2). In Europe, the total number of cancer cases that could be avoided by vaccinating girls and boys at present vaccine uptake ranged from 318 and 168 per cohort of 200,000 preadolescents (100,000 girls plus 100,000 boys) in Croatia (<20% uptake of the 9-valent vaccine) to 1904 and 467 in Estonia (<70% uptake of the 9-valent vaccine) (214). Many reports assessed the cost-effectiveness of HPV vaccination programs.

The WHO suggests that cost-effectiveness should be considered before introducing the vaccine, particularly in countries with limited resources. For national decision-making, the PRIME (Papillomavirus Rapid Interface for Modeling and Economics) model can be used to assist country-led data gathering and provide more individualized outcomes (216, 217). A report from Central

and Eastern Europe and Central Asia (CEECA) on the cost-effectiveness of the HPV-16/18 vaccine for 12-year-old girls found that the HPV-16/18 vaccination was very cost-effective in 25 of 28 countries, including Bosnia and Herzegovina (218).

Strategies to Improve HPV SCREENING and Vaccination Rates in BH

Despite marked technological improvements worldwide, BH still faces huge challenges in enhancing public health because of complex political and healthcare systems and the lack of a national cancer registry 30 years after the war. The primary goal of the cancer registry is to obtain, code, and categorize all malignancies to generate statistics on the occurrence of cancer in certain populations during a specific period and provide a system for monitoring and controlling the impact of cancer on the community. Cancer incidence statistics generated by registries can be used in a wide range of cancer control areas, including etiological research, early detection, assessment of outcomes, and overall healthcare planning (219). From all the above, we cannot even conclude which category we fall into: "the good, the bad, or the ugly."

The main strategies for reducing the prevalence of HPV infections and HPV-related malignancies should be the implementation of the HPV vaccine, adequate screening of target populations, and improvement of treatment through the introduction of new therapeutic options and the expansion of existing indications through the Federal Institute of Health Insurance. Prevention strategies are the gold standard for reducing the risk and prevalence of diseases. Therefore, it is crucial to prioritize evidence-based awareness campaigns promoting HPV vaccination to enhance cancer prevention and combat misinformation, ultimately increasing health literacy. For HPV prevention purposes, additional efforts could be made through educational workshops in schools and educational institutions, by introducing or strengthening the system of inviting and reminding about vaccination, consultations organized through youth associations or associations of cancer patients, and also STD counseling centers for the youth and persons with high-risk sexual behavior and the LGBT population. For the prevention of HPV-related malignancies, the implementation of the National Cervical Cancer Early Detection Program will provide access to cervical cancer screening, diagnostic, and treatment services. In addition to the political will to accelerate the introduction of HPV prevention programs, there is a need to build infrastructure, including high-quality cytological testing.

Role of Healthcare Policy and Education in Cancer Prevention

According to the Health Care Law, primary healthcare involves strategies that preserve and enhance the population's health, such as disease and injury prevention, treatment, and rehabilitation; identification and management of risk factors for noncommunicable diseases; youth preventive health care; immunization against infectious illnesses; rehabilitation and medical treatment; palliative care; and so on (54). All these facts suggest that healthcare providers play a valuable role and have legal liability in healthcare education among the general population as well as in high-risk groups. Doubts regarding the security and efficacy of vaccines are seen to be increasing among the public.

According to the World Health Organization, vaccine hesitancy is defined as a delay in accepting or refusing immunization regardless of the availability of vaccination services (220). Healthcare workers (HCWs) are trusted providers of medical information, yet their skepticism about vaccinations may impact vaccine coverage. A study of Croatian HCWs primarily employed in epidemiology and public health, school medicine, pediatrics, and general practice/family medicine found that 17% of primary HCWs were vaccine-hesitant, with a significant distinction between physicians and nurses (7% vs. 24.9%) (221). This finding is concerning because nurses tend to spend more time with patients, engaging in less formal interactions, and providing guidance and assistance daily. According to the 2020 Public Health Report and

2018–2019 BiH Youth Study, only 13% of young people in BiH have a university degree, 50% have a three-year secondary education, and 4% have no formal education. Data on computer literacy was collected from 1,229,972 respondents, with 38.7% declaring themselves computer illiterate (54). To ensure successful prevention, healthcare workers must have greater knowledge of and offer additional information about measures like HPV vaccines. Because vaccine hesitancy in HCWs can have a significant impact on the national vaccination program's implementation, it is critical to increase confidence among primary HCWs and address vaccination-related knowledge gaps, particularly in the nursing population, through systematic vaccination training for healthcare workers.

Future Directions

Potential Areas for Research and Policy Development

The substantial global burden of HPV-related cancers underscores the urgent need for comprehensive research in this area and effective prevention strategies. While progress has been made, significant challenges persist, particularly in countries like BH. Europe Beating Cancer Plan has initiated a comprehensive effort to eradicate HPV-related cancers through increased HPV awareness, widespread vaccine availability, and effective cervical cancer screening (222). Key goals are to achieve HPV vaccination rates of 90% for girls and to significantly increase the vaccination of boys by 2030 in Europe. These European initiatives offer valuable guidance for BH. Addressing fundamental challenges, such as establishing a functional comprehensive cancer registry, is a prerequisite for effective HPV prevention and control strategies in BH. To effectively implement and evaluate HPV prevention strategies, a central HPV vaccination registry is essential, given the current absence of comprehensive HPV prevalence data in BH. Data from the literature suggests that high vaccine costs and negative public perception have been primary obstacles to the widespread adoption of HPV vaccination programs in Central and Eastern European countries (27).

To address these challenges in BH, targeted public awareness campaigns are crucial. Similar to the prevention of other infections (e.g., COVID-19), public awareness campaigns about HPV infection, its consequences, and the importance of prevention are vital to promoting public support for HPV vaccination. These should emphasize the advantages of vaccination while proactively addressing misinformation (64). Implementing fully reimbursed vaccination programs and integrating HPV screening into national cancer plans are essential steps toward improving vaccination rates and cervical cancer prevention in BH.

Importance of Continued Surveillance and Data Collection

Continuous surveillance and robust data collection are needed for the successful implementation and evaluation of HPV prevention strategies. A comprehensive national immunization register is essential for monitoring vaccination coverage, identifying disparities, and evaluating the impact of interventions. By creating a comprehensive national HPV register it would be possible to track progress, identify challenges, and measure the impact of the interventions. For example, by identifying specific challenges such as low vaccination rates, vaccine hesitancy, or access barriers, targeted interventions can be implemented to enhance HPV prevention efforts. This approach facilitates data-driven decision-making to optimize resource allocation, target high-risk populations, and refine prevention strategies. Knowledge and experience sharing with regional and European countries can significantly enhance the effectiveness of HPV infection prevention strategies in BH. The high prevalence of high-risk HPV types 16 and 18 among younger women in BH underscores the need for a screening program prioritizing these specific subtypes. The identification of HPV in nearly half of oral and head and neck cancer cases in BH underscores the broader impact of HPV infection beyond cervical cancer (13). Eliminating

structural barriers and expanding vaccination access are important to achieving optimal HPV prevention outcomes.

Conclusions

Studies conducted in BH revealed a high HPV prevalence among women. HPV-16 has consistently been identified as the most common subtype in women with normal cervical cytology, preinvasive cervical changes, and cervical cancer. Furthermore, literature research has revealed a high HPV prevalence in HNC and colorectal cancers. HPV-related cancers have a significant public health burden, particularly in less developed countries like BH where access to prevention and screening services is limited. Despite the well-established link between HPV and cervical cancer, as well as other malignancies, a comprehensive literature search reveals that no HPV prevention or screening program has been implemented in BH. Key challenges to progress include the absence of a unified cervical cancer screening program, limited HPV vaccination coverage, and the lack of comprehensive cancer and HPV registry data. However, recent efforts to incorporate HPV prevention into national health strategies represent a positive step forward.

Final Thoughts on Improving HPV-Related Cancer Outcomes in BH

Our review offers a comprehensive overview of existing studies in BH, providing valuable insights into HPV genotypes that can guide the development of effective prevention strategies. To effectively reduce the burden of HPV-related cancers in BH, a comprehensive approach is essential. This includes prioritizing the implementation of a national HPV vaccination program and establishing a cervical cancer screening program. Additionally, investing in research and raising public awareness are required components of a successful HPV prevention strategy. By addressing these challenges and implementing evidence-based interventions, BH can significantly improve cancer outcomes and reduce the impact of HPV-related diseases on public health. Further research is necessary to explore the complete extent of HPV-related cancer in BH and to inform the development of targeted prevention strategies.

Authors' Contributions: Conception and design: AP, KT and SV; Acquisition, analysis and interpretation of data: AP, KT, LA, AF and SV; Drafting the article: AP, KT and SV; Revising it critically for important intellectual content: AP, KT, LA, AF and SV; Approved final version of the manuscript: AP, KT, LA, AF and SV.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Scientific Article
Acta Medica Academica 2024;53(3):274-285
DOI: 10.5644/ama2006-124.456

Institutional Experience of Lymphoproliferative Disorders with Initial Diagnosis Made via Fine Needle Aspiration at Otolaryngology Clinic

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Received: 7 June 2024; **Accepted**: 30 September 2024

Abstract

Background. This study characterizes lymphomas presenting as palpable head and neck masses and evaluates the role of fine needle aspiration (FNA) and flow cytometry (FC) in diagnosis. **Design.** A 5-year retrospective review of FNAs performed by pathologists in an ENT clinic identified cases with a predominant lymphoid population that lacked an epithelial component. Cytology, FC, and subsequent surgical pathology diagnoses were correlated. **Results.** Of 821 FNAs, 154 (19%) met selection criteria. Reactive lymph nodes accounted for 43% (67/154), with most diagnosed as 'negative for malignancy,' except one 'atypical' (ATY) case. Lymphoma was confirmed in 57% (87/154) of cases, categorized as ATY (55%), suspicious for lymphoma (SFM) (36%), or positive for lymphoma (PFM) (9%). Lymphoma patients were older (median 66 vs. 46 years). Thyroid and salivary gland lymphomas typically indicated systemic involvement, except for two cases of marginal zone lymphoma (MZL) in patients with Sjögren syndrome. FC alone had a sensitivity of 67.5% for detecting lymphoma, but when combined with cytology, the sensitivity increased to 100%. The combined approach maintained a specificity of 98%. More abnormal clonal cells were identified by FC in PFM cases compared to SFM or ATY cases (P=0.004). Cytologic atypia with negative FC occurred in 29% of lymphomas, including Hodgkin and diffuse large B-cell lymphoma (DLBCL). **Conclusion.** Lymphomas presenting as neck masses are diverse, with FNA playing a key diagnostic role. Cytologic atypia and FC complement each other, as some cases show minimal atypia but positive FC, while others show significant atypia with negative FC.

Key Words: Lymphoproliferative ▪ FNA ▪ Otolaryngology.

Introduction

Fine needle aspiration (FNA) represents a cornerstone in diagnostic pathology, valued for its reliability, minimal invasiveness, cost-effectiveness and adaptability across diverse clinical settings. In an otolaryngology outpatient clinic, patients presenting with neck masses often undergo this procedure as an initial step in the diagnostic workup. FNA samples are suitable for morphologic evaluation as well as ancillary studies such as immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH), molecular analysis and flow cytometry (FC), which are especially important for diagnosing and subtyping lymphoma (1). Lymphomas comprise 12% of all malignant tumors in the head and neck, ranking as the third most common malignancy after squamous cell carcinoma (46%) and thyroid carcinoma (33%) (2-4).

Hence, a meticulous evaluation for lymphoma is imperative in patients presenting with a new mass in the head and neck region. Within the context of salivary gland lesions, the diagnostic spectrum for lymphoid cell-rich FNAs extends beyond lymphoma to include a range of non-neoplastic conditions such as lymphoepithelial cyst, reactive lymph nodes and chronic sialadenitis, as well as lymphoid-rich neoplasms such as Warthin tumor (5). Despite the frequent occurrence of lymphomas in the head and neck region, the existing literature offers limited insights into their initial

presentation as palpable masses in patients seeking care at an otolaryngology clinic.

Therefore, our study endeavors to fill this knowledge gap by sharing our institutional experience of patients who presented to our outpatient otolaryngology clinic with a primary concern of mass or swelling and underwent FNA revealing a lymphoid lesion. Through the analysis of these cases, we provide a comprehensive characterization of the frequency and types of lymphoma encountered in this clinical context. Furthermore, our investigation highlights the pivotal roles played by FNA and FC in achieving accurate and precise diagnoses of lymphoproliferative disorders.

Method

A five-year retrospective search was conducted within our institutional database to identify eligible cases. Cytopathology reports from patients who underwent pathologist performed FNA of a neck mass (including cervical lymph nodes, thyroid and salivary sites) at our institution's Ear, Nose, and Throat (ENT) clinic were reviewed to identify cases which contained a lymphoid component; cases which also contained an epithelial component were excluded in order to capture pure lymphoid lesions. If applicable, FC results and subsequent surgical pathology diagnoses were recorded.

The FNA procedure adhered to established institutional protocols and was guided by palpation for superficial masses or ultrasound for deep masses. Rapid on-site assessment using air-dried, Diff-Quik-stained smears was performed by the performing pathologist at ENT clinic, and additional needle passes were taken until adequate diagnostic material was obtained. Alcohol-fixed smears were also prepared for subsequent staining with Papanicolaou stain. Needles were rinsed in separate vials containing formalin and RPMI medium, for cell block preparation and flow cytometry analysis, respectively.

For flow cytometry, fresh tissue samples underwent analysis within 24 hours using a 6-color flow cytometry approach. Neoplastic cells were initially identified through a CD45/forward scatter gating strategy, with abnormal B-cells further characterized by the expression of pan B-cell antigens and monotypic immunoglobulin light chain expression. Stained cells were acquired using a benchtop flow cytometer (FACS Canto, Becton Dickinson) and analyzed with Kaluza software (Beckman Coulter, Fullerton, CA), with fluorescence intensity measured on a logarithmic scale ranging from 100 to 104.

Ethics Statement

This study was approved by the Institutional Review Board of Thomas Jefferson University (iRISID-2023-2047).

Statistical Analysis

Data pertaining to clinical presentation, FNA cytology results, FC findings, and final surgical excision outcomes, if applicable, were collected and organized using Microsoft® Excel® for Microsoft 365 MSO (Version 2403). Descriptive analysis and one-way analysis of variance (ANOVA) were conducted using IBM SPSS Statistics 28.0 to assess the relationship between cytology diagnosis categories and the proportion of the clonal population. A chi-square test of independence was conducted to assess the association between the performance of IHC and the categorization of cytology diagnoses (atypical, positive for lymphoma, and suspicious for lymphoma).

Results

In our 5-year search of 821 cases of pathologist-performed FNA at ENT clinic, 155 (19%) fulfilled our selection criteria. Among these, 43% (67/155) were samples from benign/reactive lymph nodes; one case was characterized as "atypical cytology" (ATY) and the remainder received a "negative for malignancy" (NFM) cytologic diagnosis. The remaining 55% (86/155) of cases represented sampled lesions that were ultimately proven to be lymphoma; these were categorized on FNA as

ATY (54%, 47/87), "suspicious for malignancy" (SFM) (36%,31/86), or "positive for malignancy" (PFM) (9%,8/86). Notably, 1% (2/154) of cases exhibited marked lymphoid proliferation on cytology but were later identified as nasopharyngeal carcinoma and SMARCA4 (BRG1)-deficient high-grade tumors upon surgical resection.

The median age among patients with reactive lymph nodes was 46 years (interquartile range: 33-57), with a male-to-female ratio of 1.4:1. In contrast, lymphoma patients exhibited a median age of 66 years (interquartile range: 51-73), with a male-to-female ratio of 0.7:1. Notably, 88% (76/86) of lymphoma cases presented with a *de novo* mass, lacking any prior history of lymphoma, and two patients had a history of laryngeal squamous cell carcinoma.

FC analysis was conducted on samples from 80/86 (93%) lymphoma cases and 51/67 (76%) reactive cases. In comparative terms, the PFM category exhibited a notably higher proportion of abnormal clonal cells on FC analysis compared to both the SFM and ATY categories, as shown by ANOVA analysis ($F(2, 48) = 6.340$, $P=0.004$). Specifically, the ATY category had a mean proportion of 0.22 (SD=0.18), the PFM category had a mean proportion of 0.53 (SD=0.22), and the SFM category had a mean proportion of 0.27 (SD=0.19). However, no statistically significant difference was observed between the SFM and ATY categories ($P=0.972$, Bonferroni correction applied, with alpha adjusted to 0.017 to uphold significance levels).

When performed, IHC is typically ordered upon receipt of the cell block, with results generally available concurrently with the flow cytometry findings. The final cytologic diagnosis is rendered by integrating the cytomorphology with the results from both flow cytometry and IHC. In cases of reactive masses, predominantly lymph nodes, IHC was performed in 11 out of 67 cases (16.4%). The IHC panel for these cases primarily included lymphoid markers (CD3 and CD20), but occasionally, non-lymphoid markers (cytokeratin, TTF-1, and thyroglobulin) were performed to exclude the possibility of occult metastatic carcinoma, particularly in patients with a prior cancer history or current evidence of a mass in another anatomic location. For lymphoma cases, IHC was performed in 32 out of 87 cases (36.8%). The IHC panel for these cases typically included lymphoid markers (CD3, CD20, CD30, CD10, CD23, BCL1) and cytokeratin, especially when large atypical cells were observed in the FNA sample.

IHC was performed in 14 out of 48 ATY cases (29.2%), 9 out of 31 SFM cases (29.0%), and 5 out of 8 PFM cases (62.5%). A chi-square test of independence was conducted to evaluate the association between the performance of IHC ("performed" vs. "not performed") and the categorization of these cytology diagnoses, which revealed no statistically significant association between the two variables (Pearson Chi-Square = 3.087, df = 2, $P=0.214$). In cases of lymphoma, patients typically presented with a unilateral mass, and the duration of enlargement extended over a span of weeks to up to one year. Longer duration was notably more frequent in indolent types of lymphoma, aligning with expectations. Cervical lymphadenopathy emerged as the predominant site of lymphoma manifestation within the head and neck region, accounting for 62% of cases (53/86). Other notable sites of involvement included the parotid gland (25%), submandibular region (6%), thyroid (5%), nasal cavity (1%) and submental region (1%).

In addition to a mass lesion, 7% of patients complained of dysphagia. Remarkably, two patients presented with orbital masses concomitant with a parotid mass, with one experiencing diplopia and the other reporting painless gradual vision decline over a few days. A minority of cases, comprising only 14%, exhibited B symptoms such as weight loss, fever, night sweats or chills.

Surgical resection was performed in 73/86 (85%) lymphoma cases, facilitating histologic diagnosis, as depicted in Figure 1. Non-Hodgkin lymphoma (NHL) constituted 78% (57/73), of cases, followed by Hodgkin lymphoma (HL) at 21% (15/73). Additionally, one case of chronic myeloid leukemia (CML) involving a lymph node was observed. HL represented 25% (13/53) of lymphomas involving cervical lymph nodes, while it

occurred much less commonly in extranodal head and neck sites (5% (2/34) cases affecting the parotid and thyroid glands). Marginal zone lymphoma (MZL) was the predominant type observed in the parotid gland, with two cases identified as primary lymphomas confined to the gland and associated with Sjögren syndrome. All other lymphomas in the head and neck region were associated with systemic disease.

There were 5 cases of T-cell lymphoma, including two cases of Anaplastic Large Cell Lymphoma, ALK-negative (AN-ALCL) and one case each of angioimmunoblastic T-cell lymphoma, follicular helper T-cell lymphoma and peripheral T-cell lymphoma, not otherwise specified (NOS). For these cases, abnormal FC results were observed in all but one case, which was AN-ALCL.

In terms of diagnostic performance, the sensitivity of FC alone in identifying lymphoma stood at 67.5%, a figure that rose to 100% when combined with cytomorphologic diagnosis. Meanwhile, FC alone demonstrated a specificity of 98%, which remained consistent when combined with cytomorphology results. The positive predictive value (PPV) was 98% and the negative predictive value (NPV) was 66%. The combined sensitivity, defined as the sum of SFM cases plus PFM cases divided by biopsy-proven lymphomas, was 45% across all lymphoma subtypes. When stratified by lymphoma subtype, the combined sensitivity was 55% for non-Hodgkin B-cell lymphomas and 36% for other lymphomas (primarily HL and T-cell lymphoma). It is noteworthy that 25 cases exhibited significant cytologic atypia but concurrently showed negative FC results. These cases encompassed various lymphoma subtypes, including HL (N=14), diffuse large B-cell lymphoma (DLBCL) (N=4), large B-cell NHL (N=3), MZL (N=1), AN-ALCL (N=1) and mycosis fungoides (N=1). Examples of these lymphoma cases with false-negative FC results are illustrated in Figure 2.

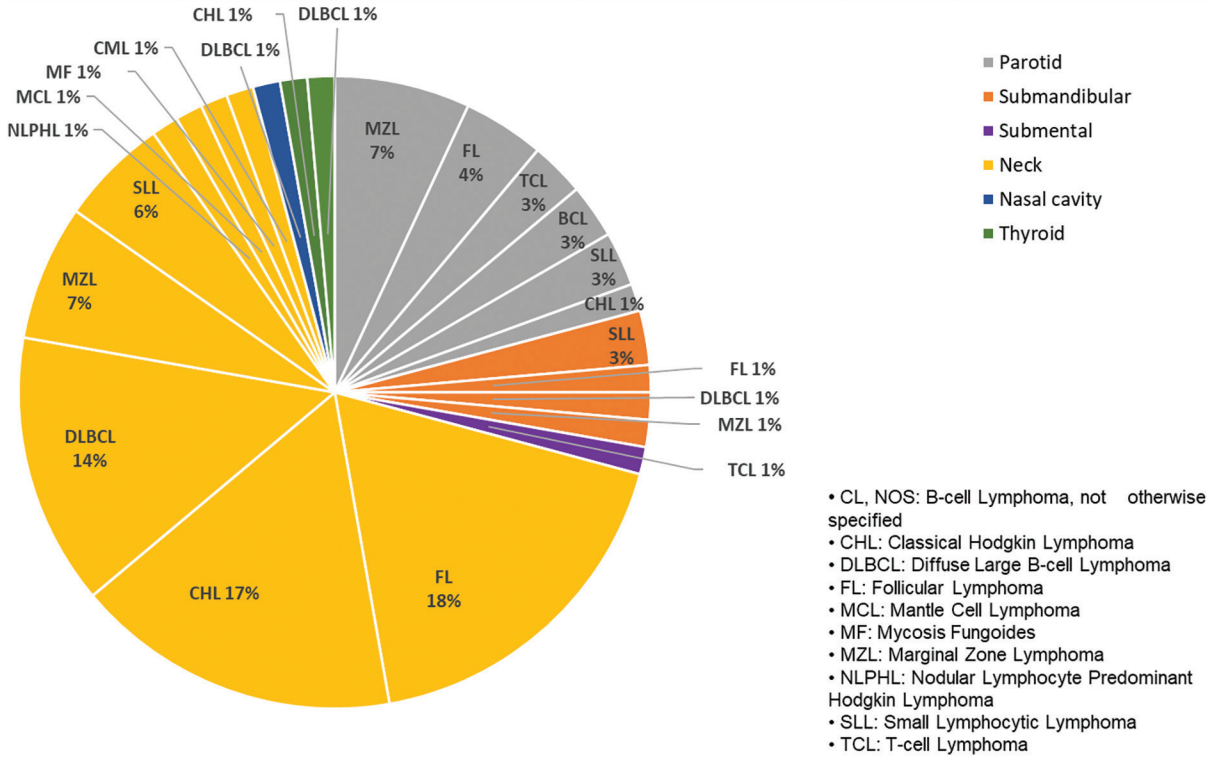

Figure 1. Final histopathologic classification of lymphomas sampled by FNA.

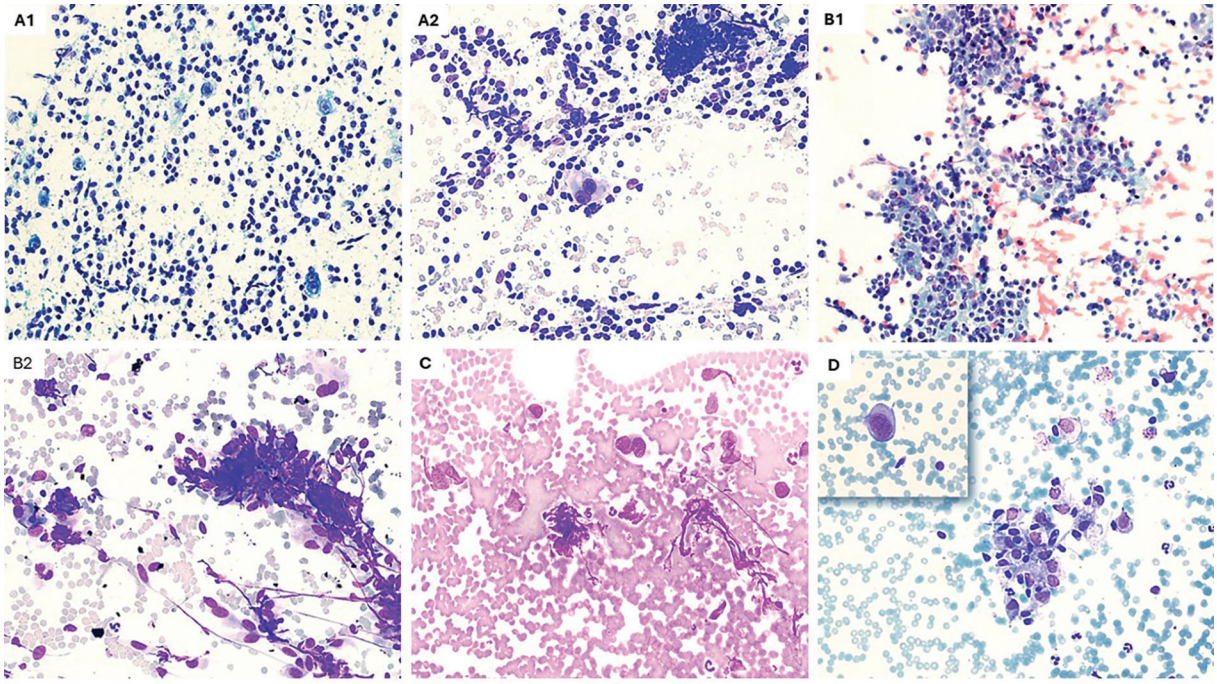

Figure 2. Examples of lymphomas exhibiting significant cytologic atypia but negative flow cytometry results. A. Classical Hodgkin lymphoma (A1. Pap stain, 400×; A2. Diff-quik, 400×). B. Diffuse large B-cell lymphoma (B1. Pap stain, 400×; B2. Diff-quik, 400×). C. Mycosis fungoides (Diff-quik, 400×). D. Nodular lymphocyte-predominant Hodgkin lymphoma [inset: large atypical lymphoid cell] (Diff-quik, 400×).

Discussion

Our study selected for patients who presented with a unilateral mass or swelling, recognized as one of the most common presenting symptoms for head and neck lymphoma (7). Notably, two patients had a previous history of laryngeal squamous cell carcinoma, which initially raised the possibility of metastasis. However, the subsequent diagnosis of lymphoma in these cases underscores the importance of keeping lymphoma on the list of differential diagnoses, regardless of previous history, and collecting additional material for ancillary study purposes.

Only 14% of our patient cohort exhibited B symptoms. This finding is consistent with existing literature indicating that only a small percentage of head and neck lymphoma patients present with constitutional or specific B symptoms (2, 6). Therefore, physicians should remain vigilant to the possibility of lymphoma when encountering a patient with a unilateral neck mass, even in the absence of B symptoms. The majority of lymphoma cases in our study were NHL, which is in line with previous findings (8). Notably, HL represented 25% of all lymphomas in lymph nodes. However, its occurrence in extranodal sites within the head and neck was relatively rare. This trend corresponds with existing literature, which indicates that HL predominantly affects lymph nodes, with only a small proportion involving extranodal areas. Conversely, NHL is known to more commonly involve extranodal sites (2, 9-11).

In our study, MZL emerged as the predominant type of extranodal NHL in the head and neck region. This finding contrasts with existing literature, which typically identifies DLBCL as the most common type (2, 12-15). However, our observation is consistent with the fact that MZL of the mucosa-associated lymphoid tissue (MALT) is the most frequent NHL affecting the parotid gland (16), which harbors the majority of extranodal lymphomas in our study. Notably, two of these cases were primary lymphomas confined

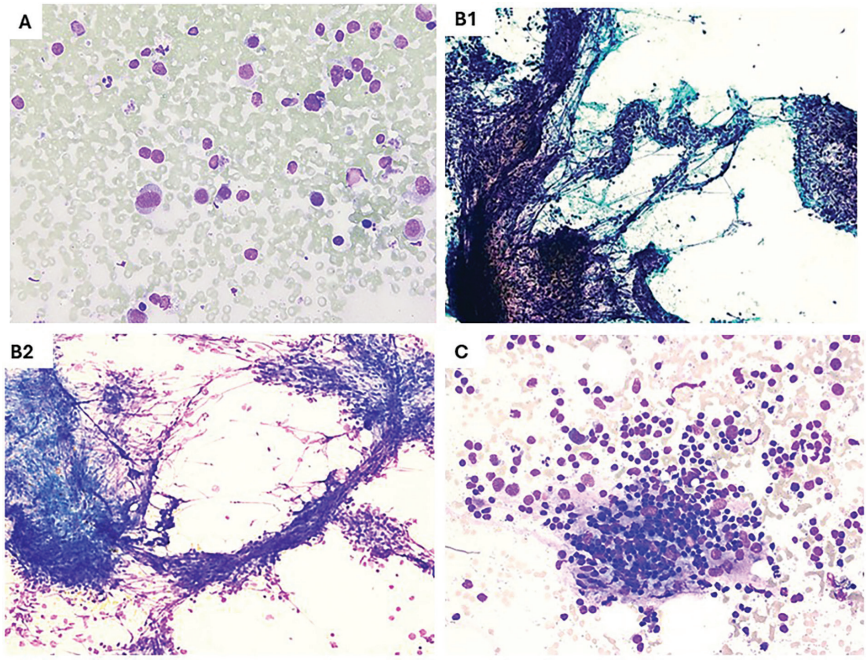

Figure 3. T-cell lymphoma examples. A: Diff-quik stained smear revealing anaplastic large T-cell lymphoma with prominent large atypical lymphoid cells (400×); B: Angioimmunoblastic T-cell lymphoma displaying atypical lymphoid tissue aggregates containing a scaffold of arborizing small vessels (B1. Pap stain, 400×; B2. Diff-quik, 400×); C: Peripheral T-cell lymphoma, not otherwise specified, showing a mixture of small, medium, and large lymphoid cells on a Diff-quik stained slide (400×).

to the parotid gland and associated with Sjögren syndrome. It is well recognized that patients with Sjögren syndrome have a significantly increased risk of lymphoma, approximately 40 times the relative risk compared to the general population (17).

FNA demonstrates variable diagnostic accuracy contingent upon the specific type of neoplasm, exhibiting higher efficacy for recurrent disease and certain aggressive primary lymphomas (18, 19). Conversely, its diagnostic accuracy for T-cell lymphomas tends to be lower. However, in our study all cases of T-cell lymphoma were successfully identified based on cytomorphology and FC and IHC results. FC analysis detected abnormal populations in all cases except for one instance of AN-ALCL, where atypia was observed via cytomorphologic evaluation (Figure 3).

Reactive follicular hyperplasia is a known diagnostic pitfall in cytopathology, as germinal center cells may be easily misinterpreted as atypical lymphoid cells (20-22). In our study, we encountered two cases of reactive follicular hyperplasia mimicking lymphoma based on limited cytology interpretation. In the first case (Figure 4A), initial suspicion of a lymphoproliferative disorder arose due to an abundance of large lymphocytes and IHC showing a predominance of CD20 over CD3-positive cells. However, final resection revealed reactive follicular hyperplasia. Similarly, in the second case (Figure 4B), FC suggested a large B-cell lymphoma based on an abnormal CD10+ B-cell population with no light chain expression. Yet, final resection unveiled a benign lymph node with follicular hyperplasia. The anomalous cellular population that lacked surface kappa or lambda expression was retrospectively identified as immunoblasts, which are commonly seen in viral infections (23), during which intense immune responses may lead to reduced or absent expression of immunoglobulin light chains (24). Upon correlating final histopathologic diagnoses with initial cytologic diagnoses, additional potential pitfalls

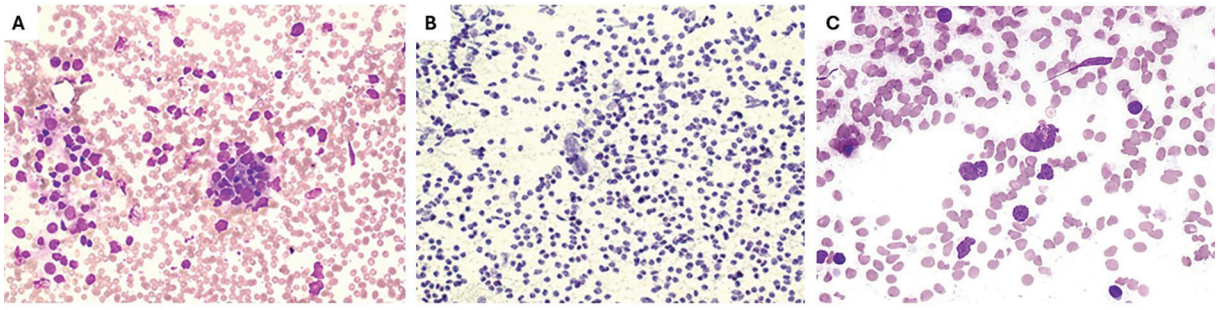

Figure 4. Entities that mimic lymphoma. A: Large atypical lymphocytes (400×); B: Abnormal B-cell population with absent light chain expression. Both cases exhibited follicular hyperplasia upon surgical excision (400×); C: Diff-quik stained smear displaying two large binucleated cells with stripped cytoplasm, resembling Reed-Sternberg (RS) cells, in a mixed inflammatory background, ultimately diagnosed as nasopharyngeal carcinoma (600×).

emerge. In one case (Figure 4C), a submandibular lymph node FNA revealed polymorphous lymphocytic population alongside rare benign salivary gland elements.

Although rare large, atypical lymphocytes resembling a Reed-Sternberg cell were identified on one smear, FC analysis showed no abnormal population, which is not unexpected given the possibility of HL. Surprisingly, the final diagnosis revealed metastatic nasopharyngeal carcinoma. The undifferentiated type of nasopharyngeal carcinoma is a well-known mimic of lymphoma, alongside malignant small round cell tumors and poorly differentiated squamous cell carcinomas (25). This case underscores the necessity of comprehensive evaluation, clinical correlation, and utilization of ancillary testing, to ensure accurate diagnosis and appropriate management. Additionally, it highlights the potential benefit of performing core needle biopsy (CNB) in addition to FNA. CNB is not typically conducted at our ENT clinic as part of the primary workup, which could be a limitation at times. At our institution, the standard approach involves performing FNA to obtain material for preparing Diff-Quik stained slides, Papanicolaou-stained slides and cell blocks. Additionally, if rapid onsite assessment reveals a lymphocyte-rich lesion, an attempt is made to collect additional material for FC analysis. This comprehensive approach has proven valuable in diagnosing and even subtyping lymphomas in certain cases.

Our analysis indicates a positive correlation between the degree of suspicion and the proportion of the clonal cell population as detected by FC. Specifically, the PFM category displayed a significantly higher proportion of abnormal clonal cells on FC analysis compared to both the SFM and ATY categories. However, no statistically significant difference was observed between the SFM and ATY categories. It remains unclear whether this observation may be related to a potential bias where cytopathologists may upgrade the diagnostic category if the percentage of clonal cells is high upon review of the concurrent FC results. Alternatively, it could suggest that a higher percentage of clonal population manifests as greater quantity and/or quality of atypia on cytomorphologic evaluation.

In lymphoma cases, IHC is particularly valuable when FC results are normal, providing critical insights when cytomorphology shows mild atypia. For instance, IHC can reveal a predominance of CD20-positive B cells or CD3-positive T cells in cases of B cell lymphoma or T cell lymphoma, respectively. CD30 is especially useful in cases where FC is negative but large, atypical cells suggest HL. Additionally, cytokeratin is often utilized to rule out metastatic carcinoma when large atypical cells are observed.

Although IHC is more frequently performed in cases ultimately categorized as "positive for lymphoma" (PFM) compared to other diagnoses, this difference was not statistically significant, indicating no association between the use of IHC and the final cytology categorization (P=0.214). This indicates that IHC does not appear to play a decisive

role in upgrading a diagnosis to PFM or SFM. Our review suggests that cases receiving these upgraded diagnoses are primarily those with significant cytologic atypia combined with positive FC results. The high incidence of ATY diagnoses is likely due to cases with positive flow results but only mild cytologic atypia. Additionally, it is important to recognize that the assessment of atypia in cytomorphology can be subjective and heavily reliant on the cytopathologist's level of expertise, which contributes to the higher rates of atypical diagnoses. IHC is more frequently ordered when FC results are negative, yet there is a presence of cytological atypia (particularly an abundance of intermediate-sized lymphocytes) or a significant clinical history that prompts the pathologist to rule out occult metastatic carcinoma. This suggests that, in reactive cases, IHC is used as an additional diagnostic tool when cytomorphological features alone are insufficient to rule out a malignant process.

To ensure standardized and replicable cytologic diagnoses of lymphoma in lymph nodes, the Sydney System offers a structured framework comprising five categories. It aims to provide essential diagnostic information and, when possible, identifies specific benign or malignant entities through ancillary testing (26). Conversely, Chong et al. proposed a stepwise approach primarily focused on morphological features, without placing significant emphasis on ancillary studies (25). In our investigation, among lesions ultimately diagnosed as lymphoma, the majority (57%; 87/154) were cytologically categorized as ATY (55%), SFM (36%) and PFM (9%). Diagnosis in most cases relied on a combination of cytomorphology, IHC on cell block, and FC results. Although ancillary studies are suggested to refine atypical and suspicious cases into definite categories (benign or malignant) (27), our cohort still observed a notable proportion of such cases. The discordance observed may stem from multiple factors, such as variability in cytopathologist experience with diagnosing lymphoid lesions. Additionally, the higher prevalence of ATY cases could be linked, at least in part, to the outcomes of concurrent FC. Specifically, in our study, 37% of cases labeled as ATY yielded normal results, 4% were deemed non-contributory due to insufficient lymphocytes, and FC was not performed in 10% of cases.

Among the lymphoma cases, 74 out of 87 underwent surgical resection, allowing for histologic diagnosis. For the remaining patients, clinical information regarding whether they underwent surgical resection with histologic diagnosis was unavailable in their electronic medical records. FNA rendered specific diagnosis in only two patients, identifying chronic lymphocytic leukemia (CLL) and DLBCL based on cytomorphology, IHC and FC. All the remaining lymphoma diagnoses were made after surgical excisional biopsy. This highlights the role of surgical biopsy as the "gold standard" for diagnosis owing to the larger quantity of tissue obtained, with preserved architectural features and increased overall diagnostic sensitivity (28-30). Based on the available evidence, there is consensus that the moderate to large benefits of employing excisional biopsies outweigh the moderate to trivial potential harms associated with using a more invasive procedure than FNA or CNB (31). However, CNB and FNA with ancillary studies can be a successful substitute for excisional biopsy in cases where excisional biopsy is not feasible (1, 32-34).

The sensitivity of FC alone in identifying lymphoma in our study was 67.5%. However, when combined with cytomorphologic evaluation, sensitivity increased to 100% in our study, which is higher than what has been reported in other studies (32, 35, 36). The overall combined sensitivity, defined as the proportion of cases with either positive or suspicious cytology among the biopsy-confirmed lymphoma cases (37), was 45%. When stratified by subtype, the combined sensitivity was 55% for non-Hodgkin B-cell lymphomas and 36% for other lymphomas, primarily HL and T-cell lymphoma. This observed difference may be influenced by the role of FC, which is often available at the time of cytology sign-out at our institution. For B-cell lymphomas, FC frequently detects clonal proliferation, providing critical evidence that supports upgrading the diagnosis from ATY to SFM

or PFM. In contrast, non-B-cell lymphomas, particularly HL, typically yield negative FC results. As a result, when cytology does not reveal a significant number of atypical cells, the diagnosis is more likely to remain classified as ATY. It is noteworthy that evaluating the performance of FNA cytomorphology alone, without the aid of flow cytometry, proved challenging in our study, as turnaround time for FC at our institution is 1-2 days, providing cytologists with concurrent FC results by the time of slide review.

In our study, 26 cases exhibited significant cytomorphologic atypia (characterized by large cell size, hyperchromatic nuclei, and irregular nuclear contours) despite concurrent negative FC results. The majority of these cases were HL (54%; 14/26) and large B-cell lymphoma (27%; 7/26). This finding is consistent with the notion in the literature that large cells are often underrepresented in FC analysis due to their low viability. Consequently, a significant proportion of large B-cell lymphomas may yield false-negative or nondiagnostic FC results (38, 39). The low sensitivity of FC can also be attributed to limitations associated with the FNA procedure itself and the histologic characteristics of lymphoma. The adequacy of diagnostic material may be influenced by the number of needle passes during the procedure, the size of the needle used, and the experience level of the aspirator (40). Additionally, features intrinsic to the lesion itself, such as sclerosis, necrosis, obscuring inflammation, or partial involvement of the lymph (41) node, may reduce the number of lesional cells available for both cytomorphologic assessment and FC analysis (29, 41, 42). To address these challenges, strategies such as careful morphological assessment and the use of IHC on cell blocks are crucial to avoid downgrading a diagnosis based solely on negative FC results.

In our study, all cases of HL were identified through cytomorphology, with each case being diagnosed at least as ATY. This contrasts with findings from other studies where HL was identified in less than half of the cases (43, 44). Notably, all HL cases in our sample yielded negative results on FC, which often struggles to detect neoplastic Hodgkin and Reed-Sternberg (HRS) cells in lymph nodes (41). This challenge has been attributed to cell lysis during preparations or cell acquisition (45). However, one study examined 53 cases of classical Hodgkin lymphoma (CHL) defined morphologically and found that HRS cells, often forming T-cell-HRS-cell rosettes, could be identified by FC with a sensitivity of 88.7% and specificity of 100% (46). Another study investigated the FC immunophenotype of T cells infiltrating HL for diagnostic assistance. The findings revealed an elevated CD4:CD8 ratio and increased CD7 expression in CD4(+) T cells, distinguishing HL from reactive lymphadenopathy. Using a CD7 mean fluorescence intensity (MFI) cutoff value from the data, this approach achieved a sensitivity of 69% and specificity of 90% for diagnosing classic HL (47).

Limitations of Study

One limitation of our study stems from our specific study design, which focused on identifying lymphocyte-rich FNA samples and then distinguishing between lymphoma and reactive cases. Consequently, we did not include "non-diagnostic" specimens that were ultimately diagnosed as lymphoma, potentially resulting in an overestimation of sensitivity for FNA performance in our analysis. The lack of CNB utilization in our practice is another potential limitation. Its adoption could have reduced the dependence on excisional biopsy and provided subtype-specific diagnoses of lymphoma in a significant number of cases, as evidenced by a median rate of 74% in a systematic review (34).

Conclusion

Our study highlights the diverse range of lymphomas that can initially present a palpable mass in the head and neck region and thus be amenable to FNA sampling. Recognizing cytologic atypia in lymphoid cells, combined with FC analysis, is crucial for early diagnosis and treatment, particularly in cases with minimal cytologic atypia but positive FC (e.g., SLL) and cases with significant cytologic atypia but negative FC (e.g., HL).

What Is Already Known on This Topic:

Fine needle aspiration (FNA) is widely recognized as a reliable, minimally invasive, and cost-effective diagnostic tool, especially for evaluating palpable masses in the head and neck region. FNA, in conjunction with flow cytometry (FC), plays a critical role in the diagnosis and subtyping of lymphomas, which account for approximately 12% of all malignant head and neck tumors. Lymphomas in this region are often secondary to systemic involvement, and while histologic examination remains the gold standard, FNA is frequently used in otolaryngology clinics as a first-line diagnostic method. Existing literature primarily focuses on the diagnostic utility of FNA in lymphomas, but the initial presentation of lymphoma as a palpable mass in patients seeking care at otolaryngology clinics remains underreported. This gap underscores the importance of institutional studies that evaluate the effectiveness of FNA and FC in diagnosing lymphoproliferative disorders in this clinical setting.

What This Study Adds:

This study provides a comprehensive evaluation of lymphomas presenting as palpable neck masses in an otolaryngology clinic. It highlights the diagnostic utility of combining FNA with flow cytometry, showing that their combined use significantly increases diagnostic sensitivity (100%) for lymphoma, with high specificity (98%). The study also emphasizes the diversity of lymphomas diagnosed, and the importance of recognizing cases where cytologic atypia may be present despite negative flow cytometry, especially in Hodgkin lymphoma.

Authors' Contributions: Conception and design: SMG and ASH; Acquisition, analysis and interpretation of data: ASH, JG and SMG; Drafting the article: ASH, JG and SMG; Revising it critically for important intellectual content: ASH, JG and SMG; Approved final version of the manuscript: ASH, JG and SMG.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2024;53(3):286-292
DOI: 10.5644/ama2006-124.448

The Efficacy of Various Orthodontic Appliances in the Treatment of Obstructive Sleep Apnea

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Received: 13 March 2024; **Accepted:** 24 July 2024

Abstract

Objective. The goal of this review was to determine the effectiveness of different types of monobloc and bibloc mandibular advancement device (MAD) devices in the treatment of all forms of obstructive sleep apnea (OSA), by reviewing the available literature. **Methods.** A systematic literature search was performed in PubMed, ResearchGate, NCBI and Google Scholar databases. The search included articles in English, published in the inclusive time period from 2000 to 2024. **Results.** A total of 13 studies were analyzed that directly compared the effectiveness of monobloc and bibloc devices. The studies were published in the period from 2000 to 2024, and included crossover and parallel randomized controlled trials, as well as cross and parallel cohort studies. Out of the 13 studies, four were classified as RCT parallel studies, six were RCT crossover studies, two cohort parallel studies, and one cohort crossover study. The duration of the studies was variable, ranging from four weeks to one year, with six studies having a so-called "washout period" between the use of monobloc and bibloc MAD devices. **Conclusion.** Both monobloc and bibloc devices show significant success rates in the treatment of mild to moderate OSA.

Key Words: Obstructive Sleep Apnea ▪ Mandibular Advancement Device ▪ Monobloc Oral Appliance ▪ Bibloc Oral Appliance.

Introduction

Obstructive sleep apnea (OSA) is a common, chronic disorder characterized by successive episodes of upper airway collapse with an increase in the airflow resistance, which leads to a decrease (hypopnea) or complete cessation of airflow (apnea) during sleep. The prevalence of the disorder in the general population varies from 3 to 7% in adult males, and 2% to 5% in adult females (1). Breathing cessation causes acute adverse effects, such as desaturation of oxyhemoglobin, vomiting, high blood pressure and heart rate, increased sympathetic activity, sleep fragmentation, etc. (2).

Risk factors for the development of obstructive sleep apnea primarily include: older age, male gender, obesity, and craniofacial anomalies, as well as anomalies of the upper respiratory pathways. The prevalence of sleep related problems, including obstructive sleep apnea, increases with age. The prevalence increases steadily until the age of 60, after which it reaches a plateau. Possible reasons for the increase in the prevalence of OSA during aging are structural changes in the parapharyngeal area, such as increased deposition of fatty tissue and lengthening of the soft palate (1). Treatment of patients with obstructive sleep apnea requires a multidisciplinary approach. Therapeutic options include continuous positive pressure therapy (CPAP), followed by weight loss, surgical interventions to the upper respiratory pathways, and intraoral orthodontic devices (3).

Intraoral devices, as a therapeutic option for OSA, are recommended for the treatment of mild and moderate OSA, as well as severe OSA in patients who do not tolerate CPAP therapy, or when CPAP therapy has proven to be unsuccessful (4). Intraoral devices can be divided into three groups:

tongue retainers (TRD); soft palate lifters (SPL) and devices for mandibular protrusion – a mandibular advancement device (MAD). SPL devices have been completely abandoned for use today, while the remaining two groups of devices are still in use. TRD device design constitutes an extraoral flexible protruding part that leads to gentle suction of the tongue under pressure, pulling the tongue forward and subsequently opening the airway during sleep (5).

The most commonly used intraoral devices are mandibular advancement devices (MAD). MADs consist of splints which are placed on the upper and lower teeth, with the aim of protruding the mandible and keeping it in a protruded position (3). This leads to the expansion of the upper airways, by lateral movement of parapharyngeal fatty deposits, as well as the forward positioning of the base of the tongue. Additionally, there are also changes in muscle activity, with the focus on relaxation of the genioglossus muscle, and activation of the masseter and submental muscles. By their action, MAD devices reduce the collapsibility of the upper respiratory pathways, resulting in a reduction in apnea episodes during sleep (4). Current research on the effectiveness of different oral devices for the treatment of OSA has conflicting opinions (6).

The goal of this review was to determine the effectiveness of different types of monobloc and bibloc MAD devices in the treatment of all forms of OSA, by reviewing the available literature.

Materials and Methods

Information Sources

For the purpose of this review, a systematic literature search was performed in PubMed, ResearchGate, NCBI and Google Scholar databases. The search was conducted using MeSh search strategies and using combined texts: obstructive sleep apnea and oral appliance, monobloc oral appliance, bibloc oral appliance, mandibular advancement device, fixed mandibular advancement device, custom-made mandibular advancement device, monobloc mandibular advancement device, and bibloc mandibular advancement devices. The search included articles in English, published in the inclusive time period from 2000 to 2024.

Selection Process

The literature review included two steps. In the first step, a literature search was performed with an overview of the available abstracts. The second step included collection of the full text of all studies that fully met the inclusion criteria. Ultimately, this review paper included a total of 13 studies directly comparing the impact of both monobloc and bibloc types of devices.

Eligibility Criteria

The studies include randomized controlled studies, nonrandomized prospective studies, clinical studies with organized data collection, and cohort studies. The inclusion criteria were: studies that evaluated the performance of two or more types of devices that had to be classified as monobloc or bibloc type; a definitive diagnosis of OSA established on the basis of polysomnography studies with an apnea-hypopnea index (AHI) value greater than five; the outcome of therapy with a MAD device assessed on the basis of a controlled polysomnographic study, and the ESS score (Epworth scale drowsiness) or SAQL score (Sleep Apnea Quality of Life). Exclusion criteria were non-English articles, case reports and review articles, different diagnostic criteria for OSA, and articles with insufficient data for analysis.

Presentation of Data

The recorded data include: the name of the authors and date of the publication of the research; study design, device design, demographic data; BMI values; the number of patients in the study; mandibular protrusion value and vertical dimensions; the degree of OSA; success of the therapy; unwanted effects of the device; acceptance of therapy; and the economic profitability of the type of

device. The success criterion is defined by AASDM (American Academy of Dental Sleep Medicine) as a reduction in the AHI value by 50% from the basal level, or a reduction in the degree of OSA.

Results

A total of 13 studies were analyzed that directly compared the effectiveness of monobloc and bibloc devices. The studies were published in the period from 2000 to 2022, and included crossover and parallel randomized controlled trials, as well as cross and parallel cohort studies. Out of the 13 studies, four were classified as RCT parallel studies, six were RCT crossover studies, two cohort parallel studies, and one was a cohort crossover study. The duration of the studies was variable, ranging from four weeks to one year, with six studies having a so-called "washout period" between the use of monobloc and bibloc MAD devices. That period implies a time period during which the subject does not use any type of MAD device, and it was used in the studies where one group of subjects used both types of devices (Table 1).

Four studies showed the equal effectiveness of both types of MAD devices by measuring the basal and control values of the AHI index (Table 2). Six studies reported the greater efficacy of monobloc MAD devices (Table 3). Three studies showed the better efficacy of the bibloc MAD device (Table 4).

Table 1. Comparison of the Studies Analyzed by Type and Duration, and the Degree of OSA*

Researchers	Year of publication	Type of study	Duration of study	Degree of OSA*
Isacsson et al. (7)	2017	Cohort parallel study	1 year	N/A‡
Isacsson et al. (8)	2019	RCT† parallel study	6 weeks	Low to moderate
Yanamoto et al. (9)	2021	RCT† crossover study	4 weeks + 2 week "washout period"	Low to moderate
Al-Dharrab (10)	2017	RCT†, crossover study	4 months + 2 week "washout period"	Low to moderate
Bloch et al. (11)	2000	RCT†, crossover study	156 days of adaptation, 1 week of use per device	N/A†
Mantia et al. (12)	2018	RCT†, crossover study	10 weeks + 2 week "washout period"	N/A‡
Umemoto et al. (13)	2019	RCT† parallel study	3 months	N/A‡
Lee WH et al. (14)	2013	Cohort parallel study	3 months	Low, moderate and severe
Geoghegan et al. (15)	2015	RCT† crossover study	12 weeks (10 weeks wear + 2 weeks acclimatization) + 2 week "washout period"	N/A‡
Zhou et al. (16)	2012	RCT† crossover study	3 months + 2 week"washout period"	Low to moderate
Sari et al. (17)	2011	RCT† parallel study	1 month	N/A‡
Tegelberg et al. (18)	2020	RCT† parallel study	1 year	N/A‡
Lettieri et al. (19)	2011	Cohort crossover study	N/A	Low, moderate and severe

*Obstructive sleep apnea; †Randomized control trial; ‡Not applicable (not stated in the study).

Table 2. Basal and Control Values of AHI*, with the Same Efficacy of Both Types of MAD†

Study	Monobloc	Monobloc	Bibloc	Bibloc
	Basal AHI*	Control AHI*	Basal AHI*	Control AHI*
Isacsson et al. (7). ($\bar{x}$)	23	12.7	22	13.8
Isacsson et al. (8). ($\bar{x}$)	25.2	12.5	26.8	12.3
Yanamoto et al. (9). ($\bar{x}$)	12.5	5.0	12.5	5.8
Al-Dharrab (10). ($\bar{x}$±SD)	25.8±4.87	5.95±2.54	25.8±4.87	6.02±2.59

*Apnea-hypopnea index; †Mandibular advancement device.

Table 3. Basal and Control Values of AHI* with Higher Efficacy of Monobloc MAD†

Study	Monobloc	Monobloc	Bibloc	Bibloc
	Basal AHI*	Control AHI*	Basal AHI*	Control AHI*
Bloch et al. (11). ($\bar{x}$±SD)	22.6±3.1	7.9±1.6	22.6±3.1	8.7±1.5
Mantia IL et al. (12). ($\bar{x}$±SD)	28.5±5.7	8.5±3.2	28.5±5.7	14.2±4.5
Umemoto et al. (13). ($\bar{x}$±SD)	21.4±5.7	14.7±9.4	20.6±11.5	11.2±9.7
Lee WH et al. (14). ($\bar{x}$±SD)	34.7±14.7	12.5±11.1	30.9±15.3	15.3±12.6
Geoghegan et al. (15). ($\bar{x}$)	21.1	5.9	21.1	15.2
Zhou et al. (16). ($\bar{x}$±SD)	26.38±4.13	6.58±2.28	26.38±4.13	9.87±2.88

*Apnea-hypopnea index; †Mandibular advancement device.

Table 4. Basal and Control Values of AHI* with Higher Efficacy of Bibloc MAD†

Study	Monobloc		Bibloc	
	Basal AHI*	Control AHI*	Basal AHI*	Control AHI*
Sari et al. (17). ($\bar{x}$±SD)	17.9±6.8	9.1±4.9	18.8±7.3	7.3±3.3
Tegelberg et al. (18). ($\bar{x}$)	23.1	11.3	25.4	8.6
Lettieri et al. (19). ($\bar{x}$±SD)	30.1±24.4	10.0±12.4	29.7±24.1	7.6±9.7

*Apnea-hypopnea index; †Mandibular advancement device.

Discussion

The success of the treatment on the basis of the AHI index,, differs between these studies. In 10 studies, the complete success of the treatment is defined as a value of AHI <5 after MAD. Therapy, or a reduction in the AHI value by 50% after MAD therapy. The results of therapy success in relation to the AHI index also differ. In 2017 and 2019, Isacsson et al. achieved equal success in both groups.

A positive response to therapy, defined as a reduction in the AHI value to less than 10 events per hour, was achieved in 61% of subjects in the monobloc group, and 56% of subjects in the bibloc group (7).

In the 2019 study, it is said that both monobloc and bibloc MAD devices led to a decrease in AHI values by 12 to 14 apneic events per hour (8). A significant improvement was recorded in the AHI index in both groups of devices by Yamamoto et al., with complete success of the therapy in almost half of the subjects in both groups (9). The Al-Dharrab study showed the same result, where both types of devices showed a reduction greater than 50% in mean AHI, which coincides with the definition of treatment success (10) (Table 2).

This study has a limitation because the sample size was relatively too small to highlight any difference between the two appliances. Five studies included in this review demonstrated the superiority of monobloc devices in lowering the AHI value (Table 3). The greater success of the monobloc devices compared to the bibloc devices was noted by Bloch et al. The definition of successful treatment in this study was a reduction in AHI values below 10 events per hour, which was achieved in 18 subjects with a monobloc device (75%), and 16 subjects with a bibloc device (67%) out of the total number of 24 subjects. Although both types of device led to a decrease in the value of the AHI index, the monobloc device resulted in statistically more significant reduction values (11). Clinical application of the results revealed reduced snoring and certain aspects of impairment in daily activities were more pronounced with the monobloc than with the bibloc device. In addition, there was a trend toward greater improvement in several objective variables of breathing and sleep disturbance with the monobloc device.

La Mantia I, Umemoto et al. and Hyun Lee et al. also demonstrated the greater success of monobloc devices in reducing the value of the AHI (12-14). In the La Mantia study, both MADs showed efficacy in improving objective parameters compared to the baseline, with a significant difference in favor of the monobloc in terms of improving AHI (12). The monobloc group had 14 subjects with a complete response to therapy, i.e. the complete success of therapy, while complete success of therapy was noted in only five subjects in the bibloc group (13). In the study by Lee WH et al. therapy success, defined as a reduction in AHI values by 50%, was noted in 77.4% of subjects in the monobloc group and 58.3% in the bibloc group (14). Greater success in reducing AHI values in the monobloc group was noted by Geoghegan et al. (15), while Zhou et al. reported an absolute decrease in AHI to less than 10 events per hour, in 68.0% of subjects in the monobloc group, compared to 56.3% in the bibloc group (16).

The greater success of the bibloc type of device was demonstrated in three studies included in this review paper (Table 4). Sari et al. demonstrated the better success of the Clearway bibloc device in lowering AHI index values on follow-up PGS analyses. The follow-up was carried out after 7 days and after one month from the start of using the device, where the second follow-up analysis showed a more significant decrease in the value of the AHI index (17). All patients subjectively reported more restful sleep with a reduction in snoring. In addition, minimum oxygen saturation increased at the end of the first week, and also increased above 90% oxygen saturation at the end of the first month in both groups.

At follow-up examinations after one year of using the MAD device, Tegelberg et al. reported the greater success of the Narval bibloc device compared to the monobloc device. Although a significant decrease in the value of the AHI index was recorded in the bibloc group, successful therapy (AHI<10) was recorded in 68% of subjects in the bibloc group and 65% of respondents in the monobloc group (18). Lettieri et al. reported a greater reduction in obstructive events in the bibloc group. In the bibloc group, the AHI value decreased by 74.4%, and in the monobloc group that value was 64.9%. Complete success of therapy, defined as AHI value reduction to less than 5 events per hour, was achieved in 57.2% of subjects in the bibloc group, or 46.9% in the monobloc group (19).

According to these data, it has been demonstrated that both types of MAD devices lead to a reduction in the AHI index values, and thus to the success of OSA therapy (20). A large number of studies point to the greater success of monobloc devices in lowering the AHI index, however, the fact that these are short-term studies should be taken into account.

Assessment of the efficacy of monobloc and bibloc device therapies is also based on the severity of obstructive sleep apnea (OSA).

Out of the 12 studies analyzed, six studies evaluated the impact of both types of devices on the treatment of mild and moderate OSA (Table 1). All the studies resulted in the conclusion that both monobloc and bibloc devices lead to a reduction in AHI values, i.e. a reduction in AHI values by 50% in both mild and moderate OSA. Isacsson et al., recorded more successful results of both types of devices in the treatment of moderate OSA (8).

The effectiveness of both monobloc and bibloc devices in the treatment of severe OSA was assessed in three of the analyzed studies. Research by Lee WH et al. showed the higher success rate of monobloc devices in the treatment of severe OSA, with a value of 86%, while the bibloc device recorded a success rate of 69.7% (14). A limitation of this study is the relatively short follow-up duration for evaluating compliance. Despite these limitations, the study may be meaningful in that it compared efficacy and compliance between mono-bloc and bi-bloc devices in the same patient population.

Lettieri et al., however, did not record the greater success of monobloc devices in the treatment of severe OSA. On the contrary, most subjects with severe OSA did not respond to monobloc device therapy, compared to a bibloc device (19). The study by Tegelberg et al., reported that both types of devices resulted in a significant reduction in values in the group of subjects with severe forms

of OSA (AHI>30), with the slightly higher efficacy of the bibloc device (18).

Preferably, the final selection of appliances should be made by dental specialists, in accordance with and adjusted to the patient, thereby introducing personalized medicine in MAD management. Cost aspects, such as appliance price and the number of return visits, are secondary factors which differ with every appliance design and per patient. Recommendations for the optimal MAD design and phenotyping of OSA patients are difficult to draw and insufficiently supported by the current literature (21, 22).

Study Limitations

It is important to acknowledge certain limitations within this review. First, the studies analyzed in this review are predominantly short-term in nature, with most having small sample sizes. Additionally, a significant portion of the studies primarily include male subjects, which may not fully represent the population affected by OSA. Given the chronic nature of OSA, necessitating lifelong therapy, there is a critical need for longer-term studies to explore the sustained effectiveness of these devices. Furthermore, due to anatomical differences in the airway between male and female populations, studies are needed that directly compare the efficacy of specific device types in both groups, as well as using larger sample sizes to enhance the robustness of the findings.

Conclusion

From the findings derived from the study's analysis regarding the efficacy of MAD devices in reducing AHI values, it can be inferred that both monobloc and bibloc devices demonstrate comparable success rates in the management of mild to moderate OSA. Nevertheless, in cases of severe OSA, the bibloc device demonstrated superior efficacy. Consequently, the initial treatment preference for mild to moderate OSA may lean towards a monobloc device, while consideration of a bibloc device may arise if the monobloc device yields unsatisfactory outcomes, or is not well-tolerated by the patient. An alternative type of MAD device may be considered as a subsequent option in the event of an insufficient response to initial MAD therapy, before consideration of CPAP therapy referral for the patient.

What Is Already Known on This Topic:
Obstructive sleep apnea (OSA) is a common, chronic disorder characterized by successive episodes of upper airway collapse, with an increase in the airflow resistance, which leads to a decrease in (hypopnea) or the complete cessation of airflow (apnea) during sleep. Oral appliance therapy with bibloc or monobloc devices is a non-invasive treatment option that offers a wide variety of oral devices for the treatment of obstructive sleep apnea.

What This Study Adds:
This review summarizes studies published between 2000 and 2024 regarding the effectiveness of MAD devices in reducing AHI. Both monobloc and bibloc devices have been shown to be effective in cases of moderate OSA, while in cases of more severe forms of OSA, bibloc devices showed greater effectiveness.

Authors' Contributions: Conception and design: AG; Acquisition, analysis and interpretation of data: AJ; Drafting the article: VDZ; Revising it critically for important intellectual content: AT; Approved final version of the manuscript: LRV.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2024;53(3):293-302
DOI: 10.5644/ama2006-124.460

Inborn Errors of Immunity: New Insights

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Received: 3 November 2024; **Accepted**: 17 December 2024

Abstract
This paper presents a comprehensive and updated overview of inborn errors of immunity (IEIs), focusing on the optimal treatment strategies. IEIs or primary immunodeficiencies (PIDs) are a heterogeneous group of approximately 500 disorders, classified into ten categories according to the affected component of the immune system. The clinical presentation varies, based on the type of the disorder and the patient's age. Early diagnosis is essential to prevent recurrent severe infections and potential organ damage. Treatment strategies, including hematopoietic stem cell transplantation, enzyme replacement therapy, thymus transplantation, or gene therapy, primarily focus to restore immune function. Emerging therapeutic approaches aiming to modify the immune response comprise small molecule inhibitors, biological therapies, and adoptive transfer of virus-specific T-cells. Given the complexity and diversity of PIDs, as well as evolving novel therapies, continuous education of the physicians on timely diagnosis and effective intervention, significantly improves patients' management and outcomes. **Conclusion.** Early diagnosis and individualized treatment plans are crucial for effectively managing IEIs. As treatment options evolve, ongoing education and the integration of new approaches are key to improving patient outcomes and quality of life.

Key Words: Inborn Errors of Immunity ▪ Bone Marrow Transplantation ▪ Gene Therapy ▪ Biological Therapy.

Introduction

Primary immunodeficiencies (PIDs) are a diverse group of inherited disorders that are typically caused by pathogenic germline variants in single genes. These alterations result in specific disruptions in the development and function of the immune system. It has been recently proposed to replace the term "PIDs" with "inborn errors of immunity" (IEIs) to emphasize the diversity of clinical presentations, including not only immune deficiencies, but also excessive or dysfunctional immune responses (1).

IEIs present clinically as increased susceptibility to infections, autoimmunity, autoinflammatory diseases, bone marrow failure, and/or malignancy. The estimated prevalence ranges from 1 to 5 per 1,000 individuals (2). Over the past two decades, advances in genomic analysis, combined with biochemical and cellular testing, have enabled a more precise identification of IEIs (3), with more than 480 classified disorders nowadays compared to approximately 150 in 2009 (4, 5). Better classification, based on the underlying molecular, cellular, and immunological mechanisms, led to significant improvements in management and outcome (4).

Many IEIs, such as severe combined immunodeficiency (SCID), have been effectively treated for several decades with hematopoietic stem cell transplantation (HSCT) (5). Recently, precision medicine strategies have emerged, including the targeted modulation of intracellular pathways affected by genetic alterations, and gene therapy using viral vectors for selected disorders (6).

The aim of this review is to provide a comprehensive and updated overview of IEIs, with a particular focus on optimal treatment strategies. By

exploring the classification, clinical presentation, and advancements in therapeutic approaches, this review highlights the importance of early diagnosis and tailored interventions in improving patient outcomes. Furthermore, it underscores the role of emerging therapies and the need for continuous education among healthcare professionals to enhance the management of these complex disorders.

Classification

The most recent classification identifies a total of 485 distinct IEIs. The International Union of Immunological Societies (IUIS) divides IEIs into nine main categories, based on the component of the immune system affected and most likely clinical presentation. A tenth category encompasses IEI phenocopies (Table 1). Each category of IEI is characterized by unique pattern of infections, autoimmunity, and/or inflammation, which aids in guidance for the initial diagnostic evaluation (2).

Category I - Combined Immunodeficiencies without Syndromic Features

This category includes combined immunodeficiencies (CIDs) that affect both cellular and humoral immunity but lack distinct syndromic features. Patients with T-cell lineage defects are predisposed to a range of viral, fungal, and bacterial infections, including opportunistic infections (e.g., *Pneumocystis jirovecii* pneumonia) and adverse reactions to live vaccines (e.g., measles, mumps, rubella, and varicella) (1). SCID is the most severe disorder within this category, typically manifesting in early childhood (7). While the most common SCID type is X-linked, autosomal recessive inheritance also exists, caused by mutations in genes such as Janus kinase 3 (*JAK3*), protein tyrosine phosphatase, and recombination activation genes *RAG1* and *RAG2* (8). Another autosomal recessive form is adenosine deaminase (ADA) deficiency, caused by mutations in the *ADA* gene, which result in the toxic accumulation of metabolites that are particularly harmful to developing lymphocytes, leading to profound defects in both T- and B-cell immunity (7).

Table 1. IUIS* Classification of Primary Immunodeficiencies

Category	Primary immunodeficiency	Genetic defects (N†)
I	Cellular and humoral immunodeficiencies	60
II	Syndromic combined immunodeficiencies	65
III	Antibody deficiencies	43
IV	Immune dysregulatory diseases	47
V	Phagocytic diseases	42
VI	Innate immunodeficiencies	71
VII	Autoinflammatory diseases	49
VIII	Complement deficiencies	36
IX	Diseases due to bone marrow failure	43
X	Phenocopies of PIDs‡	13
Total		469

*International Union of Immunological Societies; †Number; ‡Primary immunodeficiencies.

Category II - Combined Immunodeficiencies with Syndromic Features

This heterogeneous group consists of CIDs with distinctive clinical features and well-defined underlying immune system abnormalities. Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder caused by mutations in the *LYST* gene, impacting lysosomal trafficking and resulting in partial oculocutaneous albinism, immunodeficiency, and mild bleeding tendency. Around 85% of CHS patients progress to an accelerated phase or hemophagocytic lymphohistiocytosis (9). Wiskott-Aldrich syndrome (WAS) is an X-linked rare condition, caused by mutations in *WASP* gene that encodes the Wiskott-Aldrich syndrome protein (WASP), essential for B- and T-cell signaling. WAS presents with purpura, bleeding tendency, eczema, and recurrent infections. DiGeorge syndrome is characterized by congenital heart defects, craniofacial abnormalities, thymic dysgenesis or agenesis, and developmental delays. It is primarily caused by deletions in the 22q11 region or mutations in genes at chromosome 10p13 (7).

Category III - Predominantly Antibody Deficiencies

Predominantly antibody deficiencies are the most common type of PIDs, characterized by recurrent bacterial infections particularly in the upper and lower respiratory tract, such as otitis, sinusitis, and pneumonia. The genetic basis is diverse, reflecting the immunological heterogeneity of these disorders. Mutations in genes such as *BTK* (Bruton tyrosine kinase), *CD3γ*, *CD40L*, and *ZAP-70* contribute to the variability in the clinical presentation and underlying mechanisms (4). X-linked agammaglobulinemia (XLA) or Bruton agammaglobulinemia is caused by mutations in *BTK* gene on the X chromosome, that lead to a severe block in B-cell development and immunoglobulin (Ig) production (10).

Category IV - Immune Dysregulation Diseases

This group involves defects in self-tolerance mechanisms, either central or peripheral, which often lead to autoimmunity or significant lymphoproliferation. Autoimmune lymphoproliferative syndrome (ALPS) is characterized by impaired lymphocyte homeostasis. Initial manifestations typically include lymphocyte expansion with lymphadenopathy, splenomegaly, and hepatomegaly, as well as cytopenias, including thrombocytopenia and hemolytic anemia, and lymphoma in later stages. ALPS is caused by germline and somatic variants in the *FAS* gene (specifically *ALPS-FAS* and *ALPS-sFAS*), which hinder Fas/Fas ligand (FasL)-mediated apoptosis necessary for lymphocyte regulation. In addition to *FAS*, mutations in other genes, such as *FASLG* (encoding Fas ligand) and *CASP10* (encoding caspase-10), have been implicated in ALPS. These mutations disrupt the apoptotic pathways critical for immune homeostasis, leading to the accumulation of autoreactive lymphocytes and associated clinical features (11).

Category V - Phagocyte Number/Function Defects

This category includes disorders affecting the number and/or function of phagocytes. Neutrophils, essential for pathogen clearance via phagocytosis and activation of proteolytic enzymes, are the first line of immune defense. Patients with compromised phagocytosis typically suffer from severe bacterial and fungal infections. Chronic granulomatous disease (CGD) is characterized by inability of phagocytes to produce reactive oxygen species, leading to impaired microbial killing. Clinical manifestations include recurrent infections, granulomatous lesions in the lungs, liver, lymph nodes, and gastrointestinal tract, lymphadenopathy, hypergammaglobulinemia, and anemia. CGD is inherited in an X-linked and autosomal recessive pattern (12). The X-linked form of CGD is caused by mutations in the *CYBB* (Cytochrome B[-245] Beta chain) gene. The autosomal recessive forms are caused by mutations in *CYBA* (Cytochrome B[-245] Alfa chain), *NCF1* (Neutrophil Cytosolic Factor 1) and *NCF2* (Neutrophil Cytosolic Factor 2) genes (4).

Category VI - Defects in Intrinsic and Innate Immunity

This group encompasses a wide range of disorders resulting from defects in innate immune system, such as natural killer (NK) cells, Toll-like receptors (TLRs), various cytokines and other essential signaling molecules. Chronic mucocutaneous candidiasis involves persistent or recurrent *Candida* infections limited to mucous membranes, skin, and nails, due to T-cell defects. It is inherited in an autosomal dominant pattern linked to *STAT1* mutations or in an autosomal recessive pattern, most commonly linked to mutations in *AIRE* gene (13).

Category VII - Autoinflammatory Disorders

Autoinflammatory disorders are characterized by excessive activation of the innate immune system, leading to the overproduction of proinflammatory cytokines and subsequent tissue damage. Familial Mediterranean Fever is an autosomal recessive disorder caused by mutations in the *MEFV* gene which encodes pyrin (a protein found in neutrophils), resulting in recurrent fever, peritonitis, arthritis, pleuritis, skin lesions, and, in some cases, renal amyloidosis with kidney failure (14).

Category VIII - Complement Deficiencies

This group of disorders include deficiencies in the complement system, as an integral component of innate immunity. Early deficiencies in the classical component pathway (C1q, C1r, C1s, C2, C4) are associated with increased susceptibility to infections, often by encapsulated bacteria, and with autoimmune diseases such as systemic lupus erythematosus (SLE). Terminal complement component deficiencies (C5 to C9) present as recurrent meningitis, particularly with *Neisseria*. Factor H and Factor I deficiencies, affecting complement regulation, may cause atypical hemolytic uremic syndrome or increased susceptibility to *Neisseria*, depending on the underlying mutation. Hereditary angioedema results from a deficiency or dysfunction of C1 inhibitor (C1-INH), which regulates the classical and lectin complement activation, as well as kinin, procoagulant, and fibrinolytic pathways. The main manifestations are recurrent episodes of the facial, oral, and upper airway swelling (15).

Category IX - Bone Marrow Failure Defects

Bone marrow failure syndromes include genetic conditions that affect hematopoiesis, and are often accompanied by significant immune dysfunction. Fanconi anemia (FA) is autosomal recessive disorder, associated with more than 23 FA complementation genes (*FANC*) which are all involved in DNA repair. Most patients present with skeletal abnormalities or other congenital malformations, including short stature, skin, eyes, ears, heart, urinary tract, gonads, gastrointestinal tract, and central nervous system. Bone marrow failure typically occurs by the age of seven, and almost all patients experience this complication by age 40 years. There is an increased risk for developing hematological malignancies (myelodysplastic syndrome, acute myeloid leukemia) and solid tumors (squamous cell cancers of skin and head/neck/tongue, skin basal cell carcinoma, anogenital cancers) (16).

Category X - IEI phenocopies

This group includes disorders that exhibit symptoms resembling those of IEIs. They are characterized by somatic mutations (as opposed to germline mutations in IEIs) or are associated with the presence of autoantibodies (17).

Diagnosis

In children with recurrent infections, especially those confined to a single organ system, the underlying causes are often related to increased exposure to pathogens, allergies, or anatomical abnormalities, rather than immune defects. However, a significant number of PIDs remain undiagnosed or are misdiagnosed, leading to extended periods of inappropriate or ineffective treatment. Therefore, early identification and accurate diagnosis of PIDs are essential to achieve favorable patient outcomes (18).

History and Physical Examination

In a child with the suspected IEI, a detailed medical history and thorough physical examination can often help narrow the diagnosis to the specific component of the immune system. Various diagnostic models have been developed to differentiate PIDs from those with other causes of recurrent infections. These models assume that children with PIDs are more likely to experience serious, persistent, unusual and/or recurrent infections, often referred to as "SPUR" infections. The models serve as valuable tools for raising suspicion of PIDs, which in turn prompts a more focused diagnostic approach.

The European Society for Immunodeficiencies (ESID) developed guidelines for the assessment of patients with suspected PID, which were updated in 2011, and categorize these disorders into seven clinically recognizable presentation patterns (Table 2) (19). O'Sullivan and Cant proposed several key warning signs of PIDs in the first year of life, which warrant prompt evaluation and referral to an immunologist. These signs include chronic oral thrush, persistent diarrhea, failure to thrive, recurrent infections from bacterial or opportunistic pathogens, pneumonitis unresponsive to treatment, extensive skin lesions, delayed umbilical cord detachment, hepatosplenomegaly, congenital

Table 2. Clinical Presentation of Primary Immunodeficiencies

Clinical presentation	Other relevant indicators	Possible immune deficiency
Frequent ENT* and lower respiratory tract infections	Bronchiectasis	Antibody deficiency Phagocytic diseases Wiskott-Aldrich syndrome Complement deficiencies
Failure to thrive from early infancy	Persistent diarrhea, rashes, or *Candida* infections	T-lymphocyte deficiencies Severe combined immunodeficiency Neutrophil disorders
Recurrent pyogenic infections	Inflammation with poor wound recovery Chronic granulomatous inflammation due to *Aspergillus* or *Burkholderia*	Neutrophil disorders Chronic granulomatous disease
Severe and/or unusual infections	Pneumococcal meningitis Herpes simplex encephalitis	T-lymphocyte deficiencies Severe combined immunodeficiency Wiskott-Aldrich syndrome Innate immunodeficiencies
Recurrent infections with the same pathogen	Infections with meningococci or other encapsulated bacteria or with uncommon serotypes; *Candida* infections	Antibody deficiencies / Complement deficiencies (Encapsulated bacteria) T-lymphocyte deficiencies (*Candida*) Macrophage disorders / T-cell interaction defects (*Mycobacteria*)
Autoimmune or persistent inflammatory conditions	More frequently recognized as a characteristic of PID†	Common variable immunodeficiency Hemophagocytic lymphohistiocytosis
Syndromic features	PID† diagnosis becoming more common in genetic conditions	DNA repair defects Hyper-IgE‡ syndrome DiGeorge syndrome

*Ear, Nose, and Throat; †Primary immunodeficiency; ‡Immunoglobulin E.

heart defects, and a family history of PIDs, or history of early childhood deaths. Laboratory indicators such as lymphopenia and low immunoglobulin levels, and absence of a thymus shadow on X-ray should also prompt further investigations (20).

Laboratory Evaluation

The initial evaluation of PIDs should target the most likely affected component of the immune system, based on the clinical assessment. B-cell abnormalities and combined B- and T-cell defects account for the majority of PIDs and should be prioritized in the evaluation. Standard initial laboratory tests include a complete blood count (CBC) with differential, biochemical panel, immunoglobulin levels, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and urinalysis. Special attention should be given to the absolute lymphocyte count. Other findings such as anemia, thrombocytopenia, thrombocytosis, leukopenia, or leukocytosis should also be noted. The biochemical panel may reveal signs of liver or renal disease, hemolysis, and hypoalbuminemia due to protein loss or malnutrition. Elevated inflammatory markers (e.g., ESR, CRP) may point towards chronic infection or autoimmunity. Ig levels should be compared with age-appropriate reference ranges. Low Ig levels suggest antibody deficiency or combined immunodeficiencies: an IgG level <300 mg/dL, total Ig (IgG + IgM + IgA) less than 500 mg/dL, or complete absence of IgA and/or IgM in a child older than six months indicates an antibody deficiency. Significantly elevated IgE levels (>2,000 IU/mL) may indicate a monogenic atopic disorder or one of the hyper-IgE immunodeficiency syndromes. Disorders of functional antibody production can occur despite normal IgG levels, and normal Ig levels do not rule out disease. Additional tests such as microbiological cultures and imaging studies should be

performed when indicated by the initial findings (the absence of a thymus shadow on chest X-ray is a common finding in severe T-cell deficiencies) as well as human immunodeficiency virus (HIV) tests in children with a delayed onset of severe infections or unexplained lymphopenia. Total complement (CH50) is recommended for patients with a history of sepsis or *Neisseria* infections. Flow cytometry analysis detects T- and B-cell subsets, and NK-cells (21).

Genetic Testing

Genetic testing plays a crucial role in diagnosing IEIs. With the growing accessibility of next-generation sequencing (NGS) technologies, including whole exome sequencing (WES), whole genome sequencing (WGS), and targeted gene panels, genetic testing has become an integral component of the diagnostic work-up for patients suspected of having PIDs. The decreasing costs and broader availability of these technologies have led to their more widespread use, with testing being conducted earlier in the diagnostic pathway. The results of genetic tests often help determine further laboratory investigations. Targeted NGS offers a cost-efficient method to screen for mutations in known immunodeficiency-related genes. It can identify atypical presentations of established genetic defects and pinpoint the underlying cause in cases where multiple candidate genes may be involved. Additionally, most NGS panels can detect copy number variations due to their high read depth. When targeted NGS does not provide conclusive results, WES is commonly employed as the next step.

WES analyzes approximately 2 percent of the genome, focusing on the exonic regions and nearby splice sites. In contrast, WGS sequences the entire genome, including the non-coding intronic regions. Although WES is less expensive, less labor-intensive, and easier to analyze, it is less effective in identifying mutations in deep intronic or promoter regions compared to WGS. These regions are more difficult to interpret independently, so WGS is typically used in research settings and often combined with RNA sequencing to investigate alternative splicing and gene expression patterns. Both WES and WGS are valuable tools for identifying rare genetic mutations, particularly in patients with severe, early-onset conditions. However, confirming the pathogenicity of new variants in known or novel genes remains a complex challenge (21).

Diagnosis in Neonates

Diagnosing PIDs in neonates presents unique challenges due to the innate characteristics of the neonatal immune system, which can mask the clinical manifestations of immune deficiencies (22). The immune system of the newborn is structurally developed but has limited exposure to infections and weaker inflammatory responses, which makes neonates more vulnerable to infections. While most newborns remain healthy due to innate immunity and transferred maternal IgG, certain features, such as a positive family history, unusual infections, or syndromic appearance, should raise suspicion of IEI. A positive family history is particularly important, as it may indicate a genetic predisposition to PIDs and prompt early evaluation. Screening laboratory tests, including CBC with differential and Ig levels, should be conducted if the risk factors are present. Additional evaluation, such as lymphocyte immunophenotyping and T-cell receptor excision circles (TREC) analysis, are valuable for early detection of T-cell deficiencies. TRECs are circular DNA fragments produced during T-cell development, and can be detected using polymerase chain reaction (PCR) from dried blood spot on Guthrie cards. The absence of TRECs indicates defects in T-cell maturation. Therefore, the inclusion of TREC testing in newborn screening would represent significant progress in the early diagnosis of PIDs (23).

Treatment

The treatment of PIDs depends on the underlying disorder. Some PIDs manifest with subtle and intermittent signs and symptoms, while others may progress rapidly to life-threatening conditions.

Early recognition and accurate diagnosis are crucial for effective treatment (19). Children with recurrent or chronic bacterial infections (e.g., otitis, sinusitis, bronchitis, pneumonia) should be treated promptly with empiric antibiotic therapy pending culture results (7).

Immune Reconstitution

Reconstitution of immune function can be achievable in certain IEIs through various treatments, including HSCT, enzyme replacement therapy, thymus transplantation, or gene therapy (24). The range of available therapies has expanded to encompass small molecule inhibitors, biological drugs, and the adoptive transfer of virus-specific T-cells to combat viral infections in immunocompromised patients (6).

Hematopoietic Stem Cell Transplantation

Allogeneic HSCT has been a standard treatment for severe IEIs for over five decades, and remains a cornerstone for treating conditions such as SCID, WAS, hyper-IgM syndrome, CGD, familial hemophagocytic lymphohistiocytosis (FHL), severe congenital neutropenia, and other combined immunodeficiencies. However, there are numerous serious adverse effects of allogeneic HSCT, including treatment-related mortality. Graft-versus-host disease (GvHD) is a common complication that can significantly impair immune function, different organs (skin, gastrointestinal tract, liver, lungs, kidneys, eyes, and hematopoietic system), and post-transplant quality of life. Therefore, a careful evaluation of risks and benefits of HSCT for individual patients is essential. Unlike in hematological malignancies, where the goal is to eradicate immune cells, the aim of HSCT in IEIs is to achieve immune system reconstitution. Reduced intensity conditioning (RIC) regimens, such as busulfan combined with fludarabine, are recommended for non-SCID IEIs. The optimal conditioning regimen should be tailored to each specific IEI (25). A matched related or sibling donor (MRD/MSD) is a preferred donor option. However, advances in graft processing, conditioning regimens, and the prevention of post-transplant complications, have rendered HSCT from matched unrelated donors (MUD) and mismatched related donors (MMRD) suitable alternatives, with survival rates exceeding 70% (26).

Thymus Transplantation

Allogeneic thymus transplantation is a therapeutic option for patients with thymus deficiency and athymia due to complete DiGeorge syndrome, since conditioning and HSCT could exacerbate their immunodeficiency. Despite advances in techniques and treatment outcomes, thymus transplantation still carries a relatively high complication rate. Cultured thymus organoids present a new promising therapeutic approach (5).

Biological Therapy

As the ability to establish molecular diagnosis for patients with PIDs improves, there is a growing interest in targeted therapies that can replace, enhance, or modulate immune responses. Biological drugs, including monoclonal antibodies and recombinant proteins, specifically target cytokines or their receptors. Fusion receptors have significantly increased the ability to modulate the immune system by linking extracellular domains of various transmembrane proteins to other molecules. For example, treatments for CTLA-4 (Cytotoxic T-Lymphocyte-Associated Antigen 4) and LRBA (Lipopolysaccharide Responsive and Beige-like Anchor protein) deficiencies have benefited from abatacept therapy. Abatacept is a soluble fusion protein that consists of the extracellular domain of human CTLA-4. It inhibits T-cell activation and prevents autoimmune reactions mediated by regulatory T-cells (Tregs). Enzyme replacement therapy, such as for SCID due to ADA, addresses metabolic deficiencies associated with specific PIDs. Currently, for most IEIs, no approved therapies exist, and "off-label" use is based on limited data (6).

Small Molecule Inhibitors

Small molecule inhibitors are low molecular weight compounds that can easily penetrate cells

and target intracellular signaling pathways, such as JAK/STAT pathway that transmits signals downstream of various cytokines. Small molecule JAK inhibitors, ruxolitinib and tofacitinib, have demonstrated clinical improvement in patients with STAT1 or STAT3 gain-of-function syndromes. Despite the potential side effects including thrombocytopenia, elevated liver enzymes and viral infections, long-term treatment resulted in the significant amelioration of immune dysregulation (6).

Adoptive Transfer of Virus-Specific T-Cells

Adoptive transfer of virus-specific T-cells (VSTs) provides a therapeutic option in controlling viral infections in immunocompromised patients, such as cytomegalovirus, Epstein-Barr virus, and adenovirus. Derived from either stem cell donors or HLA-matched third-party donors and serving as "ready-made" therapies, VSTs have shown efficacy, particularly in patients undergoing HSCT (6).

Gene Therapy

Gene therapy (GT) has become a feasible and effective treatment for several PIDs that are restricted to hematopoietic cell lineages. GT involves the transduction of autologous hematopoietic stem cells with a vector containing the corrected gene product, which is subsequently administered to the patient as an autologous bone marrow transplant. Early phase I trials of GT using gamma-retroviral vectors to treat SCID, WAS, and CGD demonstrated immune reconstitution post-engraftment, but were complicated by multiple cases of leukemia and myelodysplastic syndrome due to insertional mutagenesis. Recent advances in vector engineering have minimized these risks. Current self-inactivating lentiviral vectors have been successfully administered in phase I trials to treat patients with X-linked SCID and ADA, WAS, and CGD without reported cases of leukemia. The concept of GT has evolved from retroviral or lentiviral gene delivery to gene editing (GE), which aims to correct disease-causing genes using techniques such as Zinc Finger Nucleases (ZFN), Transcription Activator-Like Effector Nucleases (TALEN), and CRISPR/Cas9 (CRISPR-associated protein 9). GE creates double-strand DNA breaks at specific sites, triggering endogenous repair mechanisms that permanently edit the genome through non-homologous end joining (NHEJ) or homology-directed repair (HDR). Unlike GT, GE eliminates the need for viral vectors, offering more precise gene correction with fewer complications, such as insertional mutagenesis and transgene silencing. However, GE is still experimental and is not yet widely implemented in clinical practice (27).

As our understanding of PIDs underlying mechanisms expands, new opportunities arise for research and development of targeted therapies, along with broader use of potentially curative treatments like GT. With increased survival rates, it is now crucial to establish best practice guidelines and coordinated healthcare for all adolescents with PIDs in the transition from pediatric to adult services (28).

Conclusion

IEIs represent a rapidly expanding group of genetic disorders of the immune system. Although not very rare, IEIs are frequently misdiagnosed or diagnosed late due to their complex or atypical clinical presentation, which severely diminishes patients' quality of life and survival. Timely recognition and referral to an experienced immunologist can significantly improve outcome. Therefore, continuous education of healthcare professionals on early diagnosis and advanced therapeutic strategies is crucial for minimizing complications and mortality related to PIDs.

What is Already Known on This Topic:

IEIs are a group of diverse monogenic disorders mainly characterized by deficient or dysfunctional immune system, and are associated with significant morbidity and mortality. Until recently, the mainstay of the management included prompt treatment of infections, antimicrobial prophylaxis, and Ig replacement. The advent of precision-based therapies has dramatically enhanced the quality of life and outcome of patients with IEIs. Timely referral to immunology services is critical, enabling patients to receive an accurate molecular diagnosis and targeted therapy when available.

What This Study Adds:

Lack of knowledge and awareness among healthcare professionals regarding the diversity of IEI manifestations in children and adults contributes to diagnostic and treatment delays. This review briefly describes new insights in the pathophysiology of PIDs, and discusses revolutionary treatment armamentarium, with the aim of improving recognition, timely diagnosis and management of these disorders.

Author's Contribution: Conception and design: JR and GB; Acquisition and interpretation of data: GB and JR; Drafting the article: GB; Revising it critically for important intellectual content: JR; Approved final version of the manuscript: GB and JR.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Scoping Review Protocol
Acta Medica Academica 2024;53(3):303-308
DOI: 10.5644/ama2006-124.463

Translational Research on Polygenic Risk Scores in Common Neurodegenerative Diseases - A Scoping Review Protocol*

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Received: 22 October 2024; **Accepted**: 23 December 2024

Abstract

Objective. The purpose of this protocol is to clearly describe the process for the scoping review we plan to conduct on the topic of polygenic risk scores (PRS) in common neurodegenerative diseases. We will present the review's objective, the strategy for evidence search, the data extraction and analysis procedure, and how the results will be presented. **Methods.** The inclusion criteria for the planned scoping review will focus on evidence sources that involve PRS applied to neurogenerative diseases such as Multiple sclerosis, Parkinson's disease, Alzheimer's disease, and Amyotrophic lateral sclerosis in any phase of translational research, from early development to clinical implementation. This includes its use in risk prediction, early diagnosis, prognosis, and treatment decision-making. The research questions were created based on the population, context, and concept framework. We will consider both peer-reviewed papers and grey literature published in English or German for inclusion. Two independent reviewers will search for information. **Conclusion.** The findings from the scoping review will be presented descriptively and summarized according to the research questions to illustrate the current status of translational research on PRS in common neurodegenerative diseases.

Key Words: Evidence Gaps ▪ Genetic Risk Score ▪ Nervous System Diseases.

Introduction

Neurodegenerative diseases such as multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS), are complex disorders characterized by progressive deterioration of nervous system function. These conditions pose a substantial and growing global health burden due to their chronic nature, the lack of curative treatments, and the aging population, which increases the prevalence of these diseases (1). Despite different aetiologies, a common feature of neurodegenerative diseases is chronic activation of innate immune cells within the central nervous system, and in diseases like MS, the influx of peripheral immune cells across the blood-brain barrier (2).

Even with significant progress in understanding the pathophysiology of these diseases, much remains unknown about the genetic and environmental factors that contribute to their onset and progression. Genetic predisposition plays a crucial role in many neurological disorders. Advances in genome-wide association studies have led to the identification of numerous genetic loci associated with increased risk for common neurodegenerative diseases (3-7). However, the individual effects of most genetic variants are small, and the underlying genetic architecture is highly polygenic. To address this complexity, polygenic risk scores

* Registered with Open Science Framework with the following DOI: https://doi.org/10.17605/OSF.IO/2NRGQ

(PRS) have emerged as a method for estimating an individual's overall genetic risk by combining the effects of multiple genetic variants (8). As a result, PRS offers the potential for improving risk prediction, early diagnosis, and personalized treatment by integrating genetic information into clinical decision-making.

Despite the promise of PRS, translational research in the field of neurodegenerative diseases faces several challenges that must be addressed to move from research to clinical practice. One significant challenge is the need for large, diverse datasets to ensure that PRS calculations are accurate and applicable across different populations. Most PRS models are currently based on data from individuals of European ancestry, which limits their generalizability and clinical utility for other populations (9). Moreover, PRS needs to be integrated with other risk factors, such as environmental exposures and lifestyle factors, to provide a more comprehensive risk assessment and guide more effective interventions (10). Addressing these challenges is critical for translating PRS into routine clinical tools that can improve outcomes in neurodegenerative diseases.

To assess the current state of translational research on PRS in common neurodegenerative diseases, specifically MS, PD, AD, and ALS, we are aiming to conduct a scoping review. The objective of the planned review will be to map existing literature on the clinical applicability of PRS, explore its potential benefits and limitations, and identify knowledge gaps that need to be addressed to advance the integration of PRS into routine clinical practice. By utilizing a scoping review approach, we will seek a wide range of information, including peer-reviewed articles and various forms of grey literature.

Methods and Analysis

This scoping review protocol has been registered via the Open Science Framework. The public registration is uniquely identified with the following DOI: https://doi.org/10.17605/OSF.IO/2NRGQ. The protocol was developed based on the Joanna Briggs Institute (JBI) Protocol Template (11) and in accordance with JBI methodology (12). The proposed scoping review will be conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-analyses extension for scoping review (PRISMA-ScR) (13) and the guidelines set by the JBI (14). Any modifications in the protocol during the scoping review procedure will be reported and documented in the final manuscript.

A preliminary search was conducted on MEDLINE (via PubMed), the Cochrane Database of Systematic Reviews, and JBI Evidence Synthesis, with no time restrictions applied. The search used the initial terms "genetic risk score," "polygenic risk," "neurodegenerative disease," "neurodegenerative disorder," and "review." No current or ongoing systematic reviews or scoping reviews on this topic were found.

Review Question

We formulated the following research questions:

1) What is the current state of translational research on PRS?
2) What is the evidence base for clinical implementation of PRS?
3) What is the predictive power/value/performance/accuracy of PRS?
4) What are the contexts of use of PRS?
   a. Healthcare clinical setting / laboratory / commercial entity
   b. Screening / diagnosis / prognosis / therapy

for the common neurodegenerative diseases, such as MS, PD, AD, and ALS.

Eligibility Criteria

Population

The scoping review will encompass studies involving patients with one of four common neurodegenerative diseases: MS, PD, AD, or ALS. It will also cover public opinion studies regarding the clinical use of PRS in these neurodegenerative diseases. Additionally, we will include methodological papers describing the development of PRS for the aforementioned diseases.

Concept

In the scoping review, we will be examining different concepts. The first will be related to translational research on PRS. The information will be organized according to a four-tier framework established by Khoury et al. (15). The first category, T1 studies, will encompass observational studies and clinical trials that focus on the health applications of the polygenic score. The T2 category will involve studies that evaluate the clinical utility of PRS. The T3 category will cover studies related to dissemination and implementation research of PRS, while the T4 category will address research on the population-level health impact of PRS.

Additionally, we will extract descriptive information from the evidence sources regarding the clinical implementation and predictive power of PRS in four studied neurodegenerative diseases. This information will be maped according to the type of disease and context of use.

Context

The scoping review will focus on evidence sources related to genetic testing providers, including healthcare clinical settings, laboratories, and commercial entities. The review will specifically consider the PRS test category, which encompasses risk prediction, early diagnosis, prognosis, and treatment decision-making.

Types of Evidence Sources

The scoping review will consider a wide range of peer-reviewed scientific literature, as well as grey literature. The types of eligible sources will therefore be:

- Systematic reviews or reviews of other types (16); meta-analyses
- Primary studies according to T1-T4 translation research phases (15); e.g., randomized controlled trials, non-randomized controlled studies, observational studies, dissemination and implementation research studies, outcome research studies
- Grey literature, such as guidelines, policy documents, registers, and websites

Primary sources will be excluded if already incorporated into an included evidence synthesis unless the data they contain are not otherwise reported in the evidence synthesis.

Evidence sources in English or German language will be included to broaden the search scope. This approach allows for the identification of relevant non-English papers, particularly grey literature. No time period restrictions will be applied.

Inclusion and exclusion criteria based on the population, context, and concept (PCC) framework and types of evidence sources are summarized in Table 1.

Table 1. Inclusion and Exclusion Criteria Based on Study PCC* Framework and Types of Evidence Sources

Criteria	Inclusion	Exclusion
Population	Evidence sources involving PRS† in patients with ‡MS, §PD, ‖AD, or ¶ALS	Studies presenting evidence on any related disease other than ‡MS, §PD, ‖AD, or ¶ALS
Concept	Evidence sources on PRS† according to the T1-T4 translation research phases framework established by Khoury et al. (15)	Purely methodological papers on PRS* without reference to any of the previously mentioned diseases
Context	Evidence sources related to genetic testing providers (healthcare clinical setting, laboratories, commercial entities)	-
	Evidence sources related to contexts of use of PRS† (screening, diagnosis, prognosis, therapy)	-
Types of evidence sources	Primary studies, reviews, meta-analyses, grey literature	Primary sources if already incorporated into an included review or meta-analysis
	Evidence sources in English or German	Studies not available in full form
	No time period restrictions	-

*Population, Concept, and Context; †Polygenic risk score; ‡Multiple sclerosis; §Parkinson's disease; ‖Alzheimer's disease; ¶Amyotrophic lateral sclerosis;.

Search Strategy

The search strategy will aim to locate both published and unpublished studies. An initial limited search of MEDLINE was undertaken to identify articles on the topic. The text words contained in the titles and abstracts of relevant articles, and the index terms used to describe the articles were used to develop a full search strategy for MEDLINE via PubMed (see Table 2). The search strategy, including all identified keywords and index terms, will be adapted for each included database and information source. The reference list of all included sources of evidence will be screened for additional studies.

Sources of Information

The electronic databases to be searched include:
- Cochrane Database of Systematic Reviews
- MEDLINE via PubMed
- Google Scholar
- JBI Evidence Synthesis
- JBI Evidence Implementation

Sources of Unpublished Studies/Grey Literature to Be Searched Include:

- ClinicalTrials.gov
- PHG Foundation
- Precision Health Database

Source of Evidence Selection

Evidence sources related to PCC criteria (Table 1) will be selected by two independent reviewers. Through each phase of the review, that is, screening, eligibility, and inclusion, discrepancies in study selection between the reviewers will be evaluated by calculating the inter-rater kappa coefficient. The points of disagreements will be discussed and solved to reach the acceptable level

Table 2. Search String for MEDLINE via PubMed

	Search String String
Concept 1: Neurodegenerative disease	(("Multiple Sclerosis"[Mesh] OR "Demyelinating Autoimmune Disease*"[tiab] OR MS[tiab] OR "Disseminated Sclerosis"[tiab]))
	OR
	("Parkinson Disease"[Mesh] OR "Parkinson Disease*"[tiab] OR "Paralysis Agitans"[tiab] OR "Parkinson's Disease*"[tiab] OR "Primary Parkinsonism*"[tiab])
	OR
	("Alzheimer Disease"[Mesh] OR "Alzheimer Disease*"[tiab] OR "Alzheimer Syndrome*"[tiab] OR "Alzheimer-Type Dementia*"[tiab] OR "Alzheimer Type Dementia*"[tiab] OR "Alzheimer's Disease*"[tiab] OR "Alzheimer Dementia*"[tiab] OR "Alzheimer's Disease*"[tiab] OR "Senile Dementia*"[tiab] OR "Alzheimer Type Dementia*"[tiab] OR "Alzheimer Type Senile Dementia*"[tiab] OR "Primary Senile Degenerative Dementia*"[tiab] OR "Alzheimer Sclerosis"[tiab] OR "Presenile Dementia"[tiab])
	OR
	("Amyotrophic Lateral Sclerosis"[Mesh] OR "Amyotrophic Lateral Sclerosis"[tiab] OR "ALS"[tiab] OR "Gehrig's Disease*"[tiab] OR "Gehrig Disease*"[tiab] OR "Gehrigs Disease*"[tiab] OR "Lou-Gehrigs Disease*"[tiab] OR "Charcot Disease*"[tiab] OR "Guam Disease*"[tiab])
	OR
	("Dementia"[Mesh] OR "Dementia"[tiab] OR "Amentia*"[tiab]))
	AND
Concept 2: Polygenic risk score	("Genetic Risk Score"[Mesh] OR "Genetic Risk Score*"[tiab] OR "Polygenic Risk Score*"[tiab] OR "Genetic Predisposition to Disease*"[tiab] OR "Genetic Predisposition*"[tiab] OR "Genetic Susceptibility"[tiab] OR "Genetic Susceptibilities"[tiab])

of agreement of 90% or higher (17). Following the search, all identified citations will be collated and uploaded into reference manager and duplicates removed. Titles and abstracts will then be screened for assessment against the inclusion criteria for the review. Potentially relevant sources will be retrieved in full and assessed in detail against the inclusion criteria. Reasons for exclusion of sources of evidence at full text that do not meet the inclusion criteria will be recorded and reported in the scoping review. The results of the search and the study inclusion process will be reported in full in the final scoping review and presented in a PRISMA-ScR flow diagram (13).

Data Extraction

Data extraction process will be conducted in accordance with JBI recommendations (18). Data will be extracted from papers and other evidence sources by two independent reviewers using a data extraction tool developed by the reviewers. Any disagreements between the reviewers will be resolved through discussion or with the involvement of an additional reviewer. The data extracted will include specific details about the population, concept, context, study methods and key findings relevant to the review questions. A draft extraction form is provided (see Table 3). The draft data extraction tool will be modified and revised as necessary during the process of extracting data from each included evidence source. Any modifications will be detailed in the scoping review. In addition to the data extraction form, an extraction guidance form will be developed, detailing each item to be extracted, and shared with each scoping reviewer.

Data Analysis and Presentation

The data will be analyzed and the results presented following the JBI recommendations (18). The analysis will be descriptive, and the findings will be visualized in tables and graphs. The evidence collected will be presented in accordance with the PCC framework based on the research questions. The results will be summarized using a narrative approach. Research gaps will be identified, and potential implications for future research will be discussed.

Acknowledgements: The authors would like to thanks Ms. Nana Turk, librarian at the Central Medical Library of the Faculty of Medicine, University of Ljubljana, for her kind assistance in preparing the search string.

Statement of Ethics: Not applicable. This scoping review protocol did not collect data from human participants.

Funding Sources: This study was a part of the project/program Gynecology and Reproduction: Genomics for Personalized Medicine and was financially supported by the Slovenian Research Agency (P3-0326).

Authors' Contributions: Conception and design: BP and MCS; Preliminary literature search: MCS, JS, and DžO; Drafting the article: MCS, JS, and DžO; Revising it critically for important intellectual content: BP; Approved final version of the manuscript: MCS, JS, DžO and BP.

Conflict of Interest: The authors declare that they have no conflict of interest.

Table 3. Draft Data Extraction Form

Type of evidence*	Year	Author	Title	Aim	Disease†
Ancestry of polygenic score	Translational phase‡	Type of research, methodology§	Setting‖	Context of use¶	Key findings

*Peer-reviewed papers / grey literature; †Multiple sclerosis, Parkinson's disease, Alzheimer's disease, Amyotrophic lateral sclerosis; ‡T1-T4 according to Khoury et al. (15); §Type of research: primary research, evidence synthesis, grey literature; ‖Healthcare clinical setting, laboratory, commercial entity; ¶Screening, diagnosis, prognosis, therapy

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Case Report
Acta Medica Academica 2024;53(3):309-313
DOI: 10.5644/ama2006-124.441

An Ectopic External Jugular Vein Draining into the Axillary Vein: a Rare Anatomical Variation with Clinical Implications

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Received: 11 February 2024; **Accepted:** 25 March 2024

Abstract

Objective. The external jugular vein drains a considerable part of the head and neck and constitutes a vessel implicated in various procedures in the cervical region. The aim of this study is to present an uncommon anatomical variation of the external jugular vein, and discuss the clinical implications of its presence. **Case Report.** We present a rare case of an ectopic external jugular vein terminating into the axillary vein, that we came across during routine dissection of a male cadaver of Greek origin. **Conclusion.** The venous system of the external jugular vein is used during procedures for the treatment of various conditions such as cardiac arrhythmias, hydrocephalus and defects of the head and neck. Hence, encountering the unpredictable course of a variant draining into the axillary vein may complicate these interventions, leading to multiple manipulations and undesirable results. Surgeons should be aware of the alternate anatomy of the venous system of the cervical region, and mindful of the possibility of encountering them.

Key Words: External Jugular Vein ▪ Axillary Vein ▪ Anatomical Variants ▪ Cephalic Vein ▪ Jugulocephalic Vein.

Introduction

The venous drainage of the head and neck is conducted by a subcutaneous and a deep venous system. Specifically, the subcutaneous venous system consists of the external jugular vein and the anterior jugular vein, while the internal jugular vein corresponds to the deep venous system. The external jugular vein (EJV), constituting part of the superficial venous system, drains the blood from the scalp and deep regions of the face (1, 2). The cephalic vein (CV) collects blood from the hand and radial side of the upper limb. The anatomic variability of the EJV correlates clinically with a number of interventional and surgical procedures, highlighting the importance of thorough knowledge and relevant suspicion. The common embryonic genesis with the cephalic vein conjoins with anatomic variants concerning both vessels, and the sustenance of their interconnection is considered an atavistic feature in human development (3).

We present a rare case of ectopic drainage of the external jugular vein into the axillary vein that was encountered during dissection of a male cadaver of Greek origin, that took place in the Department of Anatomy, Medical School, National and Kapodistrian University of Athens.

Case Report

During a routine dissection of an 85-year-old Caucasian male formalin-embalmed cadaver, that took place in the Hall of Dissections of the Department of Anatomy, School of Medicine, of the National and Kapodistrian University of Athens for educational and research purposes, an alternate course of the external jugular vein

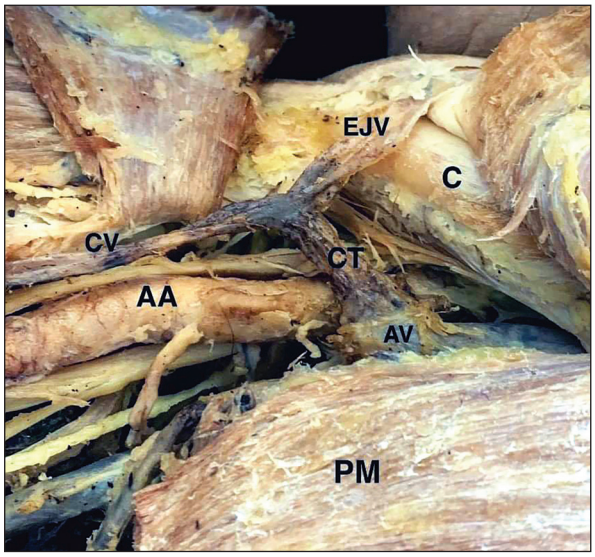

Figure 1. The region of the right deltopectoral triangle. The EJV joins the right cephalic vein with a common trunk (red arrow) that corresponds to a jugulocephalic vein that finally drains into the axillary vein. C=Clavicle; EJV=External jugular vein; CV=Cephalic vein; CT=Common trunk between CV and EJV; AA=Axillary artery; AV=Axillary vein; PM=Pectoralis major muscle.

was encountered, following the preparation of the cervical region and deltopectoral triangle on the right side. Specifically, the EJV descended superficially to the sternocleidomastoid muscle and the midpoint of the clavicle, and joined the right cephalic vein in the deltopectoral triangle, forming a common trunk. This common trunk corresponded to a jugulocephalic vein, which is a remnant that is normally present during the early stages of embryogenesis. The jugulocephalic vein passed under the pectoralis muscle and, crossing over the axillary artery, terminated into the axillary vein. A limitation of this study could be that the left jugular vein was traumatized during central line insertion when the patient was still alive and hospitalized in an intensive care unit, hence, the course of the left jugular vein could not be documented accurately.

Discussion

The external jugular vein (EJV) is traditionally formed by the junction of the posterior division of the retromandibular vein and the posterior auricular vein, at the level of the angle of the mandible. The configuration of the EJV takes place in the parenchyma of the parotid gland, and the vessel crosses the sternocleidomastoid muscle in an oblique fashion below the platysma muscle, reaching the midpoint of the clavicle. In the area of the major supraclavicular fossa, the EJV perforates the deep cervical fascia and enters the subclavian vein (1, 3).

The anatomic morphology of the EJV may present with considerable variability regarding its origin, course, tributaries and termination. Fenestrations of the EJV have been reported, i.e. the division of the vessel into two branches, which subsequently reunite to form the same vessel again (4, 5). Duplication of the EJV, on the other hand, is associated with the division of the vessel and the separate terminating points of its ensuing branches (4). Phlebectasia of the vessel has also been documented as well as absence in the form of an undivided retromandibular vein joining the internal jugular vein or as a continuation of the facial vein (4, 6). Russu et al. also presented the rare case of a EJV draining into the IJV (4).

The cephalic vein constitutes the continuation of the lateral end of the dorsal venous arch of the hand over the anatomical snuff box, and runs in the deltopectoral groove. At the deltopectoral triangle, the EJV penetrates the clavipectoral fascia and drains into the axillary vein (1, 3). Variations of the cephalic vein are indeed rare, and include its absence and merger with the subclavian vein, the internal jugular vein, the external jugular vein or the basilic vein (3, 5, 7). Communication of the cephalic vein with the external jugular vein has been recorded, where it assumes the form of the cephalic vein, following a supraclavicular course, or an anastomotic channel, uniting the terminal segment of the cephalic vein with the external jugular vein (3, 7-10). This anastomotic channel correlates to a persistent jugulocephalic vein, which represents a relic pertaining to human ontogenesis (7, 11). Święton et al. analysed 324 venographies and came across two cases of a supraclavicular cephalic vein, and 12 cases of persistent supraclavicular jugulocephalic veins (12).

During embryonic development, the superficial veins of the neck emerge from a superficial plexus of capillaries, which merge, creating larger venous stems. Initially the EJV emanates from the plexus and empties into the internal jugular vein (3, 11, 13). The cephalic vein forms as an anastomotic channel of the EJV and joins the axillary vein at a later stage of embryogenesis, while the EJV joins the subclavian vein. Subsequently, the segment crossing the clavicle, namely the jugulocephalic vein, atrophies (11, 13).

Nayak et al. encountered an anatomic variant of the external jugular vein, which communicated with the cephalic vein through an interconnecting vein (1). The communicating vein traversed the superficial surface of the clavicle, whereas the cephalic vein ran between the clavicle and the subclavius muscle, and terminated into the axillary vein (1). In contrast, the common trunk formed by the external jugular vein and the cephalic vein in this case report drained into the axillary vein. There are few reports in the literature describing the configuration of a common trunk between the cephalic vein and the EJV emptying in the subclavian vein (13, 14). Novakov et al. describe the case of a clavicular vein bifurcating into a main branch, which joined the axillary vein under the pectoralis major muscle, and a branch representing a "jugulocephalic anastomosis", which crossed the anterior surface of the clavicle reaching the EJV (3).

A thorough understanding of the morphology of the superficial veins of the neck is pertinent due to the widespread catheterization of these veins for central venous access, as well as pacemaker and defibrillator implantation (1, 12, 15). An attempt to catheterize cases where the cephalic vein communicates with the external jugular vein could result in ectopic placement of the wire, and the inability to implant the device correctly (12, 14). According to Steckiewicz et al., a supraclavicular course as well as the presence of multiple branches are considered unfavorable for the introduction of leads and subsequent implantation of a device (15). In the case described, the right cephalic vein formed a common trunk with the right external jugular vein, hence, introduction of a lead into the particular cephalic vein would result in its placement in the external jugular vein. Anatomical variations of the EJV are also relevant for catheterization and the implementation of ventriculoperitoneal shunting for treatment of hydrocephalus (16). Conducting a contrast venography prior to the procedure enables the surgeon to visualize the morphology of the veins and enhances success rates, as well as ensuring time and effort efficiency by reducing unnecessary manipulation.

Orthopedic surgeons treating clavicular fractures with concomitant blood loss should be aware of these anatomic variants since the supraclavicular course of the cephalic or external jugular vein could be interrupted by dislocated clavicular bone fragments and these vessels could be encountered during osteosynthesis (1, 2). Anastasopoulos et al. describe the course of an EJV crossing the lateral third of the clavicle, forming a common trunk with the CV, and draining into the subclavian vein (2).

The EJV may also serve as an alternative for venous outflow in free breast reconstruction, in cases where the internal thoracic vein and the thoracodorsal vein are deemed insufficient for conducting an anastomosis with the donor vessels (4, 17). Additionally, the sustenance of the EJV in the reconstruction of defects of the head and neck provides the possibility of harvesting regional flaps, such as the platysma myocutaneous flap, as the venous outflow of the part of the flap is regulated by the internal jugular vein, whereas the EJV drains the posterior part (18).

Limitation of Case Report

A limitation of this case report could be that the left external jugular vein was traumatized during central line insertion when the patient was still alive and hospitalized in an intensive care unit. As a result, the left external jugular vein of the cadaver could not be adequately depicted and documented, and no correlation in the morphology of the vessels between the two cervical sides could be established and discussed further.

Conclusion

The widespread use of the superficial veins of the neck for catheterization, venous access and reconstructive purposes renders a thorough knowledge of their configuration and anatomic variability pertinent and necessary for establishment of safe surgical dissection and interventional manipulation. This case of ectopic drainage of the external jugular vein into the axillary vein, after forming a common trunk with the cephalic vein, is extremely rare, and any attempt to utilize these vessels for the treatment of various conditions, such as cardiac arrhythmias, hydrocephalus and defects of the head and neck, may lead to undesirable results and multiple manipulations, should the surgeon proceed without suspecting the presence of anatomical variants. Preoperative imaging of the venous anatomy is suggested in order to increase the level of safety, by designing an anatomical map of the region, and revealing any relevant variants prior to any intervention.

What Is Already Known on This Topic:

Various studies have delved into the anatomical variability of the venous system of the head and neck. Moreover, the wide range of interventional and surgical procedures that involve the external jugular vein reflect its clinical significance and impact on the treatment of conditions, such as cardiac arrhythmias, hydrocephalus and defects of the head and neck.

What This Study Adds:

The aim of this report was to present an extremely rare variation of an ectopic external jugular vein draining into the axillary vein, and highlight the possible implications of performing procedures in patients carrying this anatomical particularity. The diversity of these procedures renders knowledge of the morphology of such anatomic variants indispensable for the improvement of safety and to reduce the number of iatrogenic injuries, unnecessary manipulations and inadvertent sequelae.

Authors' Contributions: Conception and design: DD, DC and TT; Acquisition, analysis and interpretation of data: DD, DC and NP; Drafting the article: DD; Revising it critically for important intellectual content: DC, FD, AS and AS; Approved final version of the manuscript: DC, TT.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
Acta Medica Academica 2024;53(3):314-319
DOI: 10.5644/ama2006-124.455

A Round Ligament Mesothelial Cyst Imitating an Inguinal Hernia in a Woman of Reproductive Age

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Received: 13 July 2024; **Accepted:** 16 September 2024

Abstract

Objective. A wide range of lesions, including a mesothelial cyst of the uterine round ligament, are included in the differential diagnosis of a groin mass. Our study emphasizes the rarity of this ailment and the significance of correct preoperative diagnosis in guiding our treatment approach. **Case Report.** A 43-year-old female patient, presented to our hospital with a five-year history of swelling and slight discomfort in the right groin. A nonreducible inguinal mass with greater projection during a Valsalva maneuver was found. An abdominal CT scan revealed a well-defined lesion located near the right horn of the uterus and extending through the inguinal canal. The patient was brought to the operating room with the clinical suspicion of an inguinal hernia. A soft mass measuring 4.5×2×1.8cm was discovered in the right round ligament, and it was surgically removed. Histopathological examination confirmed a mesothelial cystic lesion. The patient's condition, after a period of 12 months, continues to be satisfactory. **Conclusion.** Few cases of uterine round ligament mesothelial cysts have been reported worldwide. Their cause is unknown, and preoperative diagnosis is unreliable. Resection relieves symptoms, and histological examination of the surgical specimen confirms the diagnosis. Additional clinical cases are needed to create a systematic clinical approach.

Key Words: Round Ligament of the Uterus ▪ Mesothelial Cysts ▪ Inguinal Mass ▪ Inguinal Hernia ▪ Case Report.

Introduction

A mesothelial cyst of the round ligament is an uncommon developmental anomaly that is frequently linked to and mistakenly identified as an inguinal hernia (1). Multiple case reports have documented instances when a cyst was mistakenly diagnosed as a hernia in the groin area, herniation of the ovary, or malignant metastases (1-4). This clinical entity predominantly manifests in women of middle age, and tends to affect the right side (1).

This case report presents the hospital admission of a 43-year-old female patient due to the presence of a lump in her right inguinal region. Following surgery and histological confirmation, it was determined that our patient had a mesothelial cyst of the round ligament.

Case Presentation

A 43-year-old female gravida 2, para 2 patient, who had a swelling and slight discomfort in her right groin, presented to our hospital's surgical outpatient clinic. She had no known medical history and had not had any prior procedures. The inguinal lump appeared immediately after her most recent pregnancy, approximately five years before. As a result of this complaint, she had seen gynecologists and general surgeons numerous times since then, for both scheduled and emergency visits. She

received reassurances from everyone that her condition was an inguinal hernia.

On the basis of a physical examination, the skin covering the bulge appeared normal on physical examination. The patient did not experience any pain or sensitivity when the inguinal region was examined by palpation. The lump exhibited immobility, non-reducibility, and increased prominence during a Valsalva maneuver. No lump or discomfort was present in the opposite inguinal region. An assessment of the external genitalia revealed limited enlargement in the top and outer region of the right labia majora. Subsequently, an abdominal computed tomography was performed as an imaging test. The CT scan revealed a well-defined lump measuring 4.5x2cm. This lesion appeared to begin from the uterus and followed a path inside the right iliac vein, through the inguinal canal, and outside the rectus abdominis muscle, before ending in the subcutaneous fat (Figure 1, 2). Following the conclusion of the clinical and imaging evaluation, an open surgical excision of the right round ligament was planned for a few weeks later.

The patient was admitted to our clinic on the day preceding the procedure. Beyond the surgical issue, no concurrent diseases were discovered during the routine pre-operative examination. Initial optimization relies on a comprehensive history, thorough physical examination and laboratory studies. The patient underwent a bowel preparation, and was instructed to abstain from eating or drinking after midnight. During the surgical procedure, she was administered a single dose of antibiotic prophylaxis with 2 g of cefazolin intravenously. A right oblique inguinal incision was performed under general anesthesia. The superficial inguinal ring showed no abnormalities. The round ligament was abnormally enlarged. The entire right round ligament was carefully separated and removed. Upon examination, the deep inguinal ring showed no signs of an indirect hernia sac. The histologic investigation of the surgical specimen indicated the presence of a solitary layer of cuboidal cells that formed the lining of the cyst (Figure 2). These cells exhibited positive staining for calretinin and cytokeratin7 (Figure 3, 4).

After surgery, the patient was sent home on the fifth postoperative day, because of an initial occurrence of atrial flutter and the subsequent evaluation by cardiologists. At four-month follow-up, the patient's condition remained satisfactory and she reported a notably enhanced quality of life.

Discussion

Nearly 30-50% of mesothelial cysts of the round ligament of the uterus (MCURLs) are also accompanied by small inguinal hernias (1, 3, 5). The

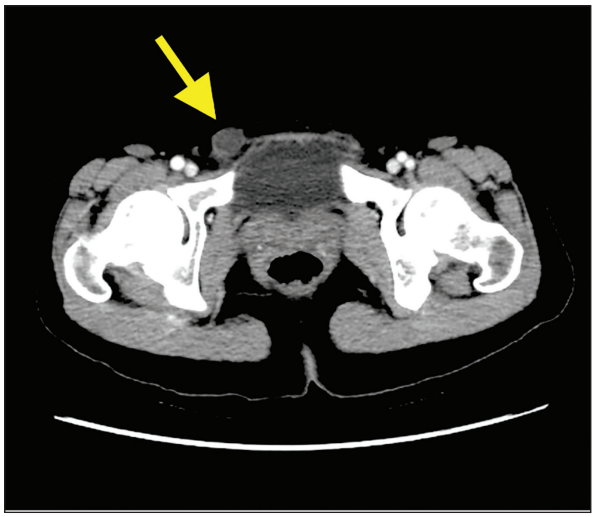

Figure 1. Axial contrast enhanced abdominal CT scan. A right inguinal cystic and solid mass (yellow arrow).

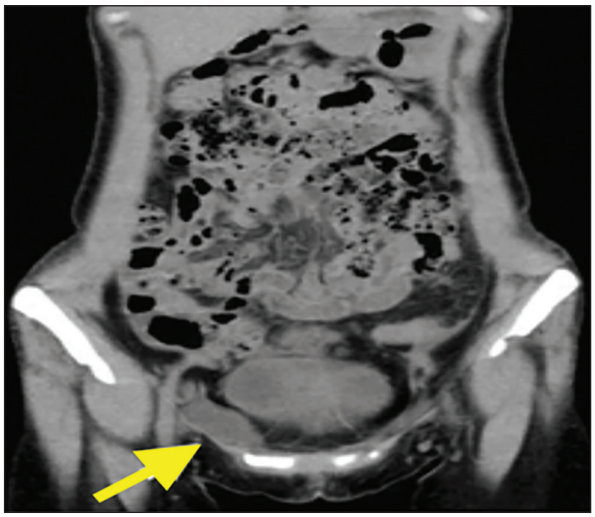

Figure 2. Coronal contrast enhanced abdominal CT scan. A well-circumscribed mass measuring 4,5×2cm (yellow arrow).

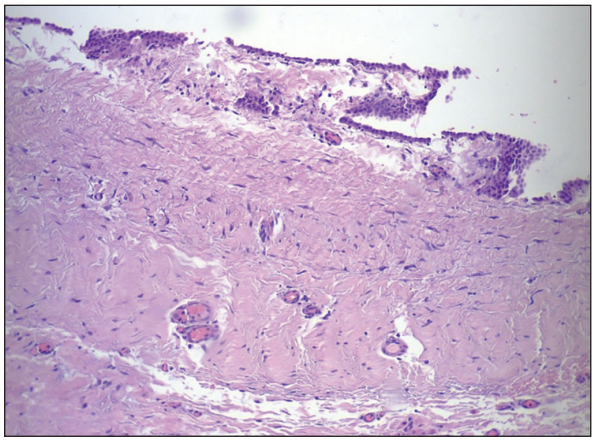

Figure 3. Histopathology of the surgical specimen. A cyst lined with a single layer of cuboidal cells (magnification 10×).

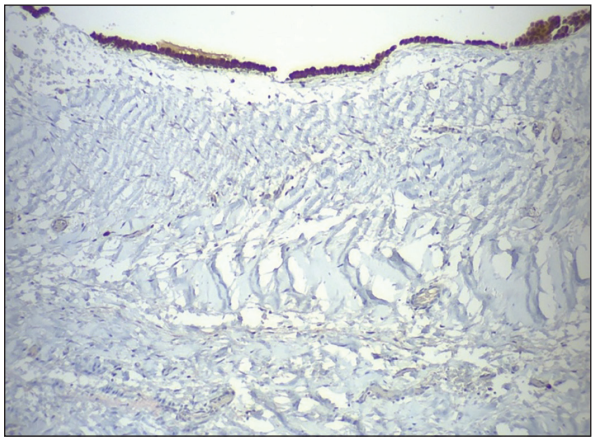

Figure 4. Immunohistochemistry of the surgical specimen. The cells stained diffusely positive for calretinin (magnification 10×).

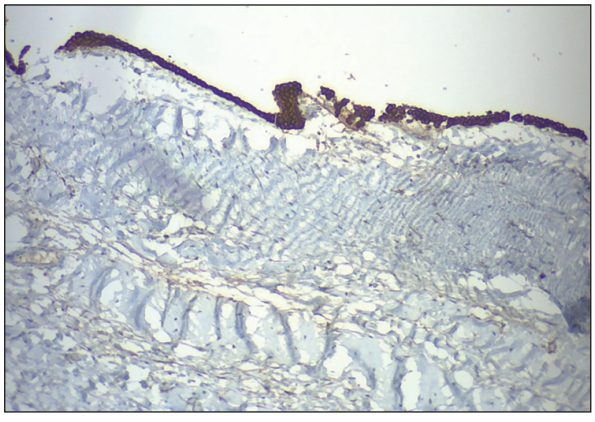

Figure 5. Immunohistochemistry of the surgical specimen. The cells stained diffusely positive for cytokeratin7 (magnification 10×).

etiology of MCURLs remains unidentified. There are three proposed theories regarding the development of MCURLs. The first theory proposes that MCURLs form when the mesothelium of the round ligament regenerates. The second theory suggests that MCURLs are a result of incomplete closure of Nuck's canal, leading to an MCURL that is essentially a cyst of Nuck's canal. The third theory proposes that MCURLs are created when embryonic, mesenchymal, and mesothelial components are incorporated during the formation of the round ligament (1-4, 6). The round ligament arises from the uterus, traverses the inguinal canal, and finishes at the region of the mons pubis and labia majora (7). From an anatomical perspective, this structure is the female equivalent of the gubernaculum testis. It consists primarily of smooth muscle fibers, connective tissue, blood vessels, and nerves, all of which are covered by a layer of mesothelial cells (8). The swelling of the inguinal region in females can be caused by various disorders, such as inguinal hernia, a tumor (lipoma, leiomyoma, sarcoma), a cyst, an abscess, lymphadenopathy, or hydrocele of the canal of Nuck (9).

Mesothelial cysts situated in the right lower quadrant can result in discomfort in the groin region, and exhibit clinical symptoms that resemble those of an inguinal hernia (1, 2, 4, 10, 11). Consequently, these conditions are frequently misinterpreted as groin hernias during the physical examination, and are only identified during the surgical procedure (4). When a person has a significant groin hernia, the surgeon may not be able to detect other issues during the procedure (11). These issues may only be identified after examining the removed tissue in a laboratory. To achieve an accurate preoperative diagnosis, it is essential to have a high level of suspicion and use imaging investigations (2).

Ultrasound is the preferred imaging technique, as it does not involve any radiation and is particularly safe for children and young women, who make up the majority of the affected population. This examination provides real-time information on intestinal peristalsis, vascular supply, and changes in size after coughing or the Valsalva

maneuver (6). Computed tomography shows the presence of a cystic mass with an uneven thickening of the wall and increased intensity of the solid areas when a contrast medium is injected intravenously. It is important to be cautious, to avoid incorrectly diagnosing the cyst as a metastatic site, especially when a primary tumor is found or when the patient has a history of cancer (3). Magnetic resonance imaging (MRI) is a costlier alternative that offers a higher level of detail regarding the surrounding anatomical structures. It is advisable only to use MRI in circumstances where the diagnosis is particularly challenging (12).

The diagnosis is established by means of histology. Our case findings suggested that the cyst was lined with a single layer of cuboidal cells that exhibit positive staining for calretinin, consistent with previous scientific publications (4, 5). After doing an extensive analysis of global literature from 1980 to 2024, we discovered that there are 35 reported cases pertaining to our particular ailment (1-6, 10, 13-17) (Table 1). The patient in our report like most in the PubMed, was under 50 years old and presented with inguinal pain but no menstrual cycle disorders. The cyst was located on the right side, consistent with the majority of cases in PubMed. Preoperative imaging included CT, but not ultrasound, which is the predominant imaging modality employed. The case was preoperatively diagnosed as inguinal hernia, which was also observed in 34% of PubMed cases. Notably, the patient underwent abdominal resection and did not have a coexisting inguinal hernia, a finding consistent with 13 cases in the literature. Laparoscopic resection, specifically TAPP (Trans Abdominal Pre-Peritoneal Repair), was used in 48% of cases.

The current research lacks adequate data to reliably recommend a specific therapy or follow-up plan. Given the benign nature of the condition, a prudent strategy would involve closely monitoring asymptomatic patients through regular ultrasound examinations. For cysts that exhibit symptoms or grow in size over time, surgical extraction is the recommended course of treatment (5). Considering the significant number of instances that utilized the TAPP procedure and the advantages of TAPP repair in comparison to open surgery, we assert that the TAPP treatment is likely the most efficacious method for eradicating MCURLs.

Table 1. The Characteristics of MCURLs in Our Case Report and Cases in PubMed

Characteristics	Our case report	Cases in PubMed (N=35)
		N; (%)
Age <50 years old	Yes	32 (91)
Symptom		
None	-	5 (14)
Inguinal pain	Yes	30 (86)
Menstrual cycle disorders	No	N/R
Location of the cyst		
Right-sided	Yes	22 (63)
Preoperative imaging		
U/S	No	21 60)
CT	Yes	12 (34)
MRI	No	3 (8)
Preoperative diagnosis		
Round ligament cyst	-	4 (11)
Inguinal hernia	Yes	12 (34)
Surgery method		
Abdominal resection	Yes	18 (51)
Laparoscopy resection	-	17 (48)
Coexisting groin hernia	No	13 (37)

N/R=Not referred; CT=Computed tomography; U/S=Ultrasonography; MRI=Magnetic resonance imaging.

Conclusion

When considering the causes of a female inguinal lump, it is important to account for the possibility of a mesothelial cyst of the round ligament. Female patients presenting with an irreducible mass in the groin region should undergo either sonographic or CT evaluation. Both the cyst and the round ligament should undergo surgical removal, as cysts that are not yet apparent within the round ligament have the potential to enlarge and result in symptoms. It is necessary to send all specimens collected during the process for pathology testing, as they may reveal any previously unnoticed pathological abnormalities.

What Is Already Known on This Topic:

Mesothelial cysts of the round ligament of the uterus commonly occur alongside small inguinal hernias in 30-50% of cases. The cause of MCURLs is still unknown. Mesothelial cysts located in the lower right quadrant can cause groin discomfort and present clinical signs that match those of an inguinal hernia. In order to obtain a precise preoperative diagnosis, it is crucial to maintain a high degree of suspicion and employ imaging investigations. Ultrasound is the primary modality for imaging, as it is non-ionizing. The diagnosis is determined through histological analysis. Surgical excision is the preferred treatment for cysts that show symptoms or increase in size over time.

What This Study Adds:

When examining the factors that contribute to the development of a lump in the female inguinal area, it is crucial to evaluate the potential presence of mesothelial cysts of the round ligament. The scarcity of recorded occurrences, along with a lack of suspicion, can lead to inaccurate preoperative diagnosis and treatment plans for the patient, as was the situation in our case. Female patients who have an irreducible mass in the groin area should receive either a sonographic or CT examination. Our study has a significant impact on the present understanding of the illness, due to the limited availability of studies that include imaging findings obtained by computed tomography. We have verified the previously documented findings on MCURLs, which indicate that they largely impact women in their middle age, predominantly manifest on the right side, and exhibit a significant rate of misdiagnosis. The existing evidence is insufficient to confidently suggest a particular treatment or course of action. We found that there are 35 documented instances related to our specific medical condition. This unequivocally illustrates the need to record more instances in order to improve our comprehension of the condition and establish a systematic approach to therapy.

Acknowledgements: The authors are immensely grateful to the patient for allowing utilization of her case data in this case report.

Authors' Contributions: Conception and design: MP, CV, KG and ND; Acquisition, analysis and interpretation of data: MP, ND, KG, DG and CV; Drafting the article: CV, MP, KG, ND and DG; Revising it critically for important intellectual content: VG, KP and DG; Approved final version of the manuscript: DG, VG and KP; All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Scientific Article
Acta Medica Academica 2024;53(3):320-327
DOI: 10.5644/ama2006-124.459

Morphometric Analysis of the Greater Palatine Foramina in the Bosnia and Herzegovina Population

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Received: 8 July 2024; **Accepted**: 16 October 2024

Abstract

Objective. The goal of this research was to examine the morphological characteristics and exact anatomical positioning of the greater palatine foramen (GPF), with reference to nearby anatomical landmarks. **Material and Method**. The research was performed on dry human skulls belonging to the Bosnian and Herzegovina population, using digital vernier calipers. The study began by noting the GPF's position relative to the maxillary molars, then measuring its distance from the median palatine suture (MPS), the incisive fossa (IF), the posterior border of the hard palate (PBHP), and the posterior nasal spine (PNS). Measurements were conducted bilaterally, and afterwards the data were analyzed using Student's t-test and Chi-squared test. A statistical significance was set at $P<0.05$. **Results**. The statistical analysis revealed that: the distance of the greater palatine foramen (GPF) from the midline is approximately 15.80±1.28 mm on the right side and 15.86±1.19 mm on the left side. The distance of the GPF from the incisive fossa measures about 40.12±2.19 mm on the right side and 40.34±2.08 mm on the left side. The GPF is positioned around 4.00±1.07 mm on the right side and 4.35±1.34 mm on the left side from the posterior border of the hard palate. Lastly, the distance from the GPF to the posterior nasal spine means 17.55±1.99 mm on the right side and 17.61±1.81 mm on the left side in the entire study population. The highest percentage of skulls (73.05%) showed the GPF positioned at the level of the third molar. **Conclusion**. The findings of this study further emphasize the variations in the location of the greater palatine foramen and underline the importance of thorough preoperative assessment in patients undergoing maxillofacial surgeries and regional block anesthesia.

Key Words: Dry Skull ▪ Greater Palatine Foramen ▪ Morphometry ▪ Clinical Anatomy.

Introduction

The greater palatine foramina (GPF) are a pair of bony foramina found at the posterior end of the hard palate close to the third molar teeth. These foramina transmit the greater palatine nerve (GPN) and vessels from the pterygopalatine fossa into the oral cavity (1).

These vital neurovascular structures could potentially sustain injuries during various surgical operations, including intraoral maxillary nerve blocks, repairs for cleft palates, and surgeries involving the maxillary sinus or molar teeth (2).

There are two intraoral methods for performing maxillary nerve blocks in maxillofacial surgeries: the high tuberosity approach and the greater palatine canal approach. Studies have demonstrated that the high tuberosity approach is often associated with issues such as inadequate anesthesia, and a heightened risk of hematoma due to its closeness to the pterygoid venous plexus. The greater palatine canal approach, which reaches the maxillary nerve through the greater palatine foramen, is generally the most effective and widely used technique, as the nerve passes through the pterygopalatine fossa (2, 3).

The use of anesthetic block on the greater palatine nerve was initially documented in 1927 (4) and has since been recommended for surgeries involving the upper molars, the maxillary sinus, and

the nasal region. However, a common challenge reported with this procedure is the difficulty in accurately locating the greater and lesser palatine foramens, which can result in inadequate anesthesia (5).

Previous studies have shown that successful palatal anesthesia relies on correctly identifying the location of the greater palatine nerve (5). This is the reason why many researchers, among them Viveka et al., have concluded that the utilization of multiple anatomical reference points, such as the incisive foramen, the midline maxillary suture, and the second and third maxillary molars, simplifies identification of the GPF (6). Due to the lack of sufficient information in traditional anatomy and anesthesiology textbooks regarding the precise location of the greater palatine foramen, our study was initiated to determine its exact positioning within the population of Bosnia and Herzegovina.

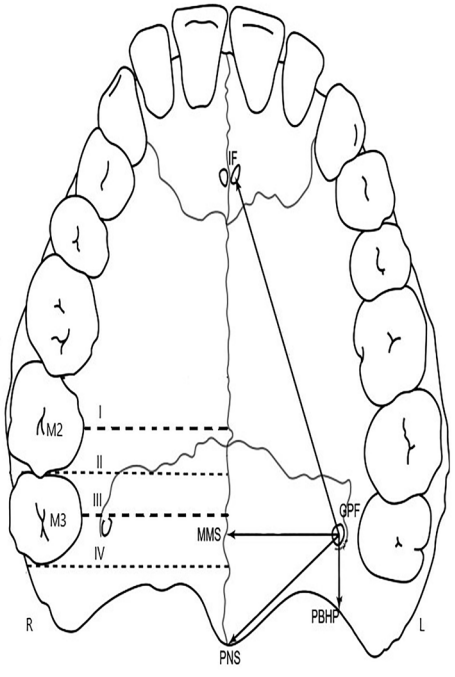
It is our goal that this research will benefit dentists and maxillofacial surgeons in their professional activities, aiming to reduce the incidence of unintended damage to the greater palatine nerves and blood vessels.

Materials and Methods

This research aimed to ascertain the location of the GPF in relation to several anatomical landmarks. The study was performed on 130 adult dry skulls (75 males and 55 females) belonging to the Bosnian and Herzegovinian population, kept at the Department of Anatomy, Faculty of Medicine, University of Sarajevo. All the skulls had fully erupted third molars and were devoid of pathological changes.

Morphometric measurements were conducted using digital vernier calipers (0-1000 mm), 0.05 mm, Metric 530-502, (Mitutoyo Corporation, Japan), with an accuracy of 0.01 mm. Each measurement was taken three times, and the mean was used for subsequent analysis. Additionally, all measurements were recorded by the same individual to reduce methodological errors. Once all the samples were measured, 20% of randomly selected samples were re-evaluated by an observer who had not been involved in the initial assessment. Interclass correlations (ICC) were calculated, showing a very high level of agreement between the evaluations (ICC = 0.92–0.96). The following measurements were taken (Schema 1):

- Distance from the greater palatine foramen (GPF) to the median palatine suture (MPS), (GPF - MPS)
- Distance from the GPF to the posterior nasal spine (PNS), (GPF - PNS)
- Distance from the GPF to the posterior border of the hard palate (PBHP), (GPF - PBHP)
- Distance from the GPF to the incisive foramen (IF), (GPF - IF)
- Location of the GPF in relation to the second (M2) and third (M3) maxillary molars



Schema 1. This illustration of the hard palate demonstrates the greater palatine foramen's location in relation to anatomical landmarks and the maxillary molars. On the left, the distances from the GPF to four major anatomical features (IF, MPS, PBHP, PNS) are shown, while the right side reveals the pooled prevalence of the GPF's position concerning the maxillary molars, (I-IV). Terminology: GPF - greater palatine foramen, IF - incisive foramen, MPS – median palatine suture, PBHP - posterior border of hard palate, PNS - posterior nasal spine; Positions: I – medial to the second maxillary molar, II – between the second and third maxillary molars, III – medial to the third maxillary molar, IV – behind the third maxillary molar.

The spatial relationship of the greater palatine foramen concerning the upper molars was recorded as being either aligned with the longitudinal axis of the maxillary second molar (I), the third molar (III), positioned between the second and third molars (II), or situated behind the maxillary third molar (IV) (7).

Statistical Analysis

All statistical analyses were performed with SPSS version 19 (SPSS Inc., Chicago, IL, USA), while data were compiled using Microsoft Excel 2020 (Microsoft Corp., Redmond, WA, USA) and displayed in tables. Descriptive analysis helped to determine mean and standard deviation. The Student's t-test and Chi-square test (χ^2) were employed to assess whether there were statistically significant differences based on sides and sex. A P-value below 0.05 was regarded as statistically significant for this research. The level of significance was evaluated using P-values, with the following classifications: P≥0.05 denotes non-significant results, P≤0.05 denotes significant results, P≤ 0.01 denotes highly significant results, and P≤0.0001 denotes very highly significant results.

Results

All the skulls that were investigated displayed one greater palatine foramen on both sides. Table 1 provides a summary of the linear measurements of the greater palatine foramen in relation to surrounding anatomical landmarks.

The mean distance from the greater palatine foramen to the median palatine suture was 15.80±1.28 mm on the right side and 15.86±1.19 mm on the left side. The mean distance to the posterior border of the hard palate was 4.00±1.07 mm on the right and 4.35±1.34 mm on the left. The distance from the greater palatine foramen to the incisive fossa measured 40.12±2.19 mm on the right and 40.34±2.08 mm on the left. For the posterior nasal spine, the distances were 17.55±1.99 mm on the right and 17.61±1.81 mm on the left. Statistically significant differences by sex were

Table 1. Distance of the Greater Palatine Foramen from Anatomical Landmarks

Measurements	Side (mm)	Sex	Mean±SD	t-value	P-value*
GPF – MPS	Right	Male	16.20±1.24	1.326	0.188
		Female	15.40±1.32		
	Left	Male	16.28±1.06	1.058	0.292
		Female	15.43±1.31		
GPF – PNS	Right	Male	18.01±2.05	1.426	0.160
		Female	17.08±1.93		
	Left	Male	18.12±1.80	1.326	0.732
		Female	17.10±1.82		
GPF – PBHP	Right	Male	4.05±1.26	8.052	0.0001
		Female	3.95±0.88		
	Left	Male	4.50±1.03	8.986	0.0001
		Female	4.19±1.64		
GPF – IF	Right	Male	41.20±1.10	2.772	0.008
		Female	39.03±3.27		
	Left	Male	41.28±1.06	2.153	0.008
		Female	39.40±3.09		

*Student's t - test; GPF=Greater palatine foramen; MPS=Median palatine suture; PNS=Posterior nasal spine; PBHP=Posterior border of hard palate; IF=Incisive foramen.

Table 2. Variations in the Position of the Greater Palatine Foramen Relative to the Upper molars.

Side	Position	Gender (N; %)		Total	Chi – square	P–value*
		Male	Female	N (%)		
Right	I	8 (57.1)	6 (42.9)	14 (10.8)	1.2625	0.738
	II	11 (64.7)	6 (35.3)	17 (13.1)		
	III	50 (52.1)	46 (47.9)	96 (73.8)		
	IV	2 (66.7)	1 (33.3)	3 (2.3)		
Left	I	8 (61.5)	5 (38.5)	13 (10.0)	2.6972	0.775
	II	12 (57.1)	9 (42.9)	21 (16.2		
	III	49 (52.1)	45 (47.9)	94 (72.3)		
	IV	1 (50.0)	1 (50.0)	2 (1.5)		

*Chi-square test; I=Medial to the second maxillary molar; II=Medial to and between the second and the third maxillary molar; III=Medial to the third maxillary molar; IV=Medial to and behind the third maxillary molar.

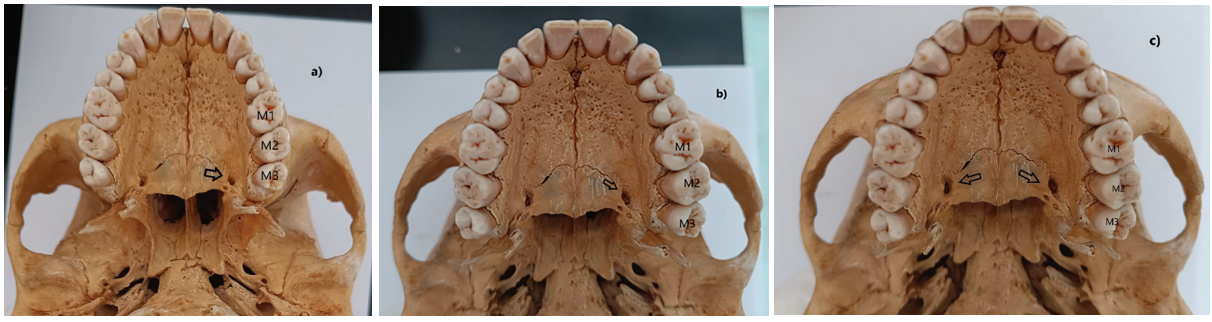


Figure 1. a) Greater palatine foramen situated medially to third maxillary molar; b) Greater palatine foramen situated medially to and between second and third maxillary molars; c) Greater palatine foramen situated medially to second maxillary molar; M1= First maxillary molar; M2=Second maxillary molar; M3=Third maxillary molar.

detected for the distances of the GPF to PBHP (P=0.0001) and the GPF to IF (P=0.008), being significantly larger in males.

The frequencies of the greater palatine foramen's positions in relation to the upper molars are summarized in Table 2. Statistical analysis of the obtained results did not show any statistical significance in the results in relation to side or sex.

The dominant position on both sides was in line with the maxillary third molar (73.05%), followed by between the second and third molars (14.64%). Collectively, these positions represented 87.7% of cases (Figures 1 and 2). Positions at the level of the second maxillary molar and behind the third maxillary molar were noted in 6.15% of cases (Figure 3).

Discussion

The greater palatine foramen (GPF) is responsible for carrying the greater palatine nerve, which innervates the posterior section of the hard palate (1). Performing an anesthetic block for this nerve is strongly recommended for surgeries related to the upper molars, the maxillary sinus, and the nasal region (3). Nonetheless, a common issue with anesthesia is the challenge of accurately identifying the location of the greater palatine foramen, often leading to insufficient anesthetic delivery (5). Matsuda first identified the GPF's location in 1927 (8), and since that time, numerous studies have aimed to establish its position variability.

Our research revealed that the mean distance from the greater palatine foramen (GPF) to the midline maxillary suture (MPS) was 15.80 mm on

the right and 15.86 mm on the left. These distances are shorter than the measurements reported in studies of Serbian (9), Thai (10), Turkish (11), Polish (12), Italian (13), and Chinese (14) populations. Conversely, our measurements exceeded those observed in Nigerian (7), South Indian (15), East Indian (16), Brazilian (17), Iraqi (18), Greek (19), and South Indian (20) populations.

Table 3 visually depicts the comparison of our findings with data from other studies.

Additionally, our study found that the mean distances from the greater palatine foramen (GPF) to the posterior margin of the hard palate were 4.00 mm on the right side and 4.35 mm on the left side. These measurements were lower than those reported for Poles (12), Iraqis (18), and Greeks (19). In contrast, they were higher than distances found in Nigerians (7), Serbs (9), Thais (10), Italians (13), East Indians (16), Brazilians (17), and South Indians (20), and similar to measurements reported for Turks (11), Chinese (14), and South Indians (15), (see Table 3).

Table 4 presents a comparison of the distances from the GPF to the posterior nasal spine (PNS) and the infraorbital foramen (IF).

In the Bosnian and Herzegovinian population, the greater palatine foramen was found to be medial to the third maxillary molar in 73.05% of cases, consistent with similar findings in Nigerians (7), Indians (7, 24), Thais (10), South Indians (15, 20), East Indians (16), Iraqis (18), individuals of Negroid descent (25), Kenyans (26), and Brazilians (27). However, Chinese populations predominantly exhibited the greater palatine foramen located between the second and third molars (14).

Interestingly, the second most common position of the greater palatine foramen in individuals of South Africans (25) and Brazilian (27) descent was distal to the third maxillary molar. Conversely, in Nigerians (7), Indians (7, 24), Thais (10), South Indians (15, 20), East Indians (16), Iraqis (18), and Kenyans (26), it was commonly found between the second and third maxillary molars (refer to Table 5 for details). The results of the present study underscore the racial variations in the location of the greater palatine foramen concerning the upper molars among different populations. This variation in positioning may be a result of ethnic influences.

Table 3. Comparison between Studies on the Distances GPF-MPS and GPF-PBHP

References	Population	GPF - MPS (mm)		GPF – PBHP (mm)	
		Right	Left	Right	Left
Present study	Bosnia and Herzegovina	15.80	15.86	4.00	4.35
Ajmani (7)	Nigerian	14.70	14.60	3.70	3.70
Radošević et al. (9)	Serbian	15.99	15.88	2.01	2.10
Methathrathip et al. (10)	Thai	16.20	16.20	2.10	2.10
Cagimni et al. (11)	Turkey	16.30	16.10	4.20	4.00
Tomaszewska et al. (12)	Polish	16.10	15.60	4.90	4.80
Gibelli et al. (13)	Italian	16.40	16.80	3.80	3.80
Wang et al. (14)	Chinese	16.00	16.00	4.11	4.11
Saralaya et Nayak (15)	South Indian	14.70	14.70	4.20	4.20
Westmoreland and Blanton (16)	East Indian	14.80	15.00	1.90	1.9 0
Lopes et al. (17)	Brazilian	15.60	15.40	3.38	3.50
Jaffar and Hamadah (18)	Iraqi	15.70	15.70	4.90	4.90
Piagkou et al. (19)	Greek	15.30	15.30	4.60	4.70
Vinay et al. (20)	South Indian	14.80	14.80	3.58	3.56

GPF=Greater palatine foramen; MPS=Median palatine suture, PBHP=Posterior border of hard palate.

Table 4. Comparison between Studies on the Distances GPF-PNS and GPF-IF

References	Population	GPF - PNS (mm)		GPF – IF (mm)	
		Right	Left	Right	Left
Present study	Bosnia and Herzegovina	17.55	17.61	40.12	40.34
Viveka and Kumar (6)	Indian	17.78	17.44	39.76	39.37
Tomaszewska et al. (12)	Polish	17.00	17.00	34.00	34.30
Saralaya et Nayak (15)	South Indian	0.00	0.00	37.20	37.30
Vinay et al. (20)	South Indian	0.00	0.00	36.60	35.70
Awad et al. (21)	Egypt	16.55	16.48	38.06	37.96
Ortug et al. (22)	Turkey	15.84	15.84	38.27	38.27
Singht et al. (23)	North Indian	13.60	13.77	37.39	37.09

GPF=Greater palatine foramen; PNS=Posterior nasal spine, IF=Incisive foramen.

Table 5. The Percentage of Opening of the GPF in Relation to the Maxillary Molars

Researchers	Population	I (%)	II (%)	III (%)	IV (%)
Present study	Bosnia and Herzegovina	10.40	14.65	73.05	1.90
Ajmani (7)	Nigerian	13.07	38.46	48.46	0.00
Ajmani (7)	Indian	0.00	32.35	64.69	2.94
Methathrathip at al. (10)	Thai	7.00	14.10	71.90	7.00
Wang et al. (14)	Chinese	17.00	48.50	33.50	0.00
Saralaya and Nayak (15)	South Indian	0.40	24.20	74.60	0.80
Westmoreland and Blanton (16)	East Indian	9.70	33.60	50.70	6.00
Jaffar and Hamadah (18)	Iraqi	12.00	19.00	55.00	14.00
Vinay et al. (20)	South Indian	3.67	19.00	76.00	1.33
Kumar et al. (24)	Indian	5.00	9.00	85.00	1.00
Langenegger et al. (25)	South Africa	1.00	3.00	62.00	34.00
Hassanali and Mwaniki (26)	Kenyan	10.40	13.60	76.00	0.00
Chrcanovic and Custodio (27)	Brazilian	0.00	6.19	54.87	38.94

I=Medial to the second maxillary molar; II=Medial to and between the second and the third maxillary molar; III=Medial to the third maxillary molar; IV=Medial to and behind the third maxillary molar.

Conclusion

This is, to our knowledge, the first investigation into the anatomical variations of the greater palatine foramen (GPF) among the population of Bosnia and Herzegovina. The results are essential for comparing Bosnian and Herzegovinian skulls with those of other ethnicities and regions. Furthermore, this information will aid anesthetists in accurately locating the GPF, thereby enhancing surgical outcomes.

What Is Already Known on This Topic:

In clinical dentistry and maxillofacial surgery, a maxillary nerve block is performed to achieve hemimaxillary anesthesia. This involves administering local anesthesia to the maxillary nerve, which is a branch of the trigeminal nerve or one of its subdivisions. The most common method for maxillary nerve block is via the greater palatine canal (GPC), which leads to the pterygopalatine fossa, where the trunk of the maxillary nerve is situated. This GPC method is favored in clinical settings because it has a high success rate and a low incidence of postoperative complications. The effectiveness of this procedure relies heavily on the position of the greater palatine foramen (GPF), found on the hard palate and serving as the entry point to the greater palatine canal (GPC). Research has indicated that the GPF exhibits various anatomical variations in its location, highlighting the necessity for detailed morphological analysis of the GPF to enhance the success of the GPC approach for maxillary nerve block.

What This Study Adds:

Despite its importance, little is known about the morphological and morphometric characteristics of the greater palatine foramen (GPF) in the population of Bosnia and Herzegovina. The previous research was conducted with the aim of obtaining the most accurate data on the position of the greater palatine foramen (GPF) in relation to anatomical landmarks in the dry skulls of the population of Bosnia and Herzegovina.

Authors' Contributions: Conception and design: AV and ET; Acquisition, analysis and interpretation of data: AV and ET; Drafting the article: AV, ET and EK; Revising it critically for important intellectual content: AV, ET; Approved final version of the manuscript: AV, ET and EK.

Acknowledgement: We thank all those who donated their bodies for scientific purposes and made it possible to conduct the present anatomical research.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2024;53(3):328-334
DOI: 10.5644/ama2006-124.465

Obturator Nerve Variations: A Narrative Review*

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Received: 5 October 2024; **Accepted:** 25 December 2024

Abstract
The aim is to understand the clinical significance of these variations in surgeries and diseases. To conduct this review, we used the PubMed database, considering factors such as the availability of full texts, the language and relevance to the topic, in order to acquire comprehensive and detailed findings. After applying our exclusion criteria, we narrowed the findings down to 11 useful results. Throughout our study, we observed significant variations concerning the obturator nerve. The nerve displays diverse paths and trajectories, leading to deviations from the commonly accepted anatomical description. Additionally, we identified variations in the division point of the obturator nerve and the resulting innervation patterns that it provides for muscles, joints, and skin. More precisely, we discovered differences regarding the path, the source and the level of composition. Moreover, the muscles innervated by the obturator nerve's anterior branch and posterior branch may vary. Furthermore, variations were observed in the innervation of both the skin and joints. **Conclusion.** Our research demonstrates that the obturator nerve is susceptible to many forms of variations. Accurate knowledge of these variations is crucial for minimizing iatrogenic complications and ensuring patient care.

Key Words: Lumbar Plexus Variations ▪ Peripheral Nerve Variations ▪ Lower Limb Innervation.

Introduction

The obturator nerve (ON) originates from the lumbar plexus located in the lower limb. It originates from the anterior division of the L2, L3, and L4 spinal nerves, and runs medially to the major psoas muscle (1-4). Eventually, the ON reaches the obturator canal, where it typically splits into an anterior and a posterior branch. The anterior branch travels behind the pectineus and adductor longus muscles, and in front of the adductor brevis muscle (1, 2). As well as providing sensation to the skin of the medial thigh and innervation to the hip joint, the obturator nerve also innervates several muscles, including the adductor longus, the gracilis the adductor magnus, and occasionally the adductor brevis and the pectineus muscle (1-3). Nevertheless, numerous variations of this nerve have been observed by the scientific community. This review aims to provide a structured summary and full description of the reported variations of the obturator nerve (ON) documented in the international literature. Our research highlights the considerable variability in the branching pattern of the ON.

In conclusion, our primary objective is to present a comprehensive understanding of the variations of the ON based on previous research and case reports.

Materials and Methods

The literature search was conducted using the PubMed database to identify original articles, case reports, and anatomical reviews related to variations of the obturator nerve. For this purpose, we

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conducted a search by using the terms "obturator nerve AND variations" in October 2023. To ensure consistency, we only considered articles written in English. Furthermore, we excluded articles whose full-text versions could not be located, and articles that did not demonstrate any obturator nerve variations. Articles were selected on the basis of their relevance to the topic, and we did not apply any publication date restrictions, as historical references might provide essential insights (5). It is important to note that we purposely avoided using other databases in order to reduce systematic errors.

We specifically selected PubMed because all the articles are peer-reviewed. A total of 57 articles were initially identified through the database search. After eliminating one duplicate, 56 articles were screened. The articles were pursued for retrieval, however, two were not accessible. The remaining 54 articles were assessed for eligibility on the basis of specific inclusion criteria related to anatomical variations of the obturator nerve (5). Among these, 43 articles were excluded: 38 were unrelated to the topic (since they did not supply any details regarding the obturator nerve or its variations), three were in a foreign language, and two did not present any relevant anatomical variations. Ultimately, 11 studies fulfilled the criteria and were included in this narrative review (Figure 1). These studies provided valuable data on the anatomical variations of the obturator nerve, contributing to the understanding of its clinical significance.

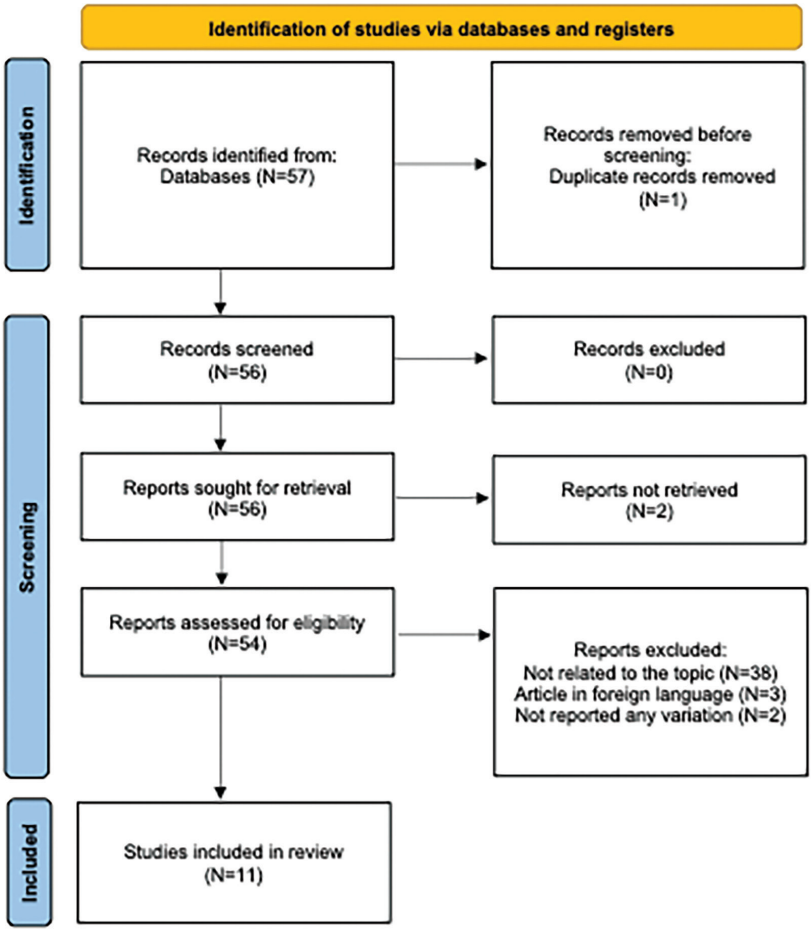

Figure 1. Flowchart describing the narrative review selection progress.

Results

The literature reviewed highlights significant anatomical variations in the obturator nerve (ON). These variations are categorized into four primary aspects: origination and morphology, division point, muscular innervation, and articular and sensory contributions.

The obturator nerve typically originates from the anterior divisions of the L2, L3, and L4 spinal nerves in most cases (4, 6). However, rare variations were identified, including origins from L3-L4, L3-L5, and in exceptionally rare cases, L1-L3 (4, 6-8). The level of emergence of the ON also displayed variability, with the majority occurring at the L5-S1 vertebral level (4). Other reported levels include S1, L5, S2 and L4-L5 (4).

In some cases, the ON travels through the obturator muscle, modifying its fibrous texture, while in other cases it is positioned either anteriorly or posteriorly in relation to the muscle (9). Moreover, the obturator nerve diverges into

anterior and posterior branches, with the branching point showing variability. This may occur as follows: inside the pelvis, prior to the obturator foramen (observed in 2%-22% of cases); within the obturator canal (noted in 23%-93% across various studies); outside the obturator canal, in the medial thigh (exemplified in 5%-64% of cases) (1, 2, 9). The division point's proximity to the superior pubic ramus, approximately ±20 mm, has clinical implications for the success of anesthesia injections administered near the obturator canal (1). Furthermore, variations concerning muscular innervation patterns of the obturator nerve are also reported.

The anterior branch primarily innervates the adductor longus, adductor brevis, and gracilis muscles. In most cases, this is the observed pattern, however, a two-branch pattern involving the adductor longus and gracilis muscles is also noted occasionally. A less common variation includes a four-branch pattern that innervates the adductor longus, adductor brevis, gracilis, and pectineus muscles (2).

Concurrent innervation of the adductor brevis by both anterior and posterior branches was also observed. The posterior branch innervates the adductor magnus and external obturator muscles. Its branching patterns varied between one, two, three or four branches (2). Rare cases reported supernumerary branches innervating anomalous muscles between the adductor brevis and adductor minimus or supplying the iliacus muscle (6, 8).

The obturator nerve's contribution to articular branches for the hip joint also displayed variability: in 61.28% of cases, a single articular branch was observed, typically originating from the common ON (61.98%), the anterior branch (19.23%), or the posterior branch (3.84%) (2). Two articular branches were observed in 20.32% of cases, with variations in their origin, while three branches were identified in 17.85% (2).

Differences in the number of articular branches (ranging from 1 to 7) and their patterns of distribution were identified, with significant implications for clinical application (2). Sensory variations included rare connections between the ON and the ilioinguinal nerve, with branches supplying the anterior scrotal skin in certain cases (3, 10). This detailed review underscores the considerable anatomical variability of the obturator nerve, emphasizing its clinical implications in surgeries and anesthetic interventions.

Discussion

Accurate documentation of anatomical variations, particularly concerning the obturator nerve, is crucial. These variations hold substantial significance in clinical practice, especially regarding surgical and anesthetic interventions. Henry et al. (2024) emphasize that systematic reviews present a systematic method for aggregating data from various studies, which guarantees the availability of high-quality evidence for clinical guidance (11). By adhering to the stringent methodologies indicated for systematic reviews, this narrative review expands on prior research to offer an in-depth insight into the variations of the obturator nerve and their importance in clinical contexts.

For educational purposes and in order to facilitate understanding, the variations will be presented as alterations relevant to the origin and morphology of the ON, variations relevant to the division point of the ON, variations relevant to muscle innervation by the obturator nerve, and finally, as variations relevant to the articular and sensory innervation by the ON.

Variations Related to the Origin and the Morphology of the Obturator Nerve

In studies using cadavers, researchers have observed many different variations in the course and formation of the obturator nerve. Specifically, studies have found variations in the course of the ON in relation to the obturator muscle. The common obturator nerve, before dividing into anterior and posterior branches, can either pass through the internal obturator muscle or above it. When it passes through the muscle, it loses its fibrous texture. This variation is almost equally distributed among the dissected samples (50% each; N=16) (9).

Additionally, there may be variations in the course of the posterior branch of the obturator nerve. According to Sim et al., in 61% (N=18) of dissected samples, the posterior branch of the obturator nerve runs anteriorly to the external obturator muscle. In some instances, the posterior branch passes through a fleshy section of the muscle with a distinct fiber separation in 22% (N=18) of cases, while in 17% (N=18), the fiber separation is unclear. In only one case, the posterior branch of the obturator nerve was separated from the muscle by fatty tissue (9).

In addition, there are variations in the origin of the obturator nerve. In most cases (around 97%, N=60 in one study, 89%, N=73 in a second study and 80%, N=181 in a third study), the obturator nerve consists of the ventral branches of the L2, L3, and L4 ventral rami. Occasionally, the obturator nerve may originate from the ventral branches of L3 and L4 ventral rami (about 3%, N=60 in one study, 5.5%, N=73 in a second study and 20%, N=181 in a third study) (4, 6, 12). In rare instances (approximately 5.5%, N=4), the obturator nerve may also derive from the ventral rami of L3, L4, and L5 (6). It is worth mentioning that, in statistical analysis, this variation seems to be unrelated to sex (12). In a single case recorded, the obturator nerve seems to derive from L1 and L2 ventral branches. These ventral branches were united and then divided into many branches, where the first of them was the obturator nerve (7). In another single case report, it is shown that the obturator nerve is formed by a combination of the L1, L2 and L3 ventral rami (8).

Furthermore, differing levels of composition variations were noted. Typically, the ON emerges at the level of the L5-S1 vertebra (60%; N=60). The ON may emerge on the level of the S1 vertebra (22%; N=60), L5 vertebra (15%; N=60), S2 vertebra (2%; N=60) or L4-L5 (2%; N=60) (4).

Variations Related to the Division Point of the Obturator Nerve

The common obturator nerve is divided into an anterior and a posterior branch. The point at which the nerve divides may occur within the pelvis, or after it passes through the obturator canal. Previous studies have not reached a consensus on the most common location of the division point. Anagnostopoulou et al. conducted cadaveric research and found that in the majority of dissections (51.78%; N=168), the division occurred after the nerve passed through the obturator canal, in the medial thigh. The division was also frequently observed within the obturator canal (23.33%; N=168) or within the pelvis, prior to the obturator foramen (21%; N=168) (2).

Iwanaga et al. found that in most cases (64.3%; N=14), the division point is observed after the obturator foramen. They also observed the significant persistence of the bifurcation point before the obturator foramen (21.4%; N=14) and within the obturator canal (14.3%; N=14) (1). In contrast, Purdhon et al. discovered that the bifurcation occurred within the obturator canal in 89% of cases (N=18) and after the obturator foramen in 11% of cases (N=18) while Zithulele et al. observed that in 2% of cadaveric dissections, the bifurcation occurred within the pelvis, 93% within the obturator canal, and 5% after exiting the canal (9, 12). However, they also demonstrated that these variations are statistically unrelated to sex (12). Additionally, it was noted that the point where the obturator nerve divides is situated approximately ±20mm from the upper or lower limit of the superior pubic ramus (1).

Variations Related to Muscle Innervation by the Obturator Nerve

Mainly, the obturator nerve provides innervation to the adductor longus, gracilis, and adductor magnus muscles. However, many studies have presented, among others, variations that relate to the muscular branches of the obturator nerve.

The first and most frequent finding is that the anterior branch of the obturator nerve appears to provide innervation, through three muscular branches, to the adductor longus, adductor brevis, and the gracilis muscle (66.6%; N=168) (2). It may also provide two branches only for the adductor

longus and gracilis muscle (28.57%; N=168) or four branches for the adductor longus, adductor brevis, gracilis and pectineus muscle (4.76%; N=168) (2). One study reports pectineus muscle innervation by the anterior branch of the obturator nerve as a rare finding, observed in only one case (12). The pectineus muscle receives its branch from the anterior branch of the obturator nerve, which descends diagonally and ends at the posterior aspect of the pectineus. It is quite common for the pectineus muscle to enclose two sources of nerve supply, both from the femoral nerve and the branch of the obturator nerve (10%, N=10) (3).

Thus, extra caution is necessary when it comes to denervating the pectineus muscle. Within the muscle, the branch of the obturator nerve that supplies the pectineus muscle is divided into two to four smaller branches, which are only found in the middle third of the muscle, and extend laterally (13). At times, there may be an anastomosis between the branches of the femoral nerve and their corresponding counterparts from the obturator nerve branches (13). It is also important to note that the branches from the femoral nerve are generally smaller in size than those from the obturator nerve. Statistical analysis reveals that there is no significant relationship between this variation and sex ($P<0.05$) (13). Additionally, it has been noted that in some cases (27.4%, N=73) the adductor brevis muscle receives innervation from both the anterior and the posterior branches of the obturator nerve (6).

Moreover, the innervation of muscles by the posterior branch of the obturator nerve also appears to vary. From the posterior branch of the obturator nerve, one branch may arise for the adductor magnus muscle (13.69%; N=168), two branches for the adductor magnus and adductor brevis muscles (60.11%; N=168), three branches for the external obturator, adductor magnus and adductor brevis muscles (19.04%; N=168) or four branches for the adductor magnus, adductor brevis, adductor longus and external obturator muscle (7.14; N=168) (2). Other research shows that in approximately 10% of cases, the posterior branch of the obturator nerve aids the anterior branch in innervating the adductor brevis (12). A single case presentation also includes a report of a small branch from the obturator nerve innervating the iliacus muscle (8).

Additionally, there have been reports of the presence of extra twigs and branches originating from the obturator nerve. These supernumerary branches are responsible for innervating the anomalous muscle, if it exists. This anomalous muscle is located between the adductor brevis and adductor minimus muscles, arising from the inferior ramus of the pubis and following a downward and lateral course, resulting in the pectineal line (23.3%, N=73). The supernumerary branch originates from the posterior branch of the obturator nerve, and supplies the anomalous muscle from its posterior aspect (6). Additional innervation of the adductor minimus by a supernumerary twig was also observed. This twig arises from the posterior branch of the obturator nerve, and it may be distributed to the anterior (24.7; N=73) or the posterior aspect (11%; N=73) of the muscle (6).

Variations Related to the Articular and Skin Innervation by the Obturator Nerve

Numerous studies have indicated that the articular branches of the obturator nerve are susceptible to variations. Firstly, the number of branches of the obturator nerve for the hip joint may vary. Typically, the obturator nerve gives rise to only one articular branch (61.28%; N=168). This branch can originate from either the common obturator nerve (61.98%; N=104) (Figure 2, 1A), the anterior branch of the obturator nerve (19.23%; N=104) (Figure 2, 1B), or the posterior branch of the obturator nerve (3.84%; N=104) (Figure 2, 3C).

In some cases, the obturator nerve may provide two articular branches for the hip joint (20.32%; N=168). In these instances, both branches can arise from the common obturator nerve (45.05%; N=34) (Figure 2, 2A), both from the posterior branch of the obturator nerve (41.17%; N=34) (Figure 2, 2B), or one from the common obturator nerve and the other from the posterior branch of the obturator nerve (22.76%; N=34) (Figure 2, 2C).

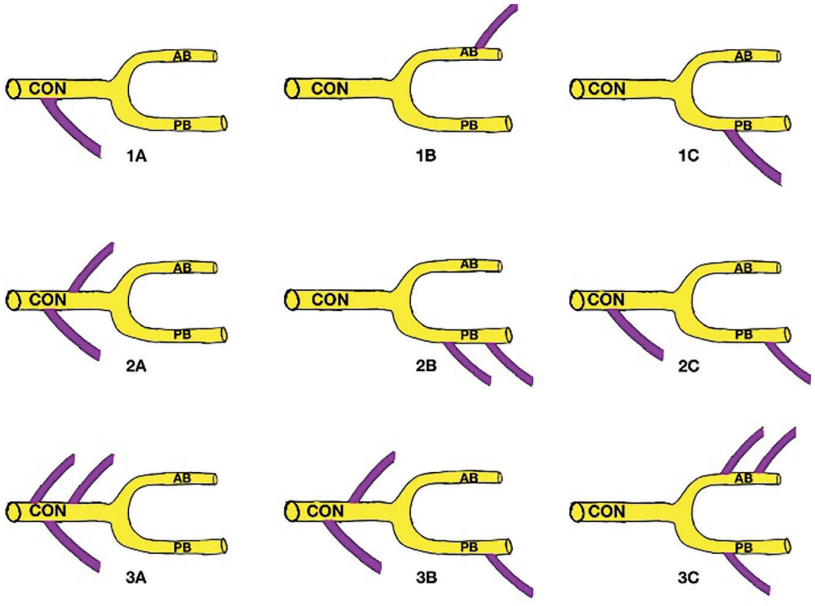

Figure 2. Illustration of variations related to the origin of the hip joint articular branch deriving from the obturator nerve.

In certain cases, the hip joint may receive three articular branches from the obturator nerve (17.85%, N=168). These three branches may be derived in different ways: all three branches deriving from the common obturator nerve (53.34%, N=30) (Figure 2, 3A); two branches deriving from the common obturator nerve and one from the posterior branch of the obturator nerve (26.66%, N=30) (Figure 2, 3B); or two branches deriving from the anterior division of the obturator nerve and one from the posterior (20%, N=30) (Figure 2, 3C) (2).

The violet-colored branch relates to the hip joint articular branch.1A: The hip joint articular branch deriving from the common obturator nerve (CON), 1B: The hip joint articular branch deriving from the anterior branch (AB) of the obturator nerve, 1C: The hip joint articular branch deriving from the posterior branch (PB) of the obturator nerve, 2A: Both hip joint articular branches deriving from the CON, 2B: Both hip joint articular branches deriving from the PB of the obturator nerve, 2C: One hip joint articular branch deriving from the CON and the other from the PB of the obturator nerve, 3A: All three hip joint articular branches deriving from the CON, 3B: Two of three hip joint articular branches deriving from the CON and the third from the PB of the obturator nerve, 3C: Two of three hip joint articular branches of the obturator nerve deriving from the AB of the obturator nerve, and the third from the PB of the obturator nerve.

In addition, it has been reported that the hip joint's articular branches originating from the obturator nerve can range from two to seven in number, with their bands starting between 9-19 mm and ending between 7-38 mm in width (10). In one reported case, it was observed that a branch originated from the anterior branch of the obturator nerve and ran in a superomedial direction in the thigh. Subsequently, it merged with the ilioinguinal nerve in front of the medial crus of the superficial inguinal ring. During this course, it produced another branch that descended along the anterior aspect of the spermatic cord. This branch divided into multiple cutaneous branches responsible for supplying the anterior scrotal skin. Such variations in nerve anatomy have significant implications in clinical practice, especially during local anesthesia procedures. Failure to recognize these variations may lead to excruciating pain for the patient (3).

Conclusions

In our review, we established that the obturator nerve is highly susceptible to variations. This finding underscores the importance of research and studies that identify and discuss morphological variations in vascular, nerve, and muscular structures, particularly in the context of surgical procedures. Recognizing these variations prior to surgery is imperative for surgeons, anesthesiologists, and plastic surgeons in order to provide optimal care to patients and minimize iatrogenic complications.

What Is Already Known on This Topic:

Many variations concerning the obturator nerve are noted throughout the literature. Research has highlighted a range of differences in the anatomy and innervation patterns of the obturator nerve. More specifically, the obturator nerve is prone to variations regarding its morphology and origin. Furthermore, the muscles and skin regions innervated by the obturator nerve may vary. Last but not least, several studies have identified variations in the articular innervation provided by the obturator nerve.

What This Study Adds:

This study provides a comprehensive overview of the anatomical variations of the obturator nerve, synthesizing findings from the existing literature. It highlights deviations in the nerve's origin, division point, and innervation patterns. By categorizing these variations, the study offers valuable insights that can help clinicians anticipate challenges during interventions involving the obturator nerve. Furthermore, this review underscores the importance of precise anatomical knowledge in minimizing iatrogenic complications and optimizing patient care, making it a crucial resource for surgeons, anesthesiologists, and medical educators.

Authors' Contributions: Conception and design: RB, V-MK and DF; Data collection: RB, V-MK, PG, NP, GT and DF; Figures design: RB and DF; Acquisition, analysis and interpretation of data: RB, V-MK, PG, NP, GT and DF; Drafting the article: RB, V-MK, PG, NP, GT and DF; Revising it critically for important intellectual content: RB, V-MK, PG, NP, GT and DF; Approved final version of the manuscript: RB, V-MK, PG, NP, GT and DF.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Literature Review
Acta Medica Academica 2024;53(3):335-342
DOI: 10.5644/ama2006-124.461

Anatomical Variations of the Vermiform Appendix

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Received: 3 October 2024; **Accepted**: 11 December 2024

Abstract

Objective. The aim of the present work is to systematically review and present the existing literature on anatomical variations of the appendix. **Methods.** Detailed research was conducted in the PubMed medical database, using the terms "Appendix" AND "Anatomical variations", and 74 articles were initially revealed. After the application of the inclusion and exclusion criteria, all the non-related articles were excluded, and thus 40 articles were finally selected. **Discussion.** The data analysis suggests that the location and form of the appendix may significantly vary among individuals. Common anatomical variations concerning its location include retrocecal, pelvic, retro-ileal, pre-ileal, prececal and paracecal appendices. The first two variants are the most common, although there is a discrepancy regarding their exact incidence. Rarely, the appendix may be intracecal, intramural, subhepatic or located in the left abdomen; mismatches of the McBurney guide point with the base of the appendix are also recorded. Concerning the appendix's form, several variations in the length, diameter, shape and number of appendages (doubling, tripling) may be present. **Conclusions.** As evident from the presentation of the results, the vermiform appendix presents a wide variety and number of anatomical variations. The latter are of particular clinical importance and should be known to doctors - especially surgeons - to avoid complications in clinical practice.

Key Words: Appendix ▪ Variations ▪ Anatomy ▪ Appendicitis.

Introduction

The vermiform appendix appears as a caudal continuation of the cecum, the first part of the ascending colon. The term 'vermiform' has a Latin origin and was attributed due to its worm-like shape. The appendix is located towards the dead end of the cecum, in the right iliac fossa, approximately 2-3 cm under the ileocecal valve. It consists of the base, the body and the apex/tip. Histologically, the structure of the appendix is composed of four layers: the mucosa, submucosa, muscularis externa and serosa. The appendix is completely covered by peritoneum, and is usually supported by a triangular peritoneal fold, the mesoappendix, along the free edge of which runs the appendicular artery. Inflammation of the appendix (appendicitis) is particularly common, 50,000 such cases are recorded annually in the UK, and is usually treated with appendectomy (1).

The purpose of this paper is to conduct a systematic bibliographic review of the anatomical variations (Position and Form) of the appendix, as well as to highlight the possible effects of these variations in clinical practice.

Materials and Methods

A systematic search using the terms "Appendix" AND "Anatomical Variations" was conducted in the PubMed database, in March 2024, from which

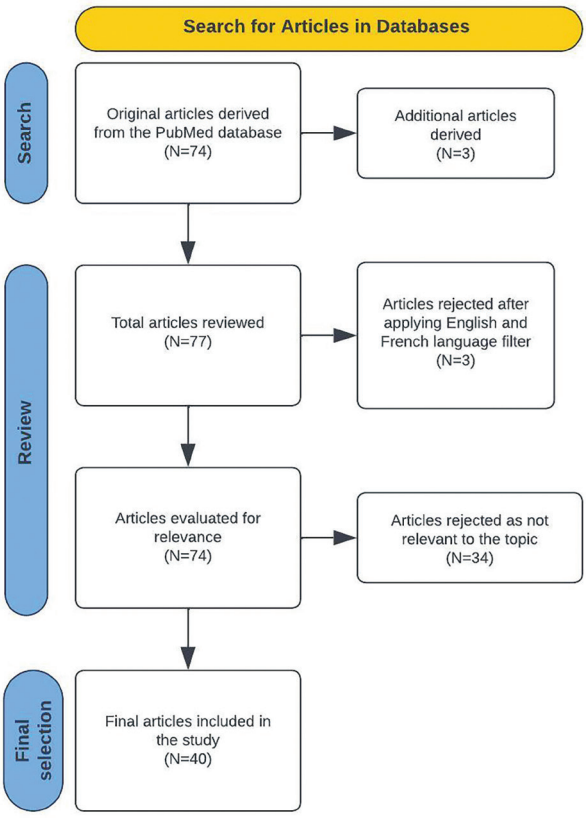

Figure 1. Flow chart of the literature search.

74 articles emerged. After the application of the selection and exclusion criteria (English and French language filter, articles relevant with the topic), 40 articles were deemed appropriate and were finally included in this work (Figure 1).

Results and Discussion

The articles included in this review (40) include case studies (26), cohort studies (8), literature reviews (5) and one meta-analysis.

The case study articles include 2 reports with a retrocecal appendix, 1 with a prececal, 1 with a paracecal, 5 with an appendix in a subhepatic location, 1 with colic, 1 with a subserosal, 1 with an intramural, 3 with left inversion of the position of appendix, 1 with pelvic, 1 in which the appendix was found inside an Amyand's hernia, 1 with the appendix in the chest, 1 with the appendix in the lumbar segment, and 1 with the appendix between the right psoas major and the right iliacus muscle. Regarding the form of the appendix, 5 cases of duplicated appendix as well as 1 case of a 23 cm long appendix were reported. The case studies involved patients aged 6 months to 85 years old. Among them, there are 3 Asians, 1 African and 3 Caucasians. The race of the remaining patients is not decisively determined.

Concerning the cohort studies, 2 assess the reliability of the McBurney's point in determining the location of the appendix, and the remaining 6 describe variations in the appendix's location. Regarding the 6 cohort studies focusing on location variations, retrocecal appendix is mentioned in all 6, subcecal in 3, paracecal in 1, pelvic in 5, retroileal in 5, preileal in 2, colic in 1, prececal in 1, intramural in 1, subhepatic in 1 and ectopic in 1. Three of the cohort studies were performed on cadaveric material from Iran, Bangladesh and Senegal, while in the remaining studies the extraction protocol of the data (anatomical, imaging, clinical or surgical) is not clearly defined.

Of the 5 literature reviews, 2 categorise variations in the location of the appendix, 1 refers to the anatomical variations in the form of the appendix, 1 focuses on the horseshoe appendix, 1 addresses the reliability of the McBurney's point as a guide point for determining the position of the appendix, 1 examines cases of duplication and triplication of the appendix.

The meta-analysis focuses on the location variants and classifies them as retrocecal, pelvic, subcecal, ileal, paracecal, prececal and subhepatic appendices. The patients studied were from Asia (30%), Africa (4%), Europe (18%), North (46%) and South (2%) America. Anatomical Variations of the Vermiform Appendix can be sorted into 2 main categories: Variations in terms of the position and Variations in terms of the form of the appendix.

Variations in Position. The appendix presents several variations in terms of its location, of which the following are frequent in the general population: the retrocecal, the prececal, the paracecal and the subcecal appendix. The retrocecal position of the appendix is the most common anatomical variant, as it is estimated to occur in about 25.4-71% of the general population (1-5). The tip of the

appendix is located posterior to the cecum (1-9). The prececal position is encountered in approximately 4% of the population and refers to the anterior (ventral) location of the appendix's tip relative to the cecum (1, 9). Additionally, the derivation of the appendix from the anterior surface of the cecum has been recorded (10). In the paracecal location, which is found in 3.1-7.5% of the general population, the body of the appendix is located between the lateral surface of the cecum and the lateral abdominal wall (1, 5, 11). In the subcecal location, the tip of the appendix is located caudally to the cecum, with an incidence of 3.5-20.3% in the general population (1-5).

Regarding the position of the appendix relative to the ileum, 2 variations can be distinguished. The retroileal appendix, with a rate of occurrence approximately 5.4-12.5%, describes an appendix whose tip is located posteriorly to the terminal ileum (2-5, 7). The pre-ileal appendix is found in 9.7-18.7% of cases and its tip is located in front of the terminal ileum (5, 7). Another frequent variant found in 16.5-30.35% of the general population is the pelvic location, in which the tip of the appendix is located caudally to the pelvic brim. The pelvic appendix may descend oriented towards the sacrum (1-5, 7, 9, 12).

The subhepatic appendix is another common anatomic variant. Both the cecum and the appendix are located below the liver, as a result of the incomplete descent of the intestinal coils during foetal development. This variant is estimated to occur in about 2.4% of the general population. This position could create significant complications in differential diagnosis of appendicitis, as the patient's symptoms may resemble those of acute cholecystitis (1, 5, 7, 8, 11, 13-17).

McBurney's point is an important guide point for determining the position of the base of the appendix. However, this point is not always accurate, as the base of the appendix may be displaced along the longitudinal or transverse axis (18-20). In fact, one of the studies reviewed found that in only 1 out of 100 subjects in the sample did the base of the appendix correspond to McBurney's point (20).

Rare variations in the position of the appendix (incidence <1%) have also been described in the literature (7, 21, 22). Such variants include intracecal, intramural and subserosal appendices, which can easily be confused (9, 21, 23, 24). In order to avoid errors in differential diagnosis, Chauhan et al. (21) and, Abramson et al. (23), proposed criteria for distinction between intracecal, intramural and subserosal appendices (Table 1). It should be noted that to date, no formal criteria have been established.

Additionally, the appendix may appear in a (para)colic position, in which the body of the

Table 1. Chauhan et al. (21) and Abramson et al. (23) Criteria for the Differential Diagnosis of Intracecal, Intramural and Subserosal Appendix

Intramural Appendix	Intracecal Appendix	Subserosal Appendix
It is located within the wall of the cecum	It is located within the wall of the cecum and can penetrate up to the cecal muscle coat.	The appendix and cecum must be distinguished as distinct and independent organs, macroscopically and microscopically
It is covered internally by the serous membrane of the cecum and externally by the peritoneum	Since local inflammation is found, this should not solely explain the fusion of the appendix with the cecum. The fusion should also exist in the absence of inflammation.	The coats of the appendix wall must be complete and not an extension of the cecal wall.
	There is no distinct mesoappendix.	There is no distinct mesoappendix.
	The base of the appendix cannot be distinguished from the cecum.	The base of the appendix must be different from the cecum's.
	The cecal tissue completely encloses the appendix.	The appendix must be completely covered by the serous membrane of the cecum.
	The vasculature tends to adapt to anatomical variations.	Intussusception, intracecal and intramural appendicitis must be ruled out.

appendix runs parallel to the ascending colon, along its lateral border (4, 22).

Other rare variations of the appendix include: localisation between the right psoas major and the right iliacus muscle (25), in the left abdomen, which is typically due to inversion of the viscera (situs viscerum inversus) or incorrect rotation of the midgut during foetal development (26-28), within an Amyand hernia (29), in the thorax, at the height of the 8th-9th thoracic vertebra (30), or in the lumbar spine (31). The last 3 extremely rare cases were associated with the presence of hernias in the respective areas (15).

Finally, combinations of the anatomical variations described above have also been recorded. A number of reports locate the appendix behind and below the cecum (9), or in retrocecal and retroperitoneal (32), retrocecal and intraperitoneal (32), posterior ileum and cecum (9), prececal and preileal (9), subcecal and prececal (9) and retrocecal positions, or attached to the posterior peritoneum and the wall of the cecum (33).

The statistical distribution of the anatomical variations of the appendix presents significant variation depending on the population under consideration. According to an anatomical study on cadaveric material (200 cadavers) in Iran, the most common variant is the pelvic position of the appendix (55.8%), followed by the subcecal (19%), the retroileal (12.5%), the retrocecal (7%) and the preileal (1.5%) location (3). According to the same survey, the retrocecal position is the most common variant in the US, Europe, Ghana and India. The retroileal location is more frequent in the Thai population, while the pelvic location is prevalent in Zambia and Nigeria (4).

In the meta-analysis included in the present review (1), 114,080 patients with acute appendicitis were studied, and it was found that retrocecal location was the most frequent variant in all races. Specifically, in Africa, the rate of occurrence of the retrocecal location of the appendix was approximately 44.8%, of the pelvic position 27.7%, subcecal 7.7%, ileal (retroileal and preileal) 13.4%, paracecal 6.2%, prececal 3% and of the subhepatic 2.2%. In Asia, the incidence of the retrocecal location was found to be 32%, of the pelvic 29.4%, subcecal 12.9%, ileal (retroileal and preileal) 15.4%, paracecal 8%, prececal 6.7% and of the subhepatic 2.1%. In Europe, the proportion of the retrocecal location of the appendix was approximately 27.6%, of the pelvic 27.1%, subcecal 17.3%, ileal (retroileal and preileal) 10%, paracecal 9.2%, prececal 4% and of the subhepatic 3.2%. In North America, the retrocecal location of the appendix was found in 24.8% of cases, the pelvic in 19.5%, the subcecal in 23.5%, the ileal (retroileal and preileal) in 18.3%, the paracecal in 7.2%, the prececal at 0.6% and the subhepatic at 0.9%. Finally, in South America the incidence of the retrocecal location was found in 36.4% of cases, the pelvic in 31.5%, the subcecal in 17.8%, the ileal (retroileal and preileal) in 16.6% and the paracecal in 6.1%, while no cases of prececal or subhepatic appendicitis were identified.

The relationship between sex and appendix location has not yet clearly identified. In a study of 80 black African cadavers (62 men, 18 women), aged 16 to 78 years (mean age 36 years), the retrocecal position of the appendix was found to be more common in women than in men (P=0.021) (9). However, both in the study of 200 Iranian cadavers (3) and in the meta-analysis mentioned above (1), no statistically significant relationship was observed (3). Overall, genetic factors, lifestyle, geographic region, race, dietary habits and, perhaps, sex are likely to influence, to a greater or lesser extent, the shape and location of the appendix in humans (Figure 2).

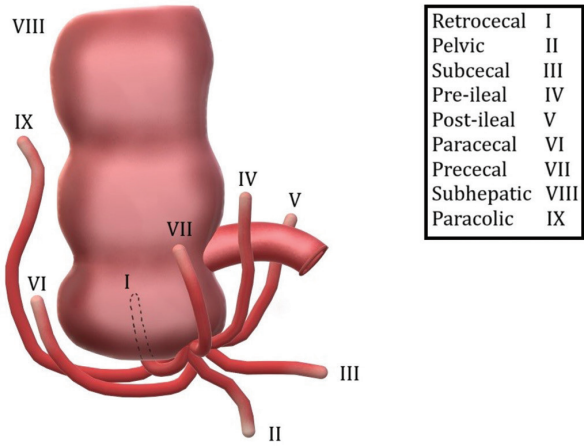

Figure 2. Variations in Position.

Table 2. Cave-Wallbridge Classification of Duplicated Appendix

Type A		Two appendixes arising from 1 cecum, with a common base and then separate.
Type B	Type B1 or Bird-like	Two appendixes arising on either side of the ileocaecal valve, from 1 cecum, but each having a separate base.
	Type B2 or Taenia-colic	One appendix found in the retrocecal position arising from the convergence of the taenia coli and a smaller second appendix along the anterior taenia at a variable distance from the first.
Type C		Two appendixes that arise from 2 cecums, which along the way unite and form a common ascending colon. Each appendix arises from a different cecum.

Variations in Form. These include variations in the number, size and shape of the appendix, as well as variations of the mesoappendix. Number variations include agenesis, and doubling and tripling of the appendix (7). Agenesis refers to the failure to form an appendix. Duplication refers to the presence of two appendixes and is one of the most studied anatomical variations (7, 34-40). According to the Cave-Wallbridge classification, duplicated appendages are distinguished into Type A, B (B1 and B2) and C (35, 36) (Table *2*).

Type B2 duplication is the most frequent variant of this category, accounting for approximately 37% of cases (38). A special case of duplication of the appendix is the horseshoe appendix. This is an especially rare variant, in which the appendix forms a horseshoe-like structure and fuses at its two ends with the cecum. Only 6 cases have been described in the international literature to date (38, 40). Triplication of the appendix is also an extremely rare variant, as only 2 cases have been described to date (38) (Figure 3).

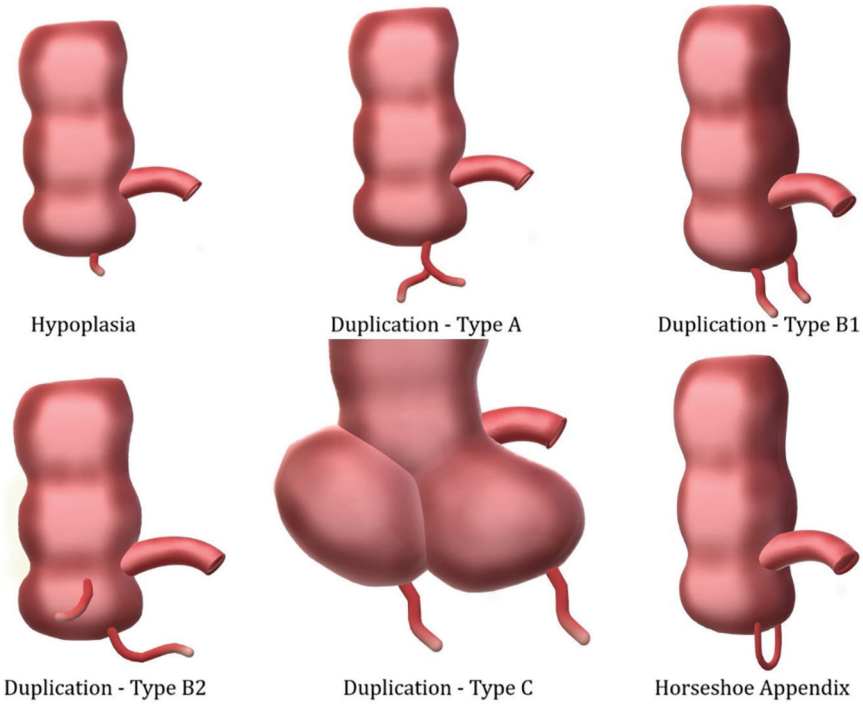

Figure 3. Variations in Form and Size variations concern the length and diameter of the appendix, where significant heterogeneity occurs. The length of the appendix ranges from 0.5 to 23 cm, with the average length between 5.3-11.7 cm. The diameter of the appendix varies from 3.2 mm to 10 mm (1-4, 8). The variant in which the appendix is less developed and smaller than normal size is referred to as hypoplasia, and is also found in the general population.

Shape variations include straight, helical and spiral appendices (Figure 4).

Variations of the mesoappendix also belong to the category of anatomical variations of the form of the appendix. Normally, the appendiceal artery runs along the free edge of the mesoappendix and, consequently, incomplete development of the mesoappendix carries the risk of inadequate perfusion of the appendix and may lead to gangrenous or perforated appendicitis (2, 3).

Concerning the possible correlation between the length/diameter of the appendix and sex/ geographic region/ race, the scientific data do not agree. The anatomical study of 200 random cadavers (153

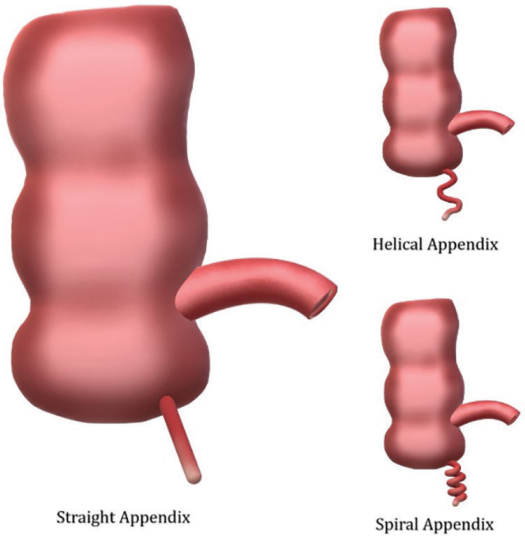

Figure 4. Variations in Shape.

men, 47 women) by the Forensic Service of Zenjan province in Iran (3), indicates a statistically significant relationship between sex and the length of the appendix (males have a longer appendix than females, P<0.01), as well as between age and the length of the appendix (maximum length at 11-19 years, P<0.001). In contrast, the meta-analysis examined (1) did not propose any statistically significant relationship between age or sex and the length of the appendix. It is highly possible that in different populations the length of the appendix is affected by different factors. Regarding race, in a study of 56 cadavers of adult men (18-67 years) from Bangladesh (13), an average length of 10.21 cm (±2.50 cm) was found, while in the study mentioned above with cadaveric material from Iran (4), an average length of 9.12 cm was found for men and 8.03 cm for women (3). In Western countries the length of the appendix tends to be shorter, with the average length ranging between 5.3 and 6.9 cm. Significant differences are also observed on the African continent. For example, the average length of the process in Kenya was found to be 7.65 cm, while in Zambia it was 11.7 cm (3).

Conclusions

In the present review study, an important attempt was made to record and present the various anatomical variations of the vermiform appendix. The most common position variations are the retrocecal, pelvic, preileal, retroileal, subcecal, paracecal and prececal. Regarding the form variations, duplication of the appendix is more frequently recorded, while agenesis, hypoplasia, and triplication constitute uncommon variations. Also, significant variation is observed in appendix's length and diameter. It should be highlighted that the clinical importance of the variants is evenly great, irrespective of their incidence in the general population. Overall, genetic factors, lifestyle, geographic region, race, dietary habits and sex are likely to influence the shape and location of the appendix in humans. Therefore, with approximately 7% of the general population experiencing acute appendicitis during their lifespan (3), knowledge of the appendix's anatomical variations is really crucial for the clinician, and especially for the surgeon, to avoid complications in surgery and clinical practice.

What Is Already Known on This Topic:
Existing literature has already underlined the great diversity regarding the anatomy of the Appendix. In terms of Position, the retrocecal location is widely considered as the most common variant, followed by the pelvic, preileal, retroileal, subcecal, paracecal and prececal locations, all of which have been extensively studied. Regarding the Form variations, duplication is the most common variant and the most studied one, while variations in length and diameter present vast heterogeneity which is affected by many different factors.

What This Study Adds:
The aim of the present study is to investigate the great variety of anatomical variations of the Vermiform Appendix and, in addition, to sum up these variations, categorising them in 2 main groups: Variations in terms of Position and Variations in terms of Form of the Appendix.

Authors' Contributions: Conception and design: AS and FS; Acquisition, analysis and interpretation of data: AS and FS; Drafting the article: AS, FS, DS and DF; Revising it critically for important intellectual content: AS, FS, DS and DF; Approved final version of the manuscript: AS, FS, DC, T S-M and TT.

Conflict of interest: The authors declare that they have no conflict of interest.

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Original Research Study
Acta Medica Academica 2024;53(3):343-351
DOI: 10.5644/ama2006-124.464

Workplace Factors Contributing to Professional Stress in Family Medicine

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Received: 30 July 2024; **Accepted:** 18 December 2024

Abstract

Objectives. The main objective was to assess the relationship between sociodemographic and occupational factors and stress levels among healthcare workers in family medicine in Bosnia and Herzegovina, using the PSS-10-BH scale. **Materials and Methods.** A cross-sectional study was conducted using the PSS-10-BH questionnaire distributed via Google Forms to primary care nurses and physicians between July and August 2022. **Results.** The study included 272 participants, with a mean age of 44.7 (±10.55), predominantly women (86.8%) and physicians (58.8%). The mean PSS-10-BH total score for the sample was 21.26 (±6.77) ranging from 3 to 36. The linear regression model indicated male gender and older age showed an association with slightly lower stress levels. Marital status and the number of children showed a slight positive association with stress levels. Occupation (B=-3.068, 95%CI: -5.442 to -0.694, P=0.012) was associated with stress, with physicians tending to report lower stress levels compared to nurses. Years of work experience (B=0.060, 95%CI: -0.190 to 0.309, P=0.636), and patient load (B=0.082, 95%CI: 0.027 to 0.137, P=0.004) were associated with higher stress levels. The results suggest that work-related variables are significant predictors of stress levels as measured by the PSS-10-BH scale in this sample of healthcare workers. The included predictors explain 10% of the variability in the outcome, indicating additional unidentified contributing factors. **Conclusion.** Occupational factors, particularly profession, work experience, and daily patient load, significantly influence stress levels in healthcare workers. Further research is needed to explore other potential influences and refine interventions aimed at managing stress in this population.

Key Words: Family Medicine ▪ Perceived Stress ▪ Occupational Stress ▪ Health Care Professionals.

Introduction

Over the past several decades, the healthcare field has been identified as a work environment that places high demands on employees at all levels. Widespread stress among healthcare workers has potential negative effects on job performance, care quality, absenteeism, job satisfaction, and healthcare professionals' mental health (1-3). Health care professionals have higher levels of psychosocial stress than other population samples and have been recognized as susceptible to burnout mainly due to work overload (4-7).

Family medicine staff function as the gatekeepers of the health system, taking care of a large number of unknown cases, primarily involving history taking and physical examination. Stress is a pervasive issue in the primary healthcare setting, and family medicine professionals face unique stressors that can impact their well-being and job performance. Many studies have explored the psychological impact, mainly on physicians and nurses, of work in the hospital setting (5-8). Fewer studies have looked at HCWs in the primary care setting, although medical staff in primary care institutions are also at risk of developing occupational

stress and its consequences. Professionals in this field face many stressors, and among the most intense are the extensive administrative tasks, lack of staff, unforeseen situations, insufficient time to examine a large number of patients, negative public perception and criticism, and unrealistic expectations from the patients, their families and the health system (9).

There are many tools for assessing stress levels, but the Perceived Stress Scale (PSS), since it was developed by Cohen, Kamarck and Mermelstein in 1983, has been widely used as a measuring instrument for self-assessment of stress levels (10). It was developed within the theoretical framework of the transactional model of stress, which emphasizes the interaction between stressful events and the individual's assessment of available coping resources (11).

In research the PSS-10 version is most often used, due to its brevity, simple application, the comprehensibility of the items, and its favorable psychometric properties. The PSS-10 bifactor model has been favored and validated in prior research in several countries with diverse participant structures. Additionally, perceived distress (PD) and perceived coping (PC) components had higher item loadings than general factors. While perceived distress and perceived coping factors exist independently, the PSS-10 is driven by the single underlying component of perceived stress (12-16).

The questions in the PSS-10 refer to feelings and thoughts related to the previous month. The same period also applied to single questions. Scoring is calculated by summation of item scores. The scale is scored using a 5-point Likert scale ranging from 0=Never to 4=Very often. Potential total scores range from 0 to 40, with a higher score indicating higher levels of perceived stress. Scores ranging from 0-13 would be considered low stress, 14-26 moderate stress, and scores ranging from 27-40 would be considered high perceived stress (15, 17).

The aim of this study was to assess the relationship between sociodemographic and occupational factors and stress levels among healthcare workers in family medicine in Bosnia and Herzegovina, using the PSS-10-BH scale.

Materials and Methods

The study was conducted as a cross-sectional survey of HCWs, physicians and nurses, working in the family medicine service in Bosnia and Herzegovina, a total of seven centers with associated outpatient clinics. Data collection took place between July and August 2022. The link for the Google Forms with questions about socio-demographic and workplace characteristics (age, gender, marital status, number of children, occupation, level of education, work experience, and the average number of patients per day) and PSS-10-BH validated questionnaire, including informed consent, was sent to the email addresses of family physicians and nurses working in public sectors throughout Bosnia and Herzegovina. The email addresses used in the study are in the database of two registered associations of family medicine in B&H. The PSS-10-BH scale was validated in the Bosnian language and had been previously used in another local study of healthcare professionals, where the reported Cronbach's alpha was 0.87 (16).

The inclusion criteria were as follows: healthcare professionals (physicians and nurses) who had been working in the family medicine department for at least one year, were employed in the public sector, voluntarily agreed to complete the questionnaire, and answered all questions in accordance with the instructions. The exclusion criteria were as follows: healthcare professionals who were on annual leave, maternity leave, or sick leave. Additionally, staff newly recruited during the study period, and those still undergoing a training program (internship) were excluded.

Ethics Statement

The study was approved by the Ethics Committee of The Association of Family Physicians No. EK-01-011-CS/22, dated May 18, 2022.

Statistical Analysis

Descriptive statistics were calculated for all variables. Continuous variables were presented as mean and standard deviation (age, work experience, and daily workload), while categorical

variables were presented as frequencies and percentages (gender, occupation, education level, marital status, number of children, and the distribution of responses to individual items on the PSS-10-BH). Linear regression was used to analyze associations between independent sociodemographic predictors (age, gender, marital status and number of children), work related predictors (occupation, work experience and daily patients load) and the PSS-10-BH value as a dependent variable. Prior to performing linear regression, preliminary analyses were conducted to evaluate the key assumptions of the method, including linearity, normality of residuals, and homoscedasticity. The scatter plot of residuals against predicted values revealed no discernible patterns or clustering, suggesting that the assumption of homoscedasticity was satisfied.

Additionally, the residuals exhibited a consistent variance across levels of the independent variables. These findings indicated that the data met the required assumptions, justifying the use of linear regression for further analysis. The possibility of a non-linear association between continuous predictors and stress was considered during preliminary analyses. Scatter plots of predictors against the PSS-10-BH scores revealed linear trends without significant curvature, justifying the use of linear regression. While non-linear relationships may exist in specific contexts, they were beyond the scope of our study. In line with contemporary statistical recommendations and to provide a more comprehensive picture of the results, we opted to use 95% confidence intervals instead of P-values. This approach allows readers to assess the effect size and precision of estimates, avoiding dichotomous thinking about statistical significance and providing a more informative presentation of our findings. The statistical analyses were performed using IBM SPSS Statistics version 27.0.0 (IBM Corp, Armonk, NY, USA).

Results

The study participants (272) were family medicine health care professionals aged 25 to 69, with a mean age of 44.7 (±10.55). Most participants were women (N=236; 86.8%) and physicians (N=160; 58.8%) with specialization in family medicine (43%). In family medicine in Bosnia and Herzegovina, 746 family medicine physicians work in the public sector. In the databases of the two Associations, there are 578 email addresses to which the questionnaire was sent. Of this number, 272 respondents gave their consent to the survey, which makes the response rate 47%. There were no subjects who did not give informed consent (negative answer to the initial/first question), all who approached and filled out the questionnaire gave informed consent. There were no incompletely filled out questionnaires. Demographic (age, gender, education level, marital status and number of children) and professional variables (profession, work experience and daily patients' workload) are presented in Table 1.

Table 1. Demographic and Work-Related Variables

Demographic & Work-Related Variables	Total (N=272)
Age	
M±SD	44.7±10.11
Range	25-69
Gender (N; %)	
Women	236 (86.8)
Men	36 (13.2)
Profession (N; %)	
Physician	160 (58.8)
Nurse	112 (41.2)
Education level (N; %)	
High school	79 (29)
University degree	76 (27.9)
Specialization	117 (43)
Marital status (N; %)	
Married	214 (78.7)
Single	58 (21.3)
Children (N; %)	
No	75 (27.6)
1	74 (27.2)
2	108 (39.7)
3	11 (4.0)
4	3 (1.1)
5	1 (0.4)
Work experience	
M±SD	17.8±10.55
Range	1-42
Self-reported daily patients' workload	
M±SD	42.42±18.06
Range	3-80

The mean PSS-10-BH total score for the sample was 21.26 (±6.77) with a range from 3 to 36. The means of the subscales Perceived Distress and Perceived Coping were 14.77 (±4.96) and 6.49 (±2.68). Individual scores on the PSS could range from 0 to 40 with higher scores indicating higher perceived stress. According to data from the literature, the PSS score can be categorized as low, moderate or high perceived stress. Among the respondents in this study, 12.5% had a low level of stress, 62.9% moderate, and almost one quarter had a high level of stress (24.6%), as measured by the PSS-10-BH score. In Table 2, the frequencies of responses to the PSS-10-BH questions are presented, divided into Perceived Distress and Perceived Coping.

The linear regression model assessing the relationship between the sociodemographic and occupational variables and stress levels, as measured by the PSS-10 scale, accounted for 10.5% of the variance in stress levels (R^2=0.105). The overall model demonstrated statistical significance (F=3.221, 95%CI: 1.123 to 5.319), suggesting that the model as a whole is significantly better than the intercept alone. The F-test does not confirm the significance of all individual predictors but rather shows that at least one predictor (variable) is significantly associated with stress levels.

Several variables exhibited statistically significant associations with stress levels. Occupation (B=-3.068, 95%CI: -5.442 to -0.694, P=0.012) was associated with stress, with physicians tending to report lower stress levels compared to nurses/technicians. Years of work experience (B=0.060, 95%CI: -0.190 to 0.309, P=0.636) showed a positive correlation, indicating that increased work experience was associated with higher stress levels. Each additional year of employment increased stress by 0.060 points on average, although this effect is not significant. Daily patient load (B=0.082, 95%CI: 0.027 to 0.137, P=0.004) also demonstrated a positive association, suggesting that a higher number of patients seen daily corresponded with increased stress levels.

Table 2. Distribution of Responses to PSS-10-BH Items, Perceived Distress and Perceived Coping

PSS-10 Item	In the last month, how often have you …	N; %				
		0*	1†	2‡	3§	4‖
Perceived distress						
Q1	… been upset because of something that happened unexpectedly?	1 (0.4)	26 (9.6)	85 (31.3)	103 (37.9)	57 (21.0)
Q2	… felt that you were unable to control the important things in your life?	10 (3.7)	55 (20.2)	95 (34.9)	76 (27.9)	36 (13.2)
Q3	… felt nervous and "stressed"?	2 (0.7)	29 (10.7)	74 (27.2)	97 (35.7)	70 (25.7)
Q6	… found that you could not cope with all the things that you had to do?	16 (5.9)	62 (22.8)	94 (34.6)	71 (26.1)	29 (10.7)
Q9	… been angered because of things that were outside your control?	2 (0.7)	34 (12.5)	85 (31.3)	80 (29.4)	71 (26.1)
Q10	… felt difficulties were piling up so high that you could not overcome them?	18 (6.6)	59 (21.7)	80 (29.4)	67 (24.6)	48 (17.6)
Perceived coping						
Q4	… felt confident about your ability to handle your personal problems?	37 (13.6)	103 (37.9)	105 (38.6)	21 (7.7)	6 (2.2)
Q5	… you felt that things were going your way?	25 (9.2)	97 (35.7)	104 (38.2)	42 (15.4)	4 (1.5)
Q7	… been able to control irritations in your life?	31 (11.4)	89 (32.7)	112 (41.2)	37 (13.6)	3 (1.1)
Q8	… felt that you were on top of things?	20 (7.4)	87 (32)	107 (39.3)	50 (18.4)	8 (2.9)

*Never; †Almost never; ‡Sometimes; §Fairly often; ‖Very often.

Table 3. Multiple Linear Regression Results for Predictors of Stress Levels Among Healthcare Workers

Model	Coefficients			t	Sig.	95% Confidence Interval for B	
	Unstandardized		Standardized				
	B	Std Error	Beta			Lower bound	Upper bound
Constant	21.404	4.526	–	4.729	0.000	12.476	30.333
Variables							
Age	-0.003	0.135	-0.004	-0.021	0.983	-0.270	0.264
Gender	-1.454	1.460	-0.070	-0.996	0.321	-4.335	1.427
Marital status	0.940	1.693	0.041	0.555	0.579	-2.400	4.281
Number of children	0.458	0.729	0.044	0.628	0.531	-0.980	1.896
Occupation	-3.068	1.203	-0.215	-2.550	0.012	-5.442	-0.694
Work experience	0.060	0.126	0.085	0.473	0.636	-0.190	0.309
Daily patients load	0.082	0.028	0.206	2.924	0.004	0.027	0.137

Dependent Variable: PSS-10-BH.

Other variables did not display statistically significant associations, but their coefficients suggested certain trends. Age (B=-0.003, 95%CI: -0.270 to 0.264, P=0.983) showed a slight negative association with stress levels. Each additional year of age slightly reduced stress, but this effect is negligible and statistically non-significant. Gender (B=-1.454, 95%CI: -4.335 to 1.427, P=0.321) indicated a tendency for males to report lower stress levels. Marital status (B=0.940, 95%CI: -2.400 to 4.281, P=0.579) contributed to a small increase in stress levels, the results suggested that married individuals may experience higher stress levels. The number of children (B=0.458, 95%CI: -0.980 to 1.896, P=0.531) showed a slight positive association with stress levels. The coefficients described here are specific to the current model and its selected predictors. The inclusion of additional variables, such as potential confounders or mediators, could influence both the strength and direction of the associations observed between the predictors and stress levels.

The results of multiple linear regression for predictors of stress levels among healthcare workers are presented in Table 3.

Discussion

In our results, a high percentage of respondents had some level of stress, but fewer had very pronounced stress (24.6%) compared to the results of the study by Dotour et al., where 49% participants were stressed, and 32% had a very high level of stress with an average PSS-10 score of 26.4 (±6.4) (18). The mean PSS-10-BH score among our subjects was significantly higher compared to a study in Poland, where it was 16.83 (±4.47), conducted among the same population, using the same tool (19).

There was a similar mean PSS-10 score for the total sample (19.0±6.89) in the study by Chakraborti et al., without any statistically significant difference between males and females (20). Likewise, our results indicate that there was no statistically significant difference in the level of stress between the genders. Being male was associated with a slight decrease in stress levels, but this was not statistically significant (P=0.321). The reason for this difference could be that the numbers of males and females in the study were not equal, as the majority of participants were women. These results are in contrast with the results of other studies regarding the level of perceived stress between genders, with a significantly higher score among female respondents (18, 21-25). In addition, the nurses had statistically significantly higher perceived stress than the physicians in our study, as well as in other studies that assessed the stress level of healthcare professionals (22, 23). The reason could be insufficient education in stress coping options, but also the increasing volume of work and

administrative duties that are introduced in recent years and represent the responsibility of the nurse.

In our results, the average PSS-10-BH score was higher in participants who had a higher number of patients per day, with a statistically significant difference between groups, as well as in the data from the 2018 Survey of America's Physicians Foundation - Practice Patterns & Perspectives. This study found that 77.8% physicians with up to 40 patients per a day, had feelings of professional burnout (24).

An often-used term in the literature is the "panel size" of patients that represents the group of patients assigned to one specific physician or clinical team. Primary care panel size has implications for patient access, physician workload, care comprehensiveness, and quality of care. The often-quoted standard panel size is 2500, but multiple studies have observed that a panel size of 2500 is not feasible because of time constraints, and results in incomplete preventive care and health care screening services (25). According to a study published in 2023 in Bosnia and Herzegovina, the average number of patients registered in the family medicine team is 1986.4 (±511), with an average of 50 patients per day (26). These data support the results of our study that workload (a large number of patients) can be one of the factors that influence the level of stress among health workers in primary health care.

In addition to these workplace characteristics that affected the PSS-10-BH score, work experience also had a statistically significant effect on the level of stress in our respondents, although it approached the limit value to meet the definition of statistical significance. Work experience is a factor that has been shown to have a significant effect on stress levels in other studies involving healthcare professionals (22, 23). A study conducted among health workers in the Department of Emergency Medicine showed levels of high stress 32.33%, and very high stress 32.38% (23). Another study conducted in Romania among health care workers in different hospital departments showed that in the total sample, the overall level of stress was average (65.7%), followed by low (32%), and high levels were found in only 2.4% of the cases (27).

Several studies suggest cut-off points, based on the median score, which represent the cut-off point between the "presence of stress" (score values greater than the median value) and "no stress" (score values lower than the median value). The threshold value used in research conducted among family doctors in France divided the perceived stress score into the categories: no stress PSS-10≤20, borderline 21-26, stress ≥27, and PSS-10≥30 high stress (18). A study conducted in Ethiopia took a score of PSS-10>20 as the cut-off for the existence of stress, without gradation (3). In the light of this gradation, our participants had a significantly higher percentage of stress compared to the results of Teshome et al., 68% (95%CI: 58.4%, 65.2%), but it was approximately equal to those from the research in France, where 81% of healthcare workers had a certain level of stress, with a mean of 26.4 (±6.4) (3, 18).

Our results are almost equivalent to the results of a study from China where the prevalence of perceived stress was 53.8%, and the results from a study conducted in Ghana from 2021 among health care professionals, using the same instrument, where 64% of the participants had moderate stress (28, 29).

A Danish study conducted in the period before the pandemic, also using the PSS-10, showed that 21% of physicians in general/family medicine had a certain level of stress, which is much lower than the 52.7% of family medicine doctors who were under stress in our study (30). This different rate of stress is a consequence of the context of the pandemic, which was reported as a source of stress in the general population, among hospital medical staff, and family medicine staff were not spared (17, 18, 23, 24, 29).

Given that, in our results, the linear regression model of the three occupational variables (occupation, work experience and daily patient load) and sociodemographic variables (age, gender, marital status and number of children) explains only 10.5% of the variability in the outcome (stress levels), suggesting that there are likely several

other strong determinants of stress that were not identified in this study. Possible additional variables that could have a more significant impact on perceived stress include: socio-economic status (income, education, and social status), work environment (support from colleagues, working conditions, and relationship with superiors), personal characteristics (personality traits, family responsibilities, health status, and level of physical activity), social support (support from friends and family), organizational culture in the workplace (rules, values and norms), and relationships with patients.

Limitations of Study

The main limitation in this research is that the sample of professionals is limited to the public health sector, so the data may not be generalizable among physicians and nurses working in the private sector. There is a possibility that different models of management and organization of work processes have a different effect on modeling perceived stress, and future studies should compare and analyze the impact of different forms of management on the stress level of healthcare workers. There is a potential selection bias because those who chose to respond to the survey may have different levels of stress compared to those who did not respond, possibly skewing the results. Variables such as individual coping mechanisms, personal life events, mental health history, and support systems were not measured, but could significantly influence stress levels. The study also did not evaluate other potentially important aspects in determining the perception of stress by PHC professionals, such as environmental risk factors, income or pre-existing chronic non-communicable diseases. Given that the linear regression model indicates the possibility of the existence of other variables that have a more significant impact on PSS-10-BH results, additional research should be conducted with identification of other factors of the work environment that can significantly influence perceived stress. The use of self-reported questionnaires can introduce bias, as participants may underreport or over-report their stress levels due to social desirability or recall bias. The Cross-Sectional Design captures data at a single point in time, which does not allow for analysis of changes in stress levels over time, or the establishment of causal relationships between variables.

Conclusion

The results suggest that work-related variables (occupation, years of work experience, and daily patient load) are significant predictors of stress levels as measured by the PSS-10-BH scale in this sample of healthcare workers in family medicine. Other variables, such as age, gender, marital status, and number of children, did not show statistically significant associations, but they indicated trends that warrant further exploration. Age and gender appeared to have a minor influence on stress, with age showing a slight negative relationship and males reporting lower stress levels. Married individuals and those with children exhibited a slight tendency toward higher stress, although these results were not significant. Overall, these findings highlight the importance of occupational factors, particularly occupation, work experience, and patient load, in predicting stress levels in healthcare workers. Further research is needed to explore other potential influences and refine interventions aimed at managing stress in this population. The results of this study can contribute to the formulation of future policies relating to the health of workers in PHC institutions, improving cost-effective actions in health promotion, and the prevention of work-related disorders in the health system. To address professional stress in family medicine, it is important to implement strategies that promote self-care, work-life balance, and support for healthcare workers.

What Is Already Known on This Topic:

Healthcare workers, particularly physicians and nurses, are known to experience high levels of professional stress due to their demanding work environments, long hours, and high patient loads. High professional stress levels can lead to burnout, job dissatisfaction, mental health issues, and a reduced quality of patient care. The PSS-10 is a widely used tool to measure perceived stress levels, providing insights into how in-

dividuals perceive their stress in response to various situations. Both professional and personal life stressors contribute to overall stress levels, impacting work performance and personal well-being.

What This Study Adds:

This study provided the detailed demographic and professional profiles of healthcare workers, highlighting age, gender, profession, marital status, number of children, daily patients load and work experience. The study, using the PSS-10-BH, identified that a significant proportion of healthcare workers experience moderate to high stress. Through regression analysis, the study identified key predictors of professional stress, including occupation, work experience, and daily patient load, providing a comprehensive understanding of factors contributing to stress in healthcare professionals.

Authors' Contributions: Conception and design: NT and ZJ; Acquisition, analysis, and interpretation of data: ZJ, NT, HE and EH; Drafting the article: NT, EH, RM, NT and SDz; Revising it critically for important intellectual content: NT, HE, ZJ and HJ; Approved final version of the manuscript: ZJ.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
Acta Medica Academica 2024;53(3):352-372
DOI: 10.5644/ama2006-124.462

Resilience in the Face of War: a Collaborative Autoethnography of a Ukrainian Refugee Student's Journey through Europe Striving to Find Oneself

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Received: 19 September 2024; **Accepted**: 10 November 2024

Abstract

Objective. This study presents the personal experience of a 19-year-old student who fled the war in Ukraine, journeyed across multiple countries, and ultimately enrolled in a university psychology program in Croatia. **Methods**. A collaborative autoethnographic approach was employed to explore the student's experience as a war refugee, traversing Europe, and beginning university life in a foreign country. Data were collected through the student's reflective writing. A thematic analysis was conducted to identify key emotional and experiential themes. **Results**. The narrative provides a comprehensive account of the student's emotional and physical journey, beginning with the onset of war in Ukraine and progressing through her travels in Romania, Bulgaria, the United Kingdom, and Ireland, before settling in Croatia. Thematic analysis revealed a complex interaction of emotions, family dynamics, community support, and the challenges of adapting to a new environment. Despite numerous obstacles, the student and her family remained hopeful and proactive in seeking a better future. The narrative also underscores the therapeutic impact of sharing personal stories. **Conclusions**. The findings highlight storytelling as a powerful medium for personal healing. Moreover, the study emphasizes the collective importance of individual narratives in fostering empathy, understanding, and connection across diverse communities. The narrative underscores the resilience of individuals and the crucial role of compassion and support in times of crisis. This resilience is not just about surviving but finding ways to thrive and contribute meaningfully despite the uncertainties and disruptions caused by the war.

Key Words: Migration ▪ Psychological Adaptation ▪ Narrative Therapy ▪ Emotional Adjustment ▪ Trauma Healing.

Introduction

Russia's invasion of Ukraine began on February 24, 2022, with missile strikes across Ukrainian cities. Although the initial plan to swiftly overturn Ukraine's government failed, Russian forces gained control of significant territories in the east and south, including Kherson and Mariupol, within weeks (1). During the early stages, around 3 million Ukrainians fled the country, and 1.85 million were internally displaced, as reported by the United Nations High Commissioner for Refugees (UNHCR) (2). As of February 2024, approximately 6.5 million Ukrainian refugees were recorded globally (3). The global response was swift and compassionate (4). In the European Union (EU), Temporary Protection status granted Ukrainians similar rights to citizens, allowing access to employment, education, and healthcare (5). Despite the support, each Ukrainian must adapt to new environments, and challenges vary by country and individual. Common hardships include the traumatic experience of fleeing war, which often leads to severe stress and anxiety (6). Oviedo et al. highlighted the initial struggles of Ukrainians, including exposure to life-threatening conditions, lack

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of sleep, food deprivation, cold, and long queues at the border. Families with children faced additional stress in keeping their children safe from trauma (6).

One of the common barriers that people confront as immigrants is the traumatic experience of leaving the country and being the victim of war (4). Traumatic experiences and the threat to life significantly impact psychological well-being (7). A study by Buchcik et al. found that 85% of 389 Ukrainian refugees in Germany that contributed to the study exhibited symptoms of anxiety and depression, which negatively affected their quality of life (8). This psychological distress was associated with a lower quality of life in refugees (9). Additionally, a prevalent part of refugees suffers from survivor syndrome guilt (10).

In those circumstances, it is harder for people to manage work and life in a new country, as they are unprepared for it (4). Many countries have organized free counselling sessions to address this challenge and help refugees' mental health. For example, in Germany, a Hamburg clinic provides psychological help and counselling for child and adolescent refugees (11). Language barriers are another significant obstacle to integration. Mastering the local language is crucial for accessing employment, education, and healthcare (12). For instance, Ukrainian students in Poland faced difficulties in adapting to higher education due to language challenges, although those studying in English fared better (13) and language challenges were a limiting factor for accessing healthcare support (10). Not knowing the native knowledge of a country may make refugee students feel somewhat excluded from the community. Luckily, most countries have established courses for Ukrainian immigrants to learn a local language for children and adults (14). Uncertainty about the duration of their stay adds further stress for refugees as they struggle to plan their futures (15). This uncertainty mainly affects higher education students, who often feel torn between pursuing their goals abroad and a strong desire to return home (13). Forced migration also disrupts refugees' sense of identity, leading to feelings of exclusion and difficulty adjusting to the host country's culture (13). Despite these challenges, many refugees have had positive experiences. Oviedo et al. reported that refugees frequently mentioned the kindness and support of volunteers and local citizens (6). Ukrainian students in Poland expressed gratitude for the welcoming environment provided by the local community (13).

Refugees rely on various strategies to cope with these challenges, including maintaining relationships, religious faith, therapy, and staying focused on daily responsibilities like work and volunteering (6). However, the influx of refugees has increased the risk of human trafficking and exploitation, with reported cases of sexual exploitation, financial fraud, and mistreatment by those offering housing, transportation, or fake visas (16-18). Although refugees face difficulties in their daily lives, this does not exclude the positive experiences immigrants might live through. This topic is usually not the focus of existing literature. Oviedo et al. found that positive experiences that prevail among Ukrainian war refugees are the kindness and willingness of volunteers and citizens of foreign countries to help. The volunteers were described in most cases as "very nice" or "nice" (6). Also, it has been recorded that Ukrainian higher education students showed gratitude for the assistance and welcoming environment that Polish people provided (13).

Here, we provide the experience of a 19-year-old psychology student from Ukraine who escaped war, moved to Croatia, and enrolled at the University of Split in Split, Croatia. This collaborative autoethnography aimed to provide a more detailed description of what a higher education student went through as an immigrant, encountering both challenging and beautiful episodes in a new environment.

Methods

Study Design

We used a qualitative research design with collaborative autoethnography. The first author (referred to as "narrator" in the manuscript) provided her experience as a narrative, describing her

individual experiences. Other authors contributed to the development of the narrator's story, prompting a more detailed description of her experiences and conducting a thematic analysis of the narrative. In autoethnography, research takes a self-reflective form that examines the researcher's viewpoint on a given topic. With this approach, the narrator aims to describe the experience from within (19).

Approach and Philosophical Stance

Given the need to understand the nuanced thoughts, feelings, and behaviours associated with the narrator's experience, a qualitative methodology was adopted. This approach facilitated the exploration of complex phenomena through immersion, interpretation, and analysis of the lived experience within the environmental setting. Specifically, an autoethnographic design was employed, reflecting a relativist ontological stance (acknowledging multiple possible realities) and a subjectivist epistemological stance (emphasizing personal experience and interpretation as sources of knowledge).

Obtaining the Story

This article is part of the Giving Voice project (20), which fosters writing research reports about the war in Ukraine. One of the project collaborators is a professor at the Faculty of Humanities and Social Sciences in Split, University of Split. He informed the narrator that there is interest within the project to publish first-person accounts about the war experiences, and the narrator accepted the invitation to write this manuscript in collaboration with academic researchers.

Data Collection

We used a twofold approach. Initially, the narrator wrote a reflective account recounting her own experiences of escaping from Ukraine after the start of the war, traveling across Europe as a refugee, and enrolling in a university in a new country. After writing the first draft, the second author (DH, academic researcher and professor; psychologist) provided comments to seek more in-depth descriptions of the experiences and feelings of the narrator. Finally, the third author (LP, academic researcher and professor; research methodologist) provided another layer of feedback and suggestions, using the additional perspective to enhance the reflective process. Feedback from the co-authors was used to explore the narrator's experiences in greater depth and cover areas of potential interest that were not covered in the initial narrative.

Ethics

The manuscript contains the narrative of the study's first author (MS). Two co-authors (LP and DH) helped the first author write the narrative and participated in the thematic analysis. The author of the narrative (MS) consented to write her story. Two co-authors (LP and DH) consented to participate in the study. Since the narrator is also an author of this manuscript and an adult (and no other human participants were involved in the study), we consulted with the institutional ethics committee, which advised that this manuscript does not need to be submitted for evaluation.

Thematic Analysis

The narrator's story was then thematically analysed. We followed the six-phase framework for the thematic analysis of Braun and Clarke (21). We began by open-coding the data. Preliminary themes were constructed to define codes relating to similar concepts. DH and LP proposed initial themes. The entire author team reviewed the suggestions and approved the final themes iteratively to ensure they accurately represented the narrator's experience.

Reflexivity

Reflexivity in this collaborative autoethnography is multi-layered and enriched by the shared

experiences of war and displacement. As co-authors, LP and DH bring their own adolescent memories of the Croatian War, resonating with the narrator's current struggles and fostering a deeper understanding of the emotional landscape. While not identical, this shared history created a unique space for empathy and nuanced interpretation. The narrator's lived experiences are central, yet the co-authors' reflections and interpretations added depth and context. The collaborative approach enhanced reflexivity, acknowledging the subjective nature of memory and interpretation while striving for a balanced, authentic portrayal of the narrator's experience.

Results

The Narrative of the Ukrainian Refugee Student

Before the war, I lived in Boryspil, Ukraine, a small town 36 km east of Kyiv. I shared a house with my brother, parents, grandparents, a cat, and two dogs in a joint household. I attended the Taras Shevchenko National University of Kyiv, pursuing a degree in psychology as a second-year student. The start of the War in Ukraine was a big surprise for me, even though people were mentioning the possibility of war. I thought those were just some unsubstantiated worries. A month before the start of the war, a psychologist I recently met recommended running away because the war was coming. Then I heard my parents talking about war a few days before it started. But I did not perceive it seriously; it all seemed to be a fictional threat to me. And I just hoped that everything would be fine. My friends were also confused, and they did not believe it either. But on the night before the war started, I felt really bad; I could not explain why. That day, I went to sleep like an ordinary student, thinking about homework and of that one boy who I met, who I thought was cute. Everything was the same, yet something felt wrong. On one side, normal life was continuing; objectively, everything was alright, and it made me think – how can anything go wrong if I am safe and at home right now? But something felt wrong – the feeling of a possible threat, like some little worry that started to grow with time. I felt a little bit of uneasiness as if I was waiting for something bad to happen. Maybe the conversations about the war that I heard made me think about its realistic possibility. As such, I was quite depressed with all the situations that were going on in the world in general, like COVID-19, for example.

The War Erupts: Confusion and Difficult Decisions

Unfortunately, my expectations were met the next day. "Did you hear that?" I asked my brother as I woke up. "Yes, there are explosions that could be heard from the nearby airport. I read about it in the news". He seemed to have been awake for a long time now. In the next few moments, my whole family was up. Initially, everyone was confused because we were uncertain about what to do next. I felt the rejection and misunderstanding of reality. My beliefs about the world and what was safe were shaken. I just sat quietly and tried to do my university assignments while my family was panicking and thinking about what to do next. There were differences in opinions, which led to minor conflicts. My father insisted on packing stuff and going to the West of Ukraine, where some friends of my parents were living, and my mum agreed. We thought it would be safer there than in the central part of Ukraine, at least for now. My father reassured us that everything would finish in a week. My parents acted fast and smart, I must say. I was just obedient at that point because I did not know what to do. The first difficult decision occurred when my grandfather refused to go with us, no matter how hard we asked him to go; he wanted to stay so he can take care of our house and dogs. I did not understand why grandfather wished to stay, but somehow, I felt he would be safe and fine. I believed in his decision and accepted it. However, we were all upset because of his decision, especially my grandmother; she and my mum tried to convince him to go with us. My father respected his decision. We had to agree with grandpa's decision, just on the condition that he try to keep himself safe. My parents asked him to leave the city when

the danger would be too close. We got in the car with some belongings and our cat. We were heading from Boryspil to Chernivtsi, hoping grandfather would be able to leave if the danger came closer. To make matters worse, my cat struggled being driven in the car. We did not give her any medicine, and she kept trying to escape from the box we placed her in. It was very hard for me to see the cat struggling.

Driving Through Kyiv

To get to our destination, we had to drive through Kyiv first. In the city, we encountered panic, heard explosions, and saw people running in all directions. I watched in awe, still unable to grasp what was happening. I could not believe my eyes because everything was happening so fast. It felt like the end of the world, something disastrous. It was very sad to see all those events. I do not remember seeing any rockets; I could only hear a sirens and explosions.

At the exit from the city, there were enormous traffic jams; people were escaping the danger that could strike at any moment. Ukrainian tanks could be seen near the road, adding to the traffic jams that extended for miles.

A Long Trip to Chernivtsi

There are 570 km between Boryspil and Chernivtski. Normally, this would take 8.5 hours of driving. However, for us, this turned out to be a very long trip. It took us two days and one night to reach the western Ukraine. We were stuck in traffic during the daytime and were sleeping in the car at night. I felt many different emotions. At first, I was sad and crying. Then, I was stressed and curious. At one point, I was okay and positive because I had my family with me, so I did not feel alone. My brother was confident that this would end in a week, and I believed him; this calmed me a bit. In the car, there was my dad, mum, grandmother, brother, me and the cat. My father was stressed, probably thinking about how to keep all of us safe. My mum and grandmother were calm but sad. The trip was tiring for everyone. Finally, we arrived at our friend's house in Chernivtsi. For the next two weeks, we lived there. We checked the news 24/7, talked to our grandfather over the phone, and occupied ourselves with anything we could find to distract ourselves from the situation. To distract myself, I worked in the garden with my family, as our friends had big fields for harvesting. Also, we kept ourselves busy with cleaning and cooking. I also listened to some lectures about first medical aid, just in case. I talked to my friends pretty often; we were all checking on each other's well-being.

An Invitation to Come to the United Kingdom

During our stay in Chernivtsi, my aunt, who is living in the United Kingdom (UK), insisted that we come to her house. She was worried about us. After days of thinking, we agreed to apply for visas for the females of our family since men were not allowed to leave the country. The original plan was that my grandmother, mother, and I would obtain visas in Romania, and then my grandmother and I would travel to my aunt's house by plane while my mother would return to my dad and brother in Chernivtsi. The plan looked good 'on paper', but in reality, it turned out to be a very different story. We completed all the visa application paperwork through the Romanian website. That process was relatively simple since the government of the UK made that easier for Ukrainians. The last step was to schedule a meeting at the visa application centre, which we couldn't do because we were not physically located in Romania. So, we decided to rent an apartment in Bucharest for a few days till we obtained the visa. And just like that, the three of us packed our things and said goodbye to everyone we had lived with for the past few weeks. I was excited about something new because being in one same place was pretty hard. Also, I wanted to help my grandmother and meet my cousin. However, at the same time, I felt like a traitor for leaving the country.

Entering Romania Aided by Countless Kind Volunteers

We planned to go to Bucharest by bus, but most of the bus lines were interrupted, so my dad decided to drive us to the border. We could see a lot of cars there when we got closer to the border. We took our stuff from the car and walked until we reached a line of people standing and waiting to cross the border. Many Ukrainian and Romanian volunteers were offering food, tea, and blankets. I still feel great respect to them because the weather was cold, and the line was too long. The experience of seeing people help others is still hard to grasp for me. I am thinking about how the world can be cruel and kind at the same time. Also, I wish I was the one who helped and not the one who ran away. We stood in that line for eight hours before we crossed the border. It was already night-time, and we were shaking as if we were about to freeze. I felt sorry for the children and older people; the cold was bitter, and the stress was tremendous. Just before crossing, we said goodbye to my dad. Despite our fear and anxiety, we held onto hope that everything would turn out well. My grandmother was not that happy to leave the country; during the trip, she would always say that it would have been better if she stayed with grandad. My mum and I were more positive because we had to think positively to succeed. Additionally, I was interested in discovering something new and challenging myself. Upon crossing the border, it was so lovely to see how volunteers warmly welcomed all Ukrainian people in Romania. Those were mostly men in red jackets; they might have been from the Red Cross or other non-profit volunteer organizations. They were standing near the border, compassionately meeting us and giving instructions on where we should go next. They established tents with food and places to sleep. Also, they provided free buses to the nearest city, so we got one to Suceava, and from that city, we had a free train journey to Bucharest.

Disappointment in Bucharest: a Month-Long Wait for the UK Visa Appointment

When we reached Bucharest, we took a taxi to the apartment we had booked previously and tried to schedule a meeting to apply for the visa. The earliest date we could get was approximately a in a month time. It was impossible to have a meeting earlier because all other terms were already booked by Ukrainians. We booked another night in an apartment and realized staying in Romania for a month would be too expensive. Also, we couldn't apply for refugee status because then we would have to stay in Romania, and we wouldn't be able to reach my aunt. We decided to go to Bulgaria, hoping the visa appointment could be quicker. My mum and I made this decision together; we always respected and helped each other during the journey. We were trying to think logically. We knew there were many places for obtaining visas, and Romania was one of the countries that shared a border with Ukraine, so we assumed that many Ukrainians must be there. Bulgaria is a little further away, so we assumed that the waiting times for visa appointments should have been shorter there.

Difficulties Reaching Bulgaria

So, the next day, we were looking for a bus going to Bulgaria's border. We discovered that the bus fares to Bulgaria were quite high, ranging from 40 to 70 euros, and we couldn't find one that went to Sofia. While looking for a bus, we met another Ukrainian woman who was also trying to get to Bulgaria. She was from Mykolaiv. She had two sons, who must have been in the army. Somebody recommended a job to her in Bulgaria, so she headed there. My mum suggested that the border was not far away and that we could take a taxi to get there. Though the taxi was still quite expensive, we managed to get near the border with it, a few kilometres from it. The next problem we faced was that the taxi driver could not take us over the bridge between the two countries. So, we had to cross the bridge on foot, as it had no pedestrian walkways. It was indeed scary, but we managed to cross the border. As we crossed the bridge and entered Bulgaria, we saw a small Red Cross tent near the border. My mother approached the volunteers and asked if we could find transportation to the nearest city. To our relief, a friendly Ukrainian man offered to

drive us to the train station. Later, we parted ways at the station with the Ukrainian woman as we took the train to Sofia.

Hospitality of Unknown People in Sofia

At the train station in Sofia, we met a very kind woman whom we contacted a few hours earlier through the site *Icanhelp.host*. That is the website where people offer accommodation to refugees from Ukraine. She offered us her apartment, but upon arriving at the apartment, we felt uncomfortable staying there with the owner. The apartment was small and not very clean, and a big dog lived with the owner. I was okay with anything, but this was uncomfortable for my mum and grandmother. The owner went to the work, so we took opportunity to do some cleaning and cooking. When the owner returned from work, we explained the situation but showed gratitude for her willingness to help. She was surprised and slightly disappointed, maybe because she wanted to help and to have someone around, but she understood us.

We thanked her for her kindness and hospitality and for driving us to the city centre. We found a hostel and immediately tried to schedule a meeting at the UK visa application centre. The waiting list was not more than a week, so with relief, we booked the appointment. We visited the Red Cross centre in Sofia, where we got some food and clothes and received information about available help, including free accommodation. We registered on the Red Cross website to request assistance in finding an apartment. The next day, we were contacted by a manager, who offered us an apartment where the only inhabitant was a cat while the owners lived in a different home. That was an excellent opportunity for us. The owners were very friendly, the apartment was cosy and comfortable, and we felt grateful for their generosity. We stayed there for approximately 2 weeks, waiting for our visas' approval and the documents that had to come from another country, where they were prepared – probably from Germany. Although the visa application process can take much time, we were lucky to obtain ours rather quickly

It is hard to understand why the procedure is so challenging, especially in the case of an emergency. The procedure to make visas is complicated in general. But when the war started, the UK was one of the countries that accepted refugees, and the country made the requirements easier to meet. Maybe the lack of knowledge makes me think it is unfair and unwise to create this lengthy process for checking people as if we were some kind of criminals. Some parts of the process seemed meaningless to me, as we had to go to the visa application centre multiple times to provide documents or for fingerprinting. However, we were lucky to have relatives in the UK compared to others who did not. The process of obtaining a visa is much simpler if you have relatives of citizens in the UK, so it must have been even more complicated for those who were not in the category. In general, it felt like we should prove that we deserve the help the British government was providing.

After getting the visa, we bought tickets to London, and the apartment owner was kind enough to drive us to the airport. I miss Bulgaria a little, especially after meeting kind people who helped us during our stay in the country. We felt accepted and supported by its society, and the citizens of Bulgaria showed us nothing but warmth and hospitality.

A Warm Welcome to the United Kingdom

London has always been a dream destination of mine. However, I never imagined that my visit would come under such unforeseen circumstances. My aunt, who is my mother's sister, lives in St Albans, a lovely town close to London. We were delighted to see my aunt, her husband, her daughter, and my other cousin, who had also been living with them for a week, having arrived from Ukraine a little earlier. Over the next week, we all lived together, having a nice time enjoying each other's company and exploring the city. My aunt gave us the best care, and we, in gratitude, helped her around the house.

Struggling to Adjust to the New Communal Living Conditions

Even though everything seemed to be fine on the surface, somehow, my mental state deteriorated. I did not feel in place. I realized that throughout the journey, I focused on helping our family reach our destination, and I hadn't paid much attention to my well-being. But, once we accomplished the goal, I felt uneasiness in my chest. Realizing where I was and what was happening knocked me off my feet. This was a late reaction to the stress. I needed time to process my feelings. I wanted a space for myself because it is usually how I cope with stress, but the problem was in that I rarely had time alone for myself. My aunt's daughter adores me and loves attention, so seeing me was a true joy for her. She wanted to spend as much time as possible with me. While I wanted to be there for her, I felt depressed and wanted to be alone. To make matters worse, her father was very strict with her, and I felt helpless. He also put pressure on me and my male cousin to start working quicker. He controlled the process of applying for jobs and criticized our resumes. Also, he used to discourage my belief and hope that the war would end soon and that I would return home.

One night, I couldn't hold back and burst into tears. I remember I was mailing my friends on my laptop. My grandmother and mother were sitting on the bed and talking and I was sitting on the floor and typing. Then my little cousin came in and talked about something. Maybe she asked if I would like to play or spend time together. I remembered I was stressed about my assignments at the university. But her attention towards me triggered something inside. And I was repetitively typing one key on the keyboard and could not stop. Then tears came down, and I was unable to control them. When my cousin noticed that, she was truly concerned and worried. I was afraid to make someone worry about me. So, when she noticed, I started crying even more. And then the attention of my grandmother and mother was on me as well. And then the whole family came to see why I was crying. I said that I missed my friends, which was part of the truth, but actually, I wanted to escape everything at once.

I couldn't stop myself from crying, and being seen in this state by others, I felt judged. When everyone was looking at me, I wanted to vanish. I did not want to turn myself into an additional 'problem'. I did not want to be seen in this state by my small cousin's father, the 'boss' of the house. When he saw me, he told me not to cry, that everything would be fine, and that I should come down because he made the apple cake and that I should try it. On one hand, he showed concern and wanted to improve the way I felt, but on the other, I did not want to talk to anyone or eat cake. I think I asked him if I could stay in the room, and he declined. I could feel the tension in the room when we were all eating the apple pie. I felt like my grandmother and the owner of the house saw me as weak or the one who wanted attention. My mum was the biggest support I received when I was in this 'down' time. She tried to comfort me, and I felt accepted. She was the biggest and most important figure for me at that point.

The Need to Escape

After that, I realized I needed to distance myself from everyone and find another place to move to and heal. I spoke to my mom, and she agreed. She knew being in that house with everyone was hard for me; she supported my intention to find my own place. We were considering different options but in the UK, the only option for accommodation was living with another family and I wanted to be by myself. Moving to another country seemed like a better option. I wanted to act right away and that was when I remembered that one of my friends was living in Ireland, having also fled the war the day it began. I contacted my friend to learn more about her experience of living in Ireland and what kind of help was available to refugees. She reassured me that there was an option for me to stay there as well. There was a great help established in Ireland for refugees; a place to stay was provided for everyone. She explained that after arriving at the airport, volunteers would assist with finding

accommodation and other necessities. I thought it was a great opportunity, and I gave my mother good arguments as to why Ireland was a good option for me. Having a friend in Ireland made me feel supported. Also, it was close enough to the UK that my relatives would not feel like I was too far away, and if I failed, I could always return back to the UK. My mother was supportive; I don't remember her being judgmental or overanalysing the situation. That very day I decided to purchase a plane ticket to Dublin, and that's how I ended up traveling alone. I did not have any detailed plan about what would happen in Ireland, but I wasn't afraid. I was just eager to get away from everything.

Joining Many Ukrainian Refugees in Ireland

Upon arriving at the airport in Dublin, I was met by a group of volunteers, as expected. What caught me by surprise was seeing a lot of Ukrainians, as I thought Ireland was too hard to reach from Ukraine. Together, we were taken to a centre, where we registered for a green card, which would allow us to stay and work in Ireland. After that, we were instructed to get onto the bus, as we were heading to another centre, where the volunteers would offer us accommodation. When I entered the large hall of the centre, I was amazed to see so many Ukrainians were sitting and waiting there. Volunteers organized people into small groups that would later be sent to different places around the country where refugees could live. They provided food and even toys to keep the children occupied while we waited. I sat for a few hours until it was my turn, and I was placed in the group that was going to the city called Kilkenny. A bus was arranged for us, and we headed towards our new home.

New Temporary Homes: A Scout Centre and a Convent in Kilkenny

Accommodation is one of the biggest issues in Ireland. Even before the war, the country couldn't provide enough housing for its citizens, and the prices were high. With the addition of refugees, the problem has only grown larger. As a result, our temporary home for the next week was a scout centre. Our group in the scout centre consisted of 40 Ukrainians, and we had a few volunteers with us who provided us with incredible care and support. They transformed the scout centre into a cosy home by setting out the beds. We were all sleeping in one room, which did not have real beds but numerous folding stretchers (Figure 1).

Figure 1. A temporary sleeping place for Ukrainian refugees in the scout centre in Ireland.

But it was not difficult for me to sleep in such beds, with all these people in one space. I think all my concerns were about how to live from now on. It felt good that no one knew me, and I could be alone for some time. And it was comfortable sleeping in that folding bed. When I lived in Ukraine, I was used to going to the mountains often and sleeping in camps, so I was used to such sleeping conditions. The volunteers also provided us with essential items like food, hygiene products, toys, and clothing. We were amazed that they even obtained washing machines.

This was the first time that I had lived separately from my family. I was actually feeling OK because I found it comforting and warm to be by myself. I felt as if I was my own boss, and it felt good. I met a lot of people, and with one woman, I got pretty close. So, in some sense, I gained a new family—this group of people who were living with me. The volunteers supported us every day and gave us any possible information they could. They worked so hard; I could see their tired faces. During our first week living in the scout centre, the volunteers wasted no time and were preparing a new house for us. They were renovating a convent, furnishing it with new furniture, and creating a kitchen and bathrooms for our use. After it was ready, we all moved to the convent. There, I shared a room with a girl that was a bit older than me, but it was nice. And for the next month, we were peacefully living in this sacred place.

Ireland Treated Refugees Very Kindly

In Ireland, we felt incredibly supported. We received financial assistance of approximately 200 Euros per week. The government provided free medical care, English language courses, education for the children, and even help finding employment or certification training. Sometimes, the locals would even hold a concert for us, and the profit would be donated to Ukraine. They also set up a shop specifically for us, where the residents donated clothes, and we could take them for free.

Ireland is a country that treated us very kindly, the citizens were very generous and supportive. They would give us everything we needed without a second thought to ensure we felt great.

During our stay at the convent, I helped volunteers and Ukrainians by translating for them, as language was a barrier for many people. Another challenge we faced was getting a job, as it required having a credit card, which took a month to obtain. However, these were minor obstacles compared to the enormous support we received. At the end of the month, living in the convent, half of our group had to move again due to the constant flow of refugees coming to Ireland. We had to free our living places for them. So, our next stop was a cosy hotel nearby, where everyone got their own room in brilliant condition. I did not have to share that room with anyone.

Worrying about My Future and My Studies

This hotel became our new home for the next month. Our little group felt like family to me because we were always talking to each other and seeing each other. We would go on excursions or to classes together. Those people were from different parts of Ukraine, mostly women, but there were also some grandparents and people from occupied territories. I am still in contact with one woman with whom I spent most of my time in Ireland. Being in my own space allowed me the opportunity to focus on myself. I searched for different opportunities to study in Ireland and received a helping hand in this process. But the more time I spent 'alone', although I still socialized with everyone, the more anxiety took over me. I became too worried about my future – I wasn't sure what to do with my studies. Back in Ukraine, I studied psychology, having finished two years of the undergraduate program. So, I was thinking about continuing my studies in Ireland. Additionally, there was an opportunity to study music professionally, as I love singing. But most universities or colleges were in other cities, and I was concerned that I would have to pay for accommodation if I moved. But I didn't have an income, nor did I have a job. My mind was going 'crazy'; the more I thought, the more I felt lost. I am sure everyone

would help me find the best option; nevertheless, I was not thinking critically and was afraid to face the future alone. From one point, I wanted to be alone, but from the other, I needed someone to help me. Maybe I was afraid to start something new that was far away from my home and family.

That's when my rescue came out of nowhere. At the end of that month, my dad gave me some great news – he had received permission to travel outside of Ukraine due to his disability status. He proposed that our entire family could relocate to another country, such as Croatia. My dad had always dreamed of living near the sea, and we had experience living in Croatia as we previously travelled there on our summer vacations. I saw this as a great opportunity and a way to escape my worries and problems. However, I did not fly directly to Croatia. We decided to first get together at our home in Ukraine. Also, I wanted to see my grandfather. I kind of missed my family. It is so interesting how, on one side, I was eager to be an adult and live life independently, but I was so afraid to do that, so I needed someone to be there for me. The following week, I bought a plane ticket to Warsaw. From there, I took a bus to Kyiv, finally returning to Ukraine.

Brief Return to Ukraine

By the time I returned to Ukraine, it had already been six months since we left the country. The atmosphere of the country had rapidly changed from the last time I was at home. It seemed like everyone felt 'dead' inside, and everything looked dull and empty. Almost all of our family was together when I returned home, except my grandmother. My mum arrived in Ukraine earlier than me because my father missed her and needed her. At the same time, my aunt in the UK needed help from my grandmother, so she stayed in the UK for another month after my mum left. But, when we all gathered back home in Boryspil, my grandmother was also on her way home as well – she missed her family too. Our whole family was finally reunited. It was a wonderful feeling just being around your loved ones. But after some time, it was time for us to move forward. Our grandmother and grandfather wanted to stay at home because it was much safer now than before, while my dad, mum, brother, and I were getting ready for a trip to another country to find a way to continue with our lives until the war ended. We got into the car and headed to Croatia.

New Beginning in Croatia

Leaving Ukraine this time, the trip was a lot easier. It was another long trip, as we had to drive for about 1500 km from Boryspil to Zagreb, but we arrived in Croatia with no troubles. When we reached Zagreb, we went to the Red Cross centre to ask for information and any possible help that we could get. They informed us that Croatia had three reception centres outside of Zagreb, where we could ask for accommodation as refugees. However, each of the centres was located away from the city of Zagreb – in the cities of Gospić, Osijek, and Varaždin. We decided to try finding an apartment ourselves. My parents preferred a place near the sea and a big city where my brother and I could continue our studies. We first tried Rijeka, a nice city near the seaside. We drove 170 km to get there from Zagreb. We entered the Red Cross centre in Rijeka to ask for help finding cheap accommodation. We were told that there was no such possibility because everything was too expensive, especially during the summer months. We didn't expect this and considered returning to Zagreb, thinking it would be easier to find accommodation there. However, we remembered that we were told in Zagreb that one of the reception centres that had information about all possible accommodations in the country was in Osijek, so we headed there. Osijek is 450 km away from Rijeka.

The people in the refugee centre in Osijek were very helpful and offered us various apartment options: a house in Plitvice Lakes, an apartment somewhere close to Osijek, and in Zagreb. Eventually, we chose a place in Zagreb, as it is a big city, the capital of Croatia, with about one million inhabitants. We were supposed to live on the first floor of a big house with another family

in Zagreb, and the owners would receive 400 Eur per month from the Croatian state for hosting our family. That's how we returned to Zagreb and met our new family and home. The family in Zagreb that owned our apartment welcomed us with open arms and were eager to help us adapt to the country and obtain the necessary documents, including residence permits and identity cards. The house was beautiful, with a big garden, and we could stay there for a year. As we now had a place to stay, it was time to look for different opportunities in Zagreb. We were happy to discover a free course for studying the Croatian language for Ukrainian refugees. There we met very nice people. One woman, a kind-hearted Croatian volunteer, helped us with everything she could. I feel great admiration for her kindness.

Seeking a University Psychology Program Where I Could Continue My Studies

After the family was settled, we also wanted to find a place where I could continue my studies. With my background in psychology, we focused our search in that direction. There was a psychology program in Zagreb where I could enrol in the third year, as I finished two years before. But the biggest obstacle was that it was taught in Croatian, and my Croatian was still not good enough to follow lectures. Luckily, a psychology program was offered in English at the Faculty of Humanities and Social Sciences in Split, at the University of Split. However, that program required a payment of 6000 Euros of tuition fee per study year, which was a big amount of money for us. We decided to try our luck and come to Split in person to ask for assistance. We thought it would be easier to explain the whole situation in person.

When we arrived to the Faculty of Humanities and Social Sciences in Split, we asked for the information we needed. A young lady from the administration approached us and explained how we could apply for the program, mentioning that we might have a chance to study for free, but we had to file a written request with supporting documents. My family was glad to hear that, and after we returned to Zagreb, we completed all the requirements. Namely, we sent the documents with my previous university grades, my exam grades after finishing school, and a motivational letter. We also enquired if it would be possible for me to study there free of charge. Then we waited anxiously for a decision to come. While waiting, we met an amazing Croatian man who had connections with different universities around Croatia. A volunteer that we met in Zagreb recommended him to me. He was involved with student affairs, so he was in contact with many professors, and was in a situation to help with my case at the University of Split.

My New Life in Split, Croatia

Luckily, my enrolment was approved, and my family and I were filled with gratitude toward the faculty members who gave me this incredible opportunity. Soon I was going with all my belongings to the Split by train, excited for the new chapter in my student life to begin. Thanks to the Croatian man we met earlier, I was offered a room in a student dormitory on campus, a comfortable and affordable option that cost 100 Eur per month at the time. And here I am today, in Split, Croatia, grateful for every support and help that brought me here.

For the first year of my studies in Split, I was living alone in my room. At first, living by myself in Split was scary; it was hard and confusing again. But with time, I gained more confidence in myself, I learned how to live by myself, and I always had support from my friends. Sometimes, I used to spend weekends with my family in Zagreb. This felt good because I was living alone, meaning dealing with my problems and taking responsibility for myself. At the same time, my parents were not that far away, and that was very comforting for me. Also, I was afraid of how my classmates and professors would react to my presence. But to my surprise, they were very welcoming and gave me enormous support. I felt accepted, and I made a lot of nice Croatian friends with whom I hang out from time to time.

I also regularly talk to my friends from Ukraine, and I participate in online sessions with a Ukrainian psychologist to support my mental health. During the first year, I was also working to pay some of my bills, but this year I decided to focus more on my studies. Studying also helped me to be distracted from what was happening in the world, and my studies were very interesting. But the biggest struggle that I would encounter from time to time is when I would think about the situation in my country and the guilt I felt for doing nothing. I miss my home very much and the old days of being with my family and living at home. But I hope these old days will return soon. So, I hope I will go back home when I finish my studies in Split in a year time. During my studies, I was also invited to participate in the Giving Voice project. When I got to know the project's ideas and developments, it melted my heart. The people were sincerely interested in the situation in Ukraine and were willing to help Ukrainians in the way they could. Mainly, project was focused on helping Ukrainians share their experience through writing. I liked the ideas that the project held, and wanted to contribute to it, with the hope that I could help my country as well. So now, I try to assist in different tasks within my abilities, such as helping with translation. And I hope the Giving Voice project will continue to grow, and so much more people will get an opportunity to be heard, as I could.

I Still Hope to Return to Ukraine Soon

My mum and dad currently live in a new apartment they rent in Zagreb. My family and I feel very accepted by Croatian people, and we are grateful for all the help and support they have given us.

Throughout our journey, fleeing the war in Ukraine, my family and I have travelled a lot and encountered different challenges, both good and bad. We have met many wonderful people, and I can say that during this time, I have seen the world from its best viewpoint. Because people from all around the world were and still are eager to help us, no matter how difficult the situation is. We know that 'the world' is on our side, and I am incredibly grateful for all the support for Ukraine. I feel lucky that my family and friends are alive and that I have been given such great opportunities. However, I also feel guilty for leaving my country and not defending it. That's a burden that I will carry with me, but I hope that in the future, I will have the opportunity to thank the world back and especially help my country.

That's my story, one of the thousands, millions of other stories, with each one of them being unique and valuable. I hope that every person who carries a big story feels safe and loved right now and that they will be given the opportunity to present their stories to the world, as well. At first, when I started writing this story, it was very hard. I did not want to bring up all those memories and just wished that everything we overcame would disappear in my mind. While writing, I cried sometimes. But I think that is a part of the therapeutic process, in which you have to face the pain of the past and live through it. So, I would say that revisiting what happened to me along this journey helped me to be a little bit more at peace with the past. In the end, I wish to thank the readers of this story for their attention, for being with me throughout this story and for going through all of the struggles that my family and I faced.

Thematic Analysis by the Co-authors

The narrator describes a journey of loss, displacement, and adaptation following the war in Ukraine, starting with the ordinary life of a university student disrupted by conflict. Thematic analysis reveals key themes, including anticipation, surprise, and unpreparedness for the war. Family dynamics shift under stress, with difficult emotions during displacement and varied coping mechanisms. Gratitude emerges for the volunteers encountered during migration, despite bureaucratic challenges. The narrator navigates internal and external conflicts, resolving them pragmatically, and adapts to new environments with hope for returning home. The healing power of sharing personal narratives is also emphasized. A thematic map (Figure 2) summarizes the themes.

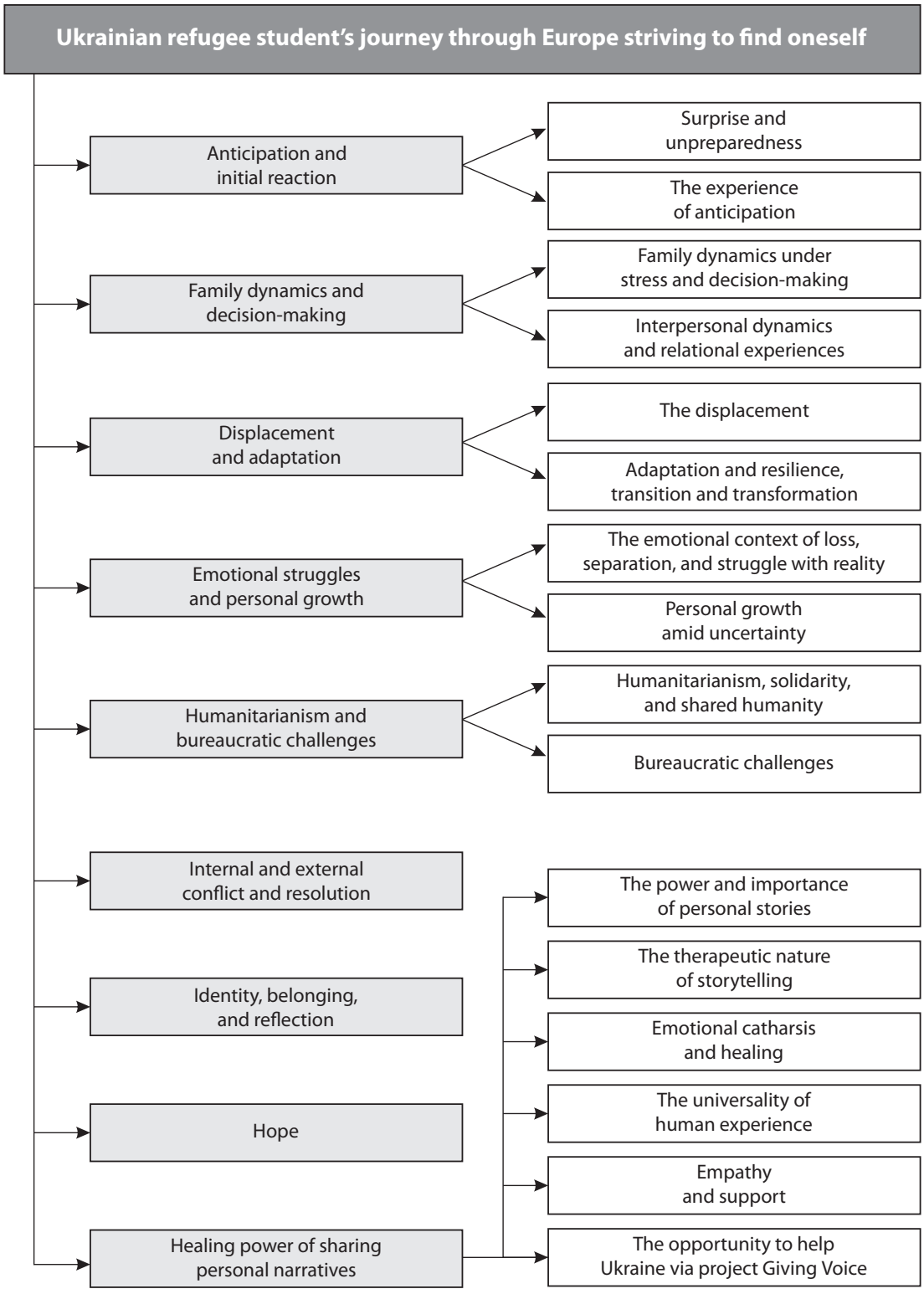

Figure 2. A thematic map of themes identified by co-authors in the narrator's story.

Themes

Anticipation and Initial Reaction

The Experience of Anticipation

The narrator describes feeling a sense of foreboding before the war, influenced by discussions and subtle indicators that something was amiss. Anticipation and uncertainty grew as the war drew closer, contrasting the normalcy of daily life.

Surprise and Unpreparedness

The narrator initially reacts to the war with disbelief and unpreparedness. Despite warnings and family discussions, the reality of war feels distant. This is contrasted by the narrator's focus on daily concerns like homework and a crush, alongside a persistent sense of unease the night before the war began.

Family Dynamics and Decision-making

Family Dynamics under Stress and Decision-making

Family plays a central role in the narrator's story. The shared household and the differing reactions and decisions among family members during the initial chaos of the war showcase the complexities of family dynamics under stress. The father's proactive approach, the mother's agreement, and the grandfather's refusal to leave the family home highlight diverse perspectives and priorities within the family.

Interpersonal Dynamics and Relational Experiences

Interpersonal dynamics within the family unit are highlighted, depicting a mix of support, conflict, and the resilience of familial bonds amidst crisis. The interactions reveal varied coping mechanisms and decision-making processes shaping the journey.

Displacement and Adaptation

The Displacement

A theme of displacement emerges as the narrator and the family make the difficult decision to leave their home in search of safety. Confusion, conflicting opinions, and a sense of urgency contribute to the turmoil experienced during the departure.

Adaptation and Resilience, Transition and Transformation

The narrative emphasizes the recurring theme of adaptation to new and challenging circumstances. It transitions from immediate displacement to a process of seeking stability in a new environment, highlighting adaptive strategies such as seeking external support, engaging in productive activities, and demonstrating resilience. Despite bureaucratic obstacles and emotional challenges encountered while navigating multiple countries, the narrator and her family ultimately achieve temporary stability, illustrating their persistent resilience in the face of uncertainty.

Emotional Struggles and Personal Growth

The Emotional Context of Loss, Separation, and Struggle with Reality

The author's emotional struggles intensify as she departs from her country and family, experiencing a range of emotions, including loss, fear, sadness, stress, and guilt. These emotions are driven not only by the immediate threat but also by broader uncertainties and a sense of helplessness. The journey to Chernivtsi, followed by relocation to the UK and Ireland, is characterized by emotional fluctuations, with significant difficulty in adjusting to the harsh realities of war and the abrupt transition from student life to seeking safety.

Personal Growth Amid Uncertainty

The narrator's journey reflects personal growth, marked by a quest for stability and a future in a

new country. Initially anxious about the future and studies, the narrator gradually develops resilience and confidence, adapting to new systems and living independently. The narrative reveals growing self-awareness, balancing independence with the need for support, and a sense of gratitude, guilt, and duty toward the homeland.

Humanitarianism and Bureaucratic Challenges

Humanitarianism, Solidarity and Shared Humanity

The narrative highlights shared human experiences, emphasizing empathy, mutual support, and resilience during crises. The narrator encounters various forms of assistance from family, volunteers, and locals, underscoring the significance of collective humanity. Support from volunteers in Romania, Bulgaria, Ireland, and Croatia, along with local and governmental assistance, provided essential resources, including housing, food, and aid in university applications. This reflects the critical role of human kindness and solidarity in fostering hope and stability in times of need.

Bureaucratic Challenges

The contrasting experiences of humanitarian aid and bureaucratic challenges are evident throughout the narrative. While volunteers and ordinary citizens provide much-needed support, the bureaucratic processes for moving across borders and obtaining visas add layers of difficulty to an already challenging situation. For example, the narrator and her family had to wait for 8 hours outside in the bitter cold to cross the Ukrainian-Romanian border. Once they arrived in Romania, they found out they would need to wait a month for the appointment to apply for a visa to enter the UK. In Bulgaria, the visa application process involved multiple visits to the visa application centre.

Internal and External Conflict and Resolution

The internal and external conflicts faced by the narrator are central to the narrative. These include the disagreement over whether to leave their grandfather behind, the struggle to find a stable living situation, and the personal conflict of feeling like a traitor for leaving Ukraine. The resolution often comes through pragmatic decisions and support from others.

Identity, Belonging, and Reflection

The narrator's personal and societal identity undergoes shifts, influenced by acts of kindness, cultural encounters, and the navigation of a complex internal landscape. The theme of belonging surfaces as the narrator struggles to adjust to life in the UK and Ireland. The feeling of being out of place and the desire to find personal space to process their emotions are indicative of the deeper search for identity and belonging in a new and foreign environment. The journey reflects a process of self-discovery and re-evaluation of values.

Hope

Despite the ongoing war and displacement, there is a persistent thread of hope. The narrator's belief in the grandfather's safety, the hopeful plans to reach the UK and Ireland, and the eventual support received in Ireland and Croatia reflect a desire for a better future amidst the uncertainty. The narrator's hope is tempered by the recognition of the unpredictable nature of the situation.

Healing Power of Sharing Personal Narratives

The Power and Importance of Personal Stories

The narrator emphasizes the significance of individual stories by asserting that each story is "*unique and valuable*." This theme underscores the idea that every person's experiences, no matter

how common they may seem, hold intrinsic worth and contribute to the rich tapestry of human existence. The narrator's hope that "*every person who carries a big story feels safe and loved*" and is given the opportunity to share their story reflects a deep respect for personal narratives and an understanding of their potential impact.

The Therapeutic Nature of Storytelling

Writing the story is portrayed as a therapeutic process. The narrator describes the difficulty of revisiting painful memories and the emotional release experienced through crying. This indicates that confronting and articulating past traumas can be crucial to healing. The act of writing serves as a means of processing and coming to terms with the past, suggesting that storytelling is not just a means of communication but also a form of self-therapy.

Emotional Catharsis and Healing

The text details the emotional journey of the narrator, who initially struggled with writing the story due to the painful memories it evoked. The process of facing and expressing these emotions is portrayed as a necessary step towards achieving "a little bit more at peace with the past." This theme highlights the concept of emotional catharsis—individuals can achieve a sense of relief and healing by reliving and expressing past traumas.

Universality of Human Experience

While the narrator's story is personal and unique, the mention of "*thousands, millions of other stories*" points to the universality of human experiences. This theme suggests that despite the uniqueness of each individual's journey, a shared human experience connects all people. This collective experience underscores the importance of empathy and understanding, as everyone carries their own set of stories and struggles.

Empathy and Support

The narrator's hope that others with significant stories "feel safe and loved" and have opportunities to share their narratives reflects a deep sense of empathy and support. This theme speaks to the need for a supportive community where individuals feel valued and safe to express their experiences. It underscores the importance of creating environments that foster emotional security and validation.

The Opportunity to Help Ukraine Via Project Giving Voice

The narrator seized the opportunity to help Ukraine by participating in the Giving Voice project, which helps authors publish research articles about the consequences of war in Ukraine. As evidenced by the phrase "it melted my heart," the narrator had a strong emotional reaction to the project's goals and initiatives. The enthusiasm for contributing to the project stems from a genuine connection to its mission, revealing a deep personal resonance with the project's objectives. Participation in the project allows the narrator to help Ukraine, compensating for her earlier feelings that she wished she was the one who helped. The project's intent to help Ukrainians share their experiences highlights an effort to give a voice to those affected by the situation in Ukraine, reinforcing empathy and support.

Discussion

The first author's narrative describes emotional experiences, family dynamics, community support, and the struggle to adapt amidst the ongoing war in Ukraine. The current phase highlights stability at the University of Split, Croatia, where the narrator received free tuition and affordable accommodation. Gratitude is expressed for the support from various individuals and systems. Despite the emotional challenges, the narrator remains hopeful for the war's end and a return to Ukraine. After the beginning of the war, the narrator's parents acted swiftly, deciding to leave their home immediately

to seek safety. They joined millions of people who fled Ukraine in the first two months of the war, which became the fastest exodus globally since World War II (22). The Ukrainian refugee crisis was much larger in scale compared to almost 2.5 million refugees that entered Europe from Syria, Iraq, and Afghanistan in 2015–2016 (23). Those who fled the devastation of Ukraine joined the estimated more than 100 million forcibly displaced individuals worldwide (23).

Lives that before the war had seemed so ordinary and predictable were turned into uncertainty of whether refugees could return to their home country or they would need to live their life in exile in one or multiple countries. The decision-making of refugees will be guided by whether and where they have families or friends, the language, cultural, and financial resources available to them, and the humanitarianism and hospitality of strangers on whom they may need to depend (22). The narrator in this story was helped by both friends and relatives and many generous strangers. After leaving their house in Boryspil, the narrator and her family were first hosted by friends in Western Ukraine. Then they left to join family in the UK. However, staying with a family in a foreign country was not without the challenges, as various expectations were imposed on the refugee relatives. In a manuscript exploring refugee flows from Ukraine, Albrecht and Panchenko observed that there is a high chance that most refugees who initially live with relatives, friends, or another private accommodation will soon face the issue of finding long-term accommodation (24).

The narrator was not content with only having her basic needs met. She also wanted to be independent and to continue her university studies in a psychology program. In 2017, there were 1.67 million higher education students in Ukraine (25). Many of those students experienced being displaced within Ukraine or fled to other countries. Ukraine is a member of the Bologna process, and the EU and UK have enabled Ukrainian nationals the right to study. Some countries committed to financially supporting Ukrainian students (24). The opening of the EU and UK borders and recognizing the importance of enabling refugees to access higher education was unprecedented and demonstrated what can become possible when there is the political will and public support (24). The narrator movingly described many psychological challenges she faced and various feelings she experienced along her journey. The ongoing war and the uncertainty of the situation of refugees may cause anxiety and stress, exacerbated by the constant information about war events. Thus, it is important to cater to the mental health and well-being of refugees, but also the host population helping them (26).

It is important to emphasize that, in the narrative, there is a notable lack of negative feelings about Russia or Russians. The narrator is positive, hopeful and optimistic, focused on herself, her personal growth, her studies and her family. It has been shown that positive expectancies, including hope, self-efficacy, and optimism, predict post-trauma resilience (27). The narrator indicated that sharing this story had a healing power. While it brought some sad memories, the writing of the story is described as therapeutic and helping the narrator make peace with the past. Multiple studies have explored expressive writing and storytelling as interventions that may help individuals cope with different types of trauma. For example, a systematic review published in 2021, which included 44 randomized controlled trials (RCTs) concluded that expressive writing may contribute to improving symptoms of post-traumatic stress disorder (PTSD) in medium to long-term (28).

In 2023, Begoteraj et al. published the results of a study that explored the effectiveness of expressive writing on the psychological distress and traumatic symptoms of migrants (29). The results of the study indicated that trauma-focused expressive writing led to an immediate improvement in phobic anxiety and positive total symptoms and improvement in somatization, global severity index, and hope for the future in migrants (29). Stickley et al. described the effect of a professionally-led creative writing program on refugees and people seeking asylum (30). The results indicated that the creative writing program helped improve educational and well-being outcomes for 144

participants. The program also helped the attendees to improve their English language; satisfaction of the attendees was extremely high, and they reported increased confidence and an increased sense of hope (30). While the primary aim of this manuscript was to document the consequences of war in Ukraine through the project Giving Voice (20), it is also immensely satisfying and practically relevant to see that sharing her story also helped the narrator to further build her resilience.

Strengths and Limitations

The main strength of an autoethnography is that of the researcher's voice (31). An autoethnography allows the narrator to eliminate the risk of misrepresentation and gives the narrator complete control over the narrative being told (31). Two co-authors collaborated with the narrator in writing this autoethnography, providing prompts and conducting thematic analysis to produce rich descriptions of the narrator's experience.

Since autoethnography is focused on the experiences of one individual, the obvious limitation is that the results may not readily be generalized to other individuals with similar life journeys.

Conclusion

The narrative provides a detailed account of the narrator's emotional and physical journey from the onset of the war in Ukraine to seeking refuge in multiple countries, finally setting in Croatia and finding a university program to continue her studies. Thematic analysis reveals a rich tapestry of emotions, family dynamics, community support, and the struggle to adapt and find hope amidst chaos. The story ultimately portrays resilience in the face of adversity. The protagonist and their family navigate numerous challenges yet remain hopeful and proactive in seeking a better future. The narrative emphasizes the importance of education, familial support, and personal determination in overcoming the obstacles posed by displacement. The narrative underscores the resilience of individuals and the crucial role of compassion and support in times of crisis. This resilience is not just about surviving but finding ways to thrive and contribute meaningfully despite the uncertainties and disruptions caused by the war. The narrator portrays storytelling as a powerful tool for personal healing and emphasizes the collective value of individual narratives in fostering a deeper understanding and connection among people.

What Is Already Known on This Topic:
The war in Ukraine, which began in February 2022, has resulted in a significant refugee crisis, with millions of Ukrainians fleeing to neighbouring countries. Research has shown that war refugees face numerous psychological and emotional challenges, including high levels of anxiety, depression, and post-traumatic stress disorder (PTSD). These difficulties are compounded by struggles with adaptation in host countries, such as language barriers, disrupted education, and uncertainty about the future. Ukrainian refugees, particularly students, report feeling isolated due to language differences, which hinder their ability to integrate into academic and social settings. While host countries have provided support, including Temporary Protection statuses, educational opportunities, and mental health services, refugees must still cope with trauma, identity changes, and social exclusion. Despite these challenges, many refugees have also experienced kindness and support from host communities, helping them navigate the difficulties of displacement and war trauma.

What This Study Adds:
This study adds to the literature by providing a detailed narrative of a Ukrainian refugee's emotional and physical journey through multiple countries, highlighting the complex interplay between emotions, family dynamics, and community support during war-induced displacement. It emphasizes the resilience and proactive efforts of the protagonist and her family in seeking a better future, despite significant challenges. The findings underscore storytelling as a therapeutic tool for personal healing and illustrate the power of individual narratives in fostering empathy and understanding. This study enhances understanding of how war refugees navigate the psychological, social, and cultural obstacles during resettlement.

Ethics Approval and Consent to Participate: The manuscript contains the narrative of the study's first author (MS). Two co-authors (LP and DH) helped the first author write the narrative and participated in the thematic analysis. The author of the narrative (MS) consented to write her story. Two co-authors (LP and DH) consented to participate in the study. Since the narrator is also an author of this manuscript and an adult (and no other human participants were involved in the study), we consulted with the institutional ethics committee, which advised that this manuscript does not need to be submitted for evaluation.

Availability of Data and Material: The narrative of the first author, which was provided in the manuscript, is the only data collected for the purpose of this study.

Acknowledgments: We would like to thank Matko Marušić and Damir Sapunar for the critical reading of the manuscript. This work is part of the Translational Research in Biomedicine (TRIBE) doctoral program project "Giving voice", which helps authors publish reports about the consequences of the War in Ukraine.

Authors' Contributions: Draft of her story: MS; Revision of the story: DH and LP; Drafted the initial version of the manuscript: MS and LP; Participated in conceptualizing the study, analysing and interpreting the data, revising, editing, and finalizing the manuscript: MS, DH and LP; Read and approved the final manuscript: MS, DH and LP.

Funding: This work was supported by the TRIBE postgraduate program at the University of Split School of Medicine, Split, Croatia. The support included voluntary time invested in preparation of the manuscript, but no direct funding.

Conflict of Interest: The authors declare that they have no conflict of interest.

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ISSN 1840-1848