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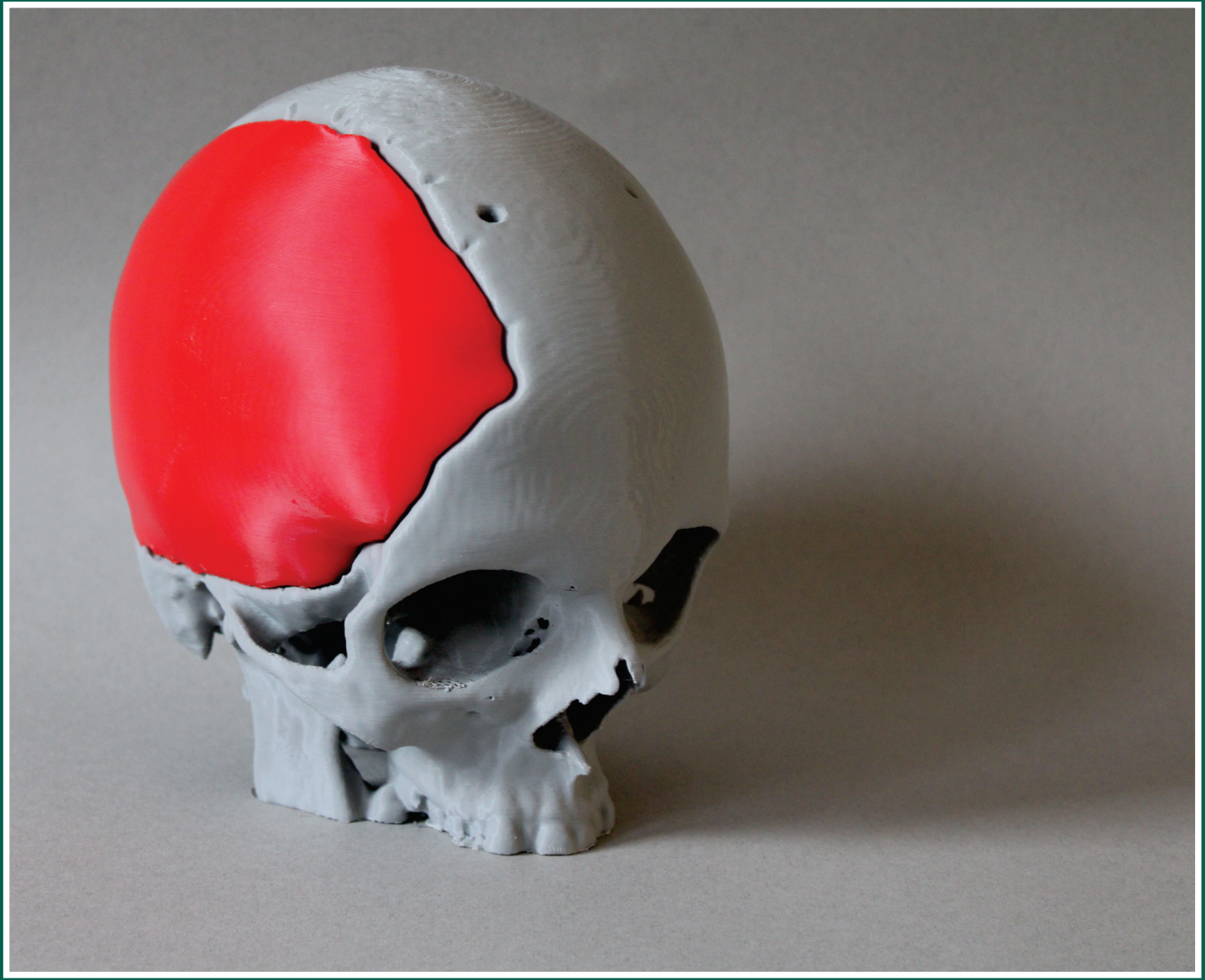

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EDITORIAL

Metabolic Associated Steatotic Liver Disease (MASLD) – Be Aware and Beware

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Abstract

Metabolic associated steatotic liver disease (MASLD) is now the most common liver and metabolic disease with rapidly rising prevalence, being among most common causes of liver transplantation, associated with liver mortality, but even more so and earlier in the course of the disease it is underappreciated independent risk factor for cardiovascular (CV) and all-cause mortality. A large body of clinical evidence suggests that MASLD is a multisystem disease whose adverse effects extend far beyond and before the liver gets seriously affected. It has a complex, independent and bidirectional relationship to all MetS components, chronic kidney disease, and CVD, being causal in one and consequential in another patient and that speaks in favor of including liver health assessment in conventional screening of this at-risk population.

Therefore, authors of this editorial call for raising awareness about this condition, write about new nomenclature that better explains what this condition is rather than what it is not, explain how novel simplified positive diagnostic criteria facilitate timely diagnosis and treatment, and offer simple algorithm for evaluation and treatment of liver steatosis in at-risk patients for non hepatologists.

Keywords: liver, metabolic syndrome, cardiometabolic risk factors.

"The *good physician treats* the *disease*,
the *great physician treats* the *patient* who has the *disease*."
Sir William Osler

MASLD – UNDERAPPRECIATED INDEPENDENT CARDIOVASCULAR-KIDNEY-METABOLIC RISK FACTOR

Metabolic associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is the most prevalent liver and metabolic disease affecting more than a quarter of global population and outnumbering diabetes mellitus and obesity together (1, 2).

Epidemiology of MASLD

MASLD prevalence according to the most recent data is 38%, with 50% increase since the previous analysis (3) and with expected growth. Recent prevalence of MASLD in the overweight and obese population is 70% and 75%, respectively (4), while among patients with type 2 diabetes (T2DM) it is 68.8%, being the highest in Eastern Europe

(80.6%). One should not forget that there is also a lean MASLD (5, 6).

MASLD and Cardiovascular-Kidney-Metabolic Factors – "Chicken-and-Egg" Situation

It is known that human liver has metabolic, nutrient storage and detoxification activities, but also complex immunological activities, all essential to maintain tissue and organ homeostasis that once disrupted lead to dysregulation that is a driver of pathology associated with chronic inflammation (7).

If not recognized or adequately treated, MASLD goes through pathological spectrum of hepatic changes from simple steatosis, steatohepatitis (MASH), advanced fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). This happens in parallel with development of insulin resistance, dysglycemia, atherogenic dyslipidemia, systemic low-grade inflammation, oxidative stress, hypercoagulability, expanded and dysfunctional adipose tissue with visceral adipose tissue deposition, gut dysbiosis, increased activity of renin-angiotensin-aldosterone system, (1), sympathetic nervous system (SNS), dysregulated function of nitric oxide synthase, that are common pathophysiological mechanisms for development of hepatic and extra-hepatic complications. And vice versa – all these pathologic processes contribute to further hepatic injury and fibrosis (8,9).

NAFLD has traditionally been considered a simple liver manifestation of metabolic syndrome (MetS). Today we know that NAFLD, renamed into MASLD, has complex and, more importantly – bidirectional relationship to all MetS components, especially T2DM, hypertension (HTN), chronic kidney disease (CKD), and cardiovascular disease (CVD), independent of other risk-factors (1, 10, 11).

It is important to understand that in constellation of different cardio-kidney-metabolic conditions, MASLD might be causal in one patient, while consequential in the other. It is therefore considered a multisystem disease whose adverse effects extend far beyond and before the liver gets seriously affected.

Complications of MASLD

A large body of clinical evidence suggests that MASLD is not at all a benign disease and is associated with liver mortality, being the most common cause of liver transplantation in the United Sates (US) among those who get listed for HCC, but even more so and earlier in the course of the disease – with the CV and cancer, especially gastrointestinal, and all-cause mortality (12, 11).

Mortality increases exponentially as the fibrosis stage increases, but it is noteworthy that all-cause mortality, unlike liver related mortality, is increasing in MASLD even before stage 1 fibrosis develops.

Due to a plethora of evidence, ESC 2021 put in their guidelines (13) that NAFLD is associated with increased risk of myocardial infarction and stroke, and in 2022 AHA also announced in their scientific statement (14) that NAFLD is a risk factor for development of ASCVD, which is the main cause of death in patients with NAFLD.

It was that same AHA statement where CKD, a previously overlooked but clinically and prognostically important feature of NAFLD and their bidirectional relationship, was also elaborated, with evidence already there and gathered through systematic review and meta-analysis (15). Updated meta-analysis (16) followed, indicating that NAFLD is significantly associated with a ~1.45-fold increased long-term risk of incident CKD stage ≥3. It was proved that MAFLD predicts CKD better than FL (fatty liver) or NAFLD (17) and that the risk increases with steatosis severity (18). Causative relationship between NASH and CKD was proved in a murine model (19) that revealed potential pathogenic mechanisms, histological changes that resembled human, together with regression of kidney damage (proteinuria, kidney dysfunction, and fibrosis) following the orthoptic liver transplantation.

It was also in 2022 that the first study investigating bidirectional and independent

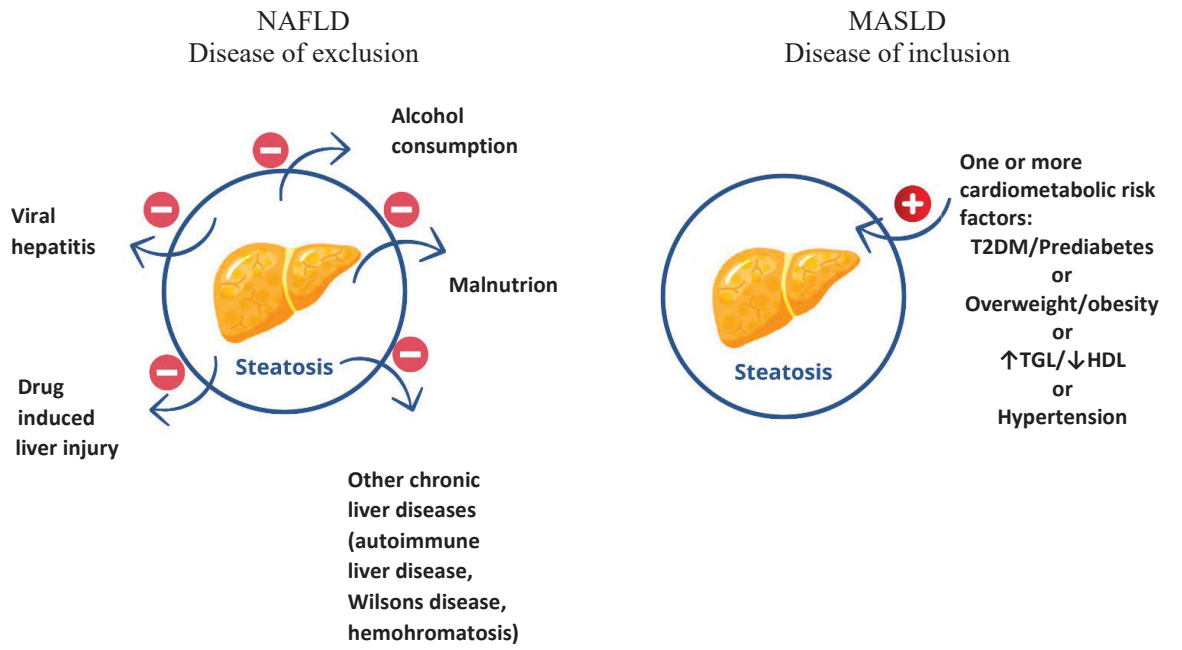

Figure 1: Difference in diagnosis of NAFLD and MASLD.

NAFLD - Non-alcoholic fatty liver disease, MASLD - Metabolic dysfunction-associated steatotic liver disease, T2DM - Diabetes mellitus typus 2, TGL - Triglycerides, HDL - Highdensity lipoprotein.

relationship between NAFLD and HTN was published (20) proving that NAFLD is associated with a 1.55-fold increased risk of incident HTN, while HTN increases the risk of incident NAFLD by 1.63-fold, and that MAFLD was significantly associated with an increase in systolic blood pressure over time compared to no or only FL (21).

Therefore, besides preventing or better control of T2DM, dyslipidemia, and other primarily metabolic conditions by timely recognizing and treating MASLD, it may serve as an important aspect in prevention, slowing or reversing of CVD, CKD and HTA, while control of all those conditions will on the other hand prevent or ameliorate MASLD and liver fibrosis.

New Nomenclature and Diagnostic Criteria – Keep Simple and Carry On

It was in 2020 when the term MAFLD was proposed to replace NAFLD to better reflect what that condition is rather than what it is not, unrelated to presence or absence of other causes of liver disease (22). Multi-society effort of three large pan-national liver associations, including patient advocates, was put into developing consensus about a new name to solve problems of exclusionary nature of the previous one, more appropriately describe this disease, and reduce the potential stigma associated with the terminology (23). One of the most significant differences was the removal of exclusion of concurrent liver disease previously required to establish the diagnosis (6, 24), but physicians are encouraged to identify coexistent etiologies of liver injury and treat them in a holistic manner (23) (Figure 1).

It is important to stress that years of work put into research of NAFLD are not in vain for it is proved that 98% of the existing registry cohort of patients with NAFLD would fulfill the new criteria for MASLD (24), the performance of the most commonly used NITs (Noninvasive Tests) is similar, and MASLD correlates similar (5) or even better than NAFLD (25) with clinical profiles and mortality rates. Therefore, the change in nomenclature was not at all purely semantic.

Important steps that should follow are simple diagnostic algorithms of in-risk populations together with therapeutic options, and authors of this editorial are offering some suggestions (Figure 2). New society guidelines and algorithms are released or upcoming (26, 24,

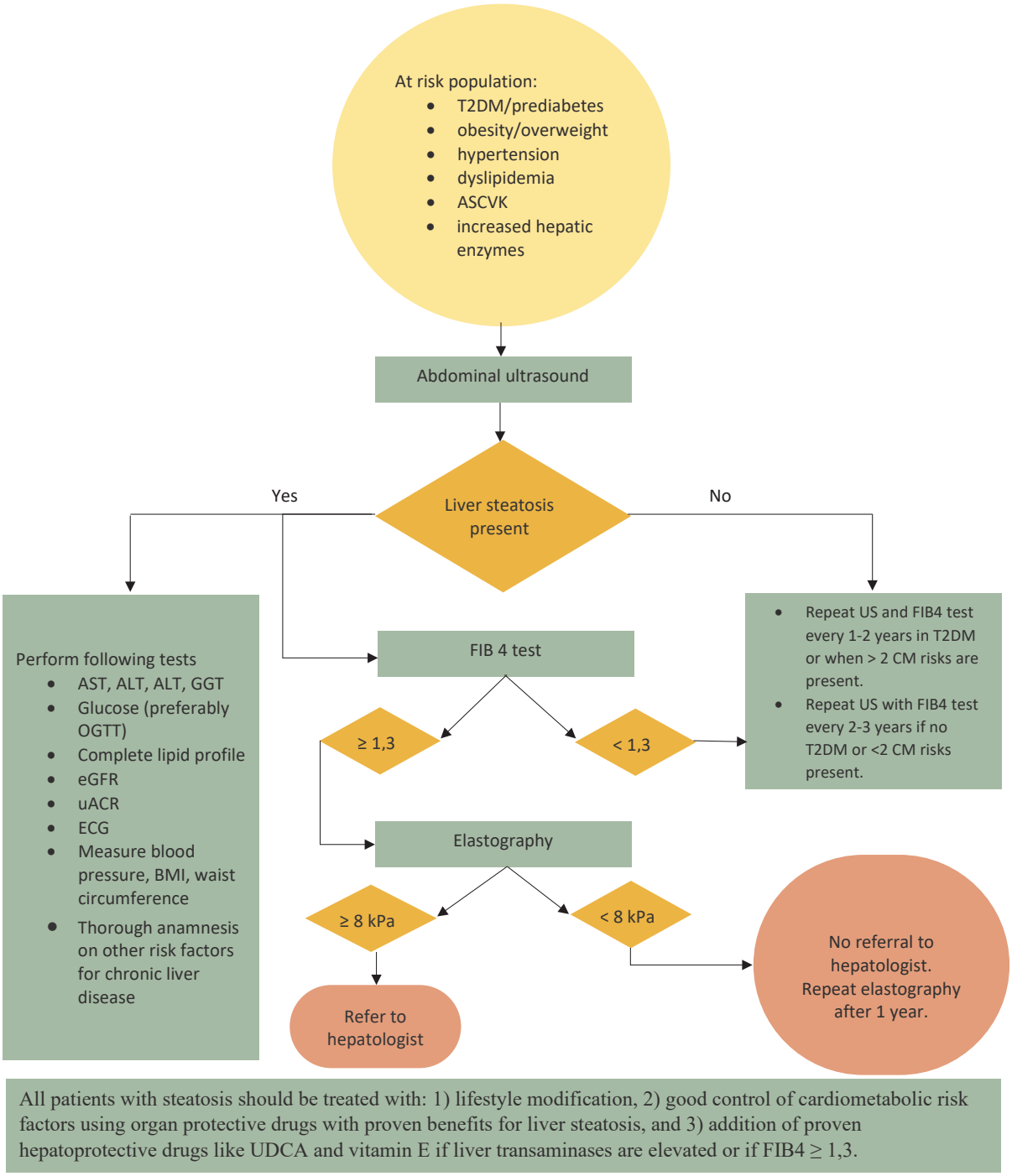

Figure 2: Algorithm of evaluation and treatment of liver steatosis in at risk patients or with known liver steatosis for primary care physicians and non hepatologists.
T2DM - Diabetes mellitus type 2, eGFR - Estimated glomerular filtration rate (online calculator, preferably CKD EPI – chronic kidney disease epidemiology collaboration), CM - Cardiometabolic, ASCVK - Atherosclerotic cardiovascular disease, AST - Aspartate aminotransferase, ALT - Alanine aminotransferase, GGT - Gamma glutamyl transpeptidase, OGTT - Glucose tolerance test, uACR - Urine albumin-creatinine ratio, ECG - electrocardiogram, FIB 4 - Fibrosis 4 (online calculator for liver fibrosis), US - Ultrasound, UDCA - ursodeoxycholic acid.

27), but with all the knowledge we gathered on MASLD, it is important to create guidelines that are not "hepatopetal" but put emphasis on bidirectional relationship of MASLD with its risk-factors and complications for it has important diagnostic and therapeutic implications.

Therapeutic Directions

Once MASLD is diagnosed, we should try to explain it first as being a cause or a consequence of co-existent conditions. That interplay is often not straight-forward, but one should find the most plausible explanation for

MASLD in each patient in order to treat the patient and not the separate conditions by tailoring therapy individually, avoiding unnecessary polypharmacy, and using modern disease-modifying multi-organ protective therapy. Such drugs that are common denominators of many of these conditions and have proven benefits for treating MASLD are: sodium-glucose transport protein 2 (SGLT2) inhibitors, glucagon-like peptide-1 agonists (GLP-1a), dipeptidyl peptidase 4 (DPP-4) inhibitors, metformin, angiotensin converting enzyme (ACE) inhibitors/ angiotensin receptor blockers (ARBs), statins (28, 29).

The expert opinion is to also introduce proven hepatoprotective drugs such as vitamin E, but more importantly – ursodeoxycholic acid that not only improves liver function, but independently from that also corrects metabolic abnormalities and provides extra-hepatic organ-protection (30, 31).

Lifestyle modifications are undoubtedly the cornerstone of therapy while on the other end stands resmetirom as the only MASH-targeted medication recently approved by the Food and Drug Administration in the United States for treating stage 2-3 fibrosis (6) for data suggest benefits of regression of fibrosis even by one stage, and even more profound in later stages (11).

CONCLUSIONS

MASLD has gone a long way from fatty liver, as it was first described by Thomas Addison in 1800s (32), then nonalcoholic fatty liver (NAFLD) that was being perceived as accidental finding on ultrasound and complicated to diagnose, to the present day when it is being recognized as not only hepatic but even more so – important independent extra-hepatic, especially CV risk factor. MASLD has received attention in academic circles, but the information was not articulated in parallel to the general healthcare practitioners in whom it is priority to raise awareness. It is much easier now, when academic society recognizes and embraces cardiovascular-kidney-metabolic (CKM) syndrome to include liver health assessment in conventional screening of in-risk population. Diagnosing MASLD by novel simplified positive criteria is practical and simple and this could help first-contact physicians to timely identify and treat this otherwise silent condition which, if left untreated, may cause major extra-hepatic adverse events, long before it causes hepatic ones. Since MASLD has bidirectional relation to its risk factors, being a causal condition to one patient while consequential to the other, there is and will probably not be single solution or drug for treating it. Only by abandoning organ-specific and embracing multidisciplinary holistic and individualized approach to treatment of our patients we can count on improvements in all outcomes.

Perhaps we should not be too modern and should go back to what Sir William Osler, often referred to as The Father of Modern Medicine, has once taught us.

Author Contribution: Conceptualization, Formal Analysis, Methodology, Writing – Original Draft, and Writing – Review & Editing were carried out by MAH and MBD.

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ORIGINAL RESEARCH

Antimicrobial Resistance Of Streptococcus Pneumoniae Among Preschool-age Children

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Abstract

Introduction. This research aimed to investigate the prevalence of S. pneumonia isolates among preschool-age children and analyze the susceptibility and resistance patterns of these isolates to investigated antimicrobial drugs before and after the onset of the Coronavirus disease 2019 (COVID-19).

Methods. The data for this study were gathered retrospectively over a two-year period (1 January 2018 – 31 December 2018 and 1 January 2022 – 31 December 2022) at the Public Health Institution "Health Center Brcko", Bosnia and Herzegovina. In the observed period, a total of 2287 swabs were performed.

Results. Among the total 214 clinical samples with isolated S. pneumoniae, 68% belonged to male patients. 47% of those samples were collected within the age group of 0-2 years. Multiresistance was identified in 92 isolates. Before the Covid-19 pandemic, S. pneumoniae isolates exhibited the highest sensitivity to cefuroxime and ceftriaxone, (99.1%), while after the Covid-19 pandemic, the highest sensitivity was demonstrated to norfloxacin (99.0%).

Conclusion. It is essential to use antibiotics rationally to prevent the further increase of resistance, especially multidrug resistance, in S. pneumoniae.

Keywords: antimicrobial resistance, Streptococcus pneumoniae, children

INTRODUCTION

Streptococcus pneumonia is one of the most prevalent opportunistic pathogens globally (1). This Gram-positive pathogen is a commensal of the human nasopharynx and plays a significant role as a causative agent in pneumonia, otitis media, sepsis, and meningitis worldwide. The incidence of the disease is highest at the extremities of life, affecting very young children and the elderly. According to the World Health Organization, Streptococcus pneumonia is responsible for the deaths of over 500,000 children annually on a global scale (2).

Pneumococci are transmitted between individuals through close contact and aerosols. Colonization is deemed a prerequisite for disease, although numerous colonized individuals may remain asymptomatic (3).

It is associated with high morbidity, mortality (causes 11% of deaths in children aged under 5 years) and global economic burden (4, 5). Despite advancements in treatment that led to a decrease in the case-fatality ratio of pneumococcal meningitis, it still stood at 25–27% in Europe and the Americas in 2015. In Africa, during the same year, the rate was notably higher, reaching as high as 61% (6).

The majority of pneumococci possess capsules, featuring surfaces made up of intricate polysaccharides, that play a crucial role in determining the pathogenicity of pneumococci and serve as the foundation for their classification into serotypes. As of today, more than 100 serotypes of S. pneumoniae have been identified (7). S. pneumoniae has developed increased resistance to multiple classes of antibiotics, which adds complexity to the treatment of pneumococcal infections (8, 9). According to the previous study in our country by Karcic et al., pneumococcus exhibited the highest resistance to erythromycin, clindamycin, and trimethoprim-sulfamethoxazole (10).

This research aimed to investigate the prevalence of S. pneumoniae isolates across various hospital and outpatient samples among preschool-age children and to analyze the susceptibility and resistance patterns of S. pneumoniae isolates to investigated antimicrobial drugs before and after the onset of the Covid-19 pandemic.

MATERIALS AND METHODS

Patients and Study Design

The data for this study were gathered retrospectively over a two-year period (1 January 2018 – 31 December 2018 and 1 January 2022 – 31 December 2022) at the Public Health Institution "Health Center Brcko", Bosnia and Herzegovina.

Methods

Clinical specimens included a nasal swab, throat swab, and ear swab. In the observed period, a total of 2287 swabs were performed. All samples were collected and transported to the Department of Microbiology following standard protocols for obtaining clinical material for microbiological examination. Hospital samples were taken from the Department of Pediatrics at the Public Health Institution "Health Center Brcko", and out-of-hospital samples were taken on an outpatient basis.

In the processing of the collected samples, three Petri dishes were employed: one for the Optochin test, and the other two for assessing resistance or sensitivity to specific antibiotics.

The optochin test serves to identify S. pneumoniae, an alpha-hemolytic streptococcus that typically exhibits sensitivity to optochin, while other types of alpha-hemolytic streptococci tend to be resistant. After incubation, the zone of growth inhibition is examined. If the diameter of the zone is ≥14 mm, the tested isolate is deemed sensitive to optochin. Conversely, if the zone of inhibition is less than 14 mm, it is likely not S. pneumoniae. In the case of resistance (where the diameter of the zone equals the disk's diameter), a deoxycholate lysis test is necessary, as a few pneumococci may exhibit resistance to optochin. Positive and negative controls are also essential in this process.

The antibiogram is conducted through the disc diffusion method, wherein antibiotic-soaked discs are placed on agar media that has been inoculated with the tested pathogen. Subsequently, there is an incubation period of 18 hours ± 2 hours, after which the diameter of the zone of inhibition around each disc is measured.

The antibiogram follows EUCAST recommendations. Based on the readings of the diameter of the growth inhibition zone, the tested strain is categorized as either S (Susceptible) or R (Resistant). The following

antibiotics were used to assess sensitivity/resistance in S. pneumoniae: amoxiclav, cefuroxime, cefixime, ceftriaxone, cefpodoxime, erythromycin, clindamycin, tetracycline, norfloxacin, chloramphenicol, and trimethoprim/sulfamethoxazole.

Multidrug Resistance (MDR) refers to bacteria's resistance to three or more antibiotics, encompassing at least one antibiotic from three or more distinct antibiotic groups.

The Ethics Committee of the Public Health Institution "Health Center Brčko" provided consent and approval for the retrospective use of data from the Department of Microbiology. The study was conducted in accordance with the Convention on Human Rights and the Helsinki Declaration on the Rights of Patients in Biomedical Research.

Statistical Methods

The results underwent analysis through standard statistical methods, employing the SPSS computer program for statistical analysis (SPSS-Statistical Package for Social Sciences) version 21.0. The findings were presented as both absolute numbers and percentage values. The analysis of categorical variables involved the use of the Chi-square test or Fisher exact test. Statistical significance was set at a p-value of <0.05.

RESULTS

This research was retrospective, spanning a two-year period: 2018 (prior to the Covid-19 pandemic) and 2022 (post the Covid-19 pandemic). During this time frame, a total of 214 antibiogram results from clinical samples, with S. pneumoniae isolation, were analyzed and processed – comprising 109 results from 2018 and 105 from 2022.

Among the total 214 clinical samples, 145 (68%) belonged to male patients.

Among the 214 S. pneumoniae samples analyzed, 100 (47%) pertain to patients within the age group of 0-2 years, while 114 (53%) were from patients within the age group of 3-6 years. The largest number of S. pneumoniae isolates was obtained from nasal swabs (185), followed by throat swabs (28) and ear swabs (1).

Out of the total 214 S. pneumoniae isolates, multiresistance was identified in 92 isolates. Among these, 17 isolates demonstrated resistance to three antibiotics from distinct drug groups. Specifically, 7 isolates exhibited simultaneous resistance to erythromycin, clindamycin, and tetracycline, 3 isolates were resistant to erythromycin, clindamycin, and trimethoprim-sulfamethoxazole, 2 isolates to erythromycin, clindamycin, and cefixime, and the remaining 5 isolates displayed resistance to different antibiotics concurrently.

In 24 samples, simultaneous resistance to 4 antibiotics was observed, with the highest number (10) displaying resistance to erythromycin, clindamycin, tetracycline, and cefixime. Additionally, 7 isolates exhibited resistance to erythromycin, clindamycin, tetracycline, and trimethoprim-sulfamethoxazole, 5 isolates to erythromycin, clindamycin, trimethoprim/sulfamethoxazole and cefixime.

Moreover, resistance to 5 antibiotics was identified in 41 isolates. Among these, 37 isolates demonstrated resistance to erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethoxazole, and cefixime.

Furthermore, 8 isolates exhibited resistance to 6 antibiotics. Among these, 4 isolates were resistant to erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethoxazole, cefixime, and norfloxacin, while the other 4 isolates showed resistance to erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethoxazole, cefixime, and chloramphenicol.

Regarding resistance to 7 antibiotics, this was observed in two samples, both displaying resistance to erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethoxazole, cefixime, chloramphenicol, and norfloxacin.

Before the Covid-19 pandemic, S. pneumoniae isolates exhibited the highest sensitivity to cefuroxime and ceftriaxone, recording 108 (99.1%), while the lowest sensitivity was observed for trimethoprim/sulfamethoxazole, with 45 isolates (43.1%) (Figure 1).

After the Covid-19 pandemic, Streptococcus pneumoniae isolates demonstrated the highest sensitivity to norfloxacin, recording 104 (99.0%), while the lowest sensitivity was observed for erythromycin, with 63 isolates (61.9%) (Figure 2).

A significant difference was identified in the frequency of sensitivity of S. pneumoniae isolates to cefuroxime, cefixime, clindamycin, tetracycline, norfloxacin and trimethoprim/sulfamethoxazole before and after the Covid-19 pandemic. (Table 1).

DISCUSSION

Streptococcus pneumoniae is a significant pathogen that can lead to the development of pneumococcal disease. This disease primarily affects individuals with weakened immune systems, young children (typically up to two years of age), and the elderly. Immunocompromised children with underlying health conditions are particularly vulnerable to developing severe forms of the disease, along with various complications, resulting in a higher mortality rate. The occurrence and frequency of S. pneumoniae infections in children raise particular concern, especially in developing countries where vaccination programs may not be well-established, despite the availability of vaccines against S. pneumoniae for an extended period.

Developing countries are currently grappling with a notable surge in antimicrobial drug resistance, primarily stemming from unauthorized sales and unregulated use of antibiotics. The resistance of Streptococcus pneumoniae isolates to antimicrobial drugs shows substantial variation from one country to another. Nevertheless, numerous studies highlight a prevalent and concerning frequency of resistance among pneumococcal isolates. This emphasizes the urgent need for improved regulation and awareness to curb unauthorized antibiotic use and address the growing challenge of antimicrobial resistance (11).

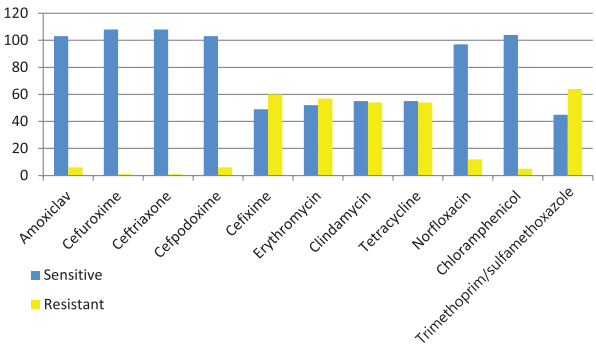

Figure 1. Sensitivity and resistance of Streptococcus pneumoniae to antibiotics before the COVID-19 pandemic

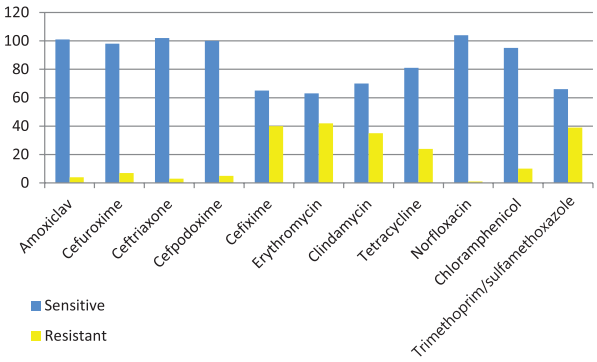

Figure 2. Sensitivity and resistance of Streptococcus pneumoniae to antibiotics after the COVID-19 pandemic

Table 1. Sensitivity and resistance to specific antibiotics before and after the pandemic

	before COVID-19		after COVID-19		p value
	sensitivity	resistance	sensitivity	resistance	
cefuroxime	99.10%	0.90%	93.30%	6.70%	0.029
cefixime	45%	55%	61.90%	38.10%	0.013
clindamycin	50.50%	49.50%	66.70%	33.30%	0.016
tetracycline	50.50%	49.50%	77.10%	22.90%	0.001
norfloxacin	89%	11%	99.05%	0.95%	0.002
trimethoprim/sulfamethoxazole	41.30%	58.70%	62.90%	37.10%	0.002

COVID-19 Coronavirus disease 2019; p - level of significance

In our study, the highest frequency of resistance among beta-lactam antibiotics was observed with cefixime, a third-generation cephalosporin commonly used for oral treatment. In 2018, resistance to cefixime was noted in 55% of cases, while after the pandemic, in 2022, resistance was recorded in 38.1% cases.

For cefuroxime, a second-generation cephalosporin, resistance was documented in 0.9% cases in 2018, and it increased to 6.7% in 2022, indicating a significant rise in resistance after the pandemic. Ceftriaxone, a third-generation cephalosporin for parenteral use, showed resistance in 0.9% of isolates before and 2.9% after the pandemic, suggesting a slight increase in resistance to ceftriaxone. Resistance to cefpodoxime, a third-generation cephalosporin for oral use, was recorded in 5.5% of patients before and 4.8% after the pandemic. Resistance to amoxiclav was 5.5% before the pandemic and 3.8% after the pandemic in our study.

Studies conducted over the years in various regions have reported high levels of resistance to penicillin in Streptococcus pneumoniae. For example, in China, the reported resistance was 88.3%, while in Russia, Nigeria, Canada, and Ethiopia, resistance levels ranged from 28% to 26.1% and 17.5%, respectively. In Central Africa and Tunisia, the incidence of penicillin resistance was slightly lower at 6% and 1.2%, respectively. The notable resistance to penicillin, as indicated by these studies, suggests that the empirical use of penicillin for treating suspected pneumococcal infections is no longer recommended (11-17).

In the presented study, where a combination of amoxicillin and clavulanic acid was used, a significantly lower frequency of resistance was observed compared to studies focusing on penicillin resistance.

The frequency of resistance to cephalosporins was found to be significantly lower in the mentioned studies compared to penicillin resistance. This suggests that cephalosporins may retain effectiveness against pneumococcal infections in regions where penicillin resistance is high (12-17).

In our study, resistance to erythromycin among Streptococcus pneumoniae isolates was recorded at 52.3% in 2018 and 40% in 2022. Comparatively, higher levels of resistance were reported in other regions, such as Canada (100%), China (95.2%), and Ethiopia (59.6%), while lower levels were observed in Russia (26%) and Pakistan (29.7%) (11, 13, 15, 18).

Resistance to clindamycin was observed in 49.5% of patients in 2018 and 33.3% in 2022 in our study. Comparatively, a higher rate of resistance was reported in a similar study conducted in China (95.8%), while in Canada, resistance was recorded in 40.6% of patients.

Regarding tetracyclines, our study showed a high rate of resistance, with tetracycline resistance found in 49.5% of patients in 2018 and 22.9% in 2022. Other similar studies also reported a significantly higher degree of resistance to tetracyclines, such as in China (93.6%) and Nigeria (73.5%), while resistance to tetracyclines in Ethiopia was recorded in 38.6% of isolates (11,12, 14, 15).

In our study, resistance to norfloxacin was reported in 11% of subjects in 2018 and it decreased to 1% in 2022. Comparatively, a low rate of resistance to levofloxacin was also observed in other countries, such as Germany (0.2%) and India (6%). However, Italy recorded a much higher rate of resistance to levofloxacin at 29%. These variations underscore the importance of regional and local surveillance to monitor antibiotic resistance patterns and guide appropriate treatment strategies (19-21).

As for chloramphenicol, the study reported resistance in 4.6% of subjects in 2018, increasing to 9.5% in 2022. Notably, a significantly higher rate of resistance to chloramphenicol was recorded in Nigeria (60%). In contrast, Central Africa (18.9%) and Ethiopia (17.5%) reported lower rates of resistance than Nigeria but higher than what was observed in our study (11, 14).

In our study, resistance to trimethoprim/sulfamethoxazole was observed in 58.7% of respondents in 2018, which decreased to 37.1% in 2022. Comparatively, higher rates of resistance to trimethoprim-sulfamethoxazole were found in other regions, such as Nigeria (96.2%), Pakistan (86.6%), Central Africa (69%), China (66.7%), and Russia (57%) (12-14, 16, 18).

Multidrug resistance (MDR), defined as infections caused by bacteria resistant to multiple antibiotics, poses a significant challenge as it limits the options for effective antimicrobial therapy. In our study, out of a total of 214 isolates, 92 showed resistance to three or more antibiotics, highlighting the prevalence of multidrug-resistant strains of Streptococcus pneumoniae. These strains commonly exhibited resistance to erythromycin, clindamycin, tetracycline, cefixime, and trimethoprim/sulfamethoxazole. Additionally, the results revealed a consistent pattern of co-resistance, particularly with erythromycin, clindamycin, and tetracycline.

Addressing the issue of multidrug resistance requires comprehensive strategies, including prudent antibiotic use, improved surveillance, and the development of new therapeutic approaches to combat infections caused by resistant strains.

The empiric treatment of pneumococcal infection often involves a combination of two or more antibiotics, and it may require a longer duration of therapy, especially for invasive forms of pneumococcal disease. Such situations can contribute to the development of resistance to multiple antibiotics in patients.

Interestingly, in our study, a decrease in resistance to a greater number of antibiotics was observed in 2022 compared to 2018. As stated by Meng et al., the resistance rates of S. pneumoniae to erythromycin, clindamycin, and tetracycline were maintained at a high level (> 85%) over the 4-year period (2018-2021); no penicillin-, moxifloxacin- or vancomycin-resistant strains were detected (22).

CONCLUSION

Despite extensive efforts to alleviate the impact of pneumococcal disease, it persists as a significant public health concern. The emergence of resistance to commonly used antimicrobials, observed on a global scale, adds complexity to the treatment of pneumococcal infections.

According to the results of the study, there is a need for the rational use of antibiotics to prevent the further escalation of resistance, particularly multidrug resistance, in Streptococcus pneumoniae.

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Declaration of Patient Consent: Written, informed consent was obtained from all volunteers in the study.

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ORIGINAL RESEARCH

Correlation Between Homocysteine and Lipid Parameters in Patients with End-Stage Renal Disease

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Abstract

Introduction. Chronic kidney insufficiency presents a progressive decline in renal function. Long-term hemodialysis treatments lead to biochemical parameter imbalances like an altered level of homocysteine (Hcy). Hcy can contribute to oxidative stress and endothelial damage, which promotes the accumulation of low-density lipoprotein (LDL) cholesterol and the development of atherosclerosis. It can also affect lipoprotein metabolism and trigger inflammatory processes that alter the lipid profile, especially increasing triglycerides and lowering high-density lipoprotein (HDL) cholesterol.

Methods. This cross-sectional, clinical and descriptive-analytical study included 83 patients, 40 patients on hemodialysis, and 43 subjects in healthy control group.

Results. The hemodialysis group had significantly higher Hcy values compared to the control group of seemingly healthy subjects ($p<0.05$). The control group had significantly higher values of total cholesterol, LDL and very low-density lipoprotein (VLDL) compared to the hemodialysis patients ($p<0.05$). There was non significant correlation between Hcy and lipid parameters in the hemodialysis group, neither in the control group.

Conclusion. Patients undergoing hemodialysis had significantly higher total homocysteine levels compared to the control group. However, total homocysteine did not demonstrate an association with lipid status parameters in either the hemodialysis patients or the healthy subjects.

Keywords: homocysteine, hemodialysis, cholesterol, triglycerides, metabolism.

INTRODUCTION

End-stage renal disease (ESRD) represents the final stage of chronic kidney disease (CKD) and is associated with high cardiovascular morbidity and mortality (1). Cardiovascular disease is the leading cause of death in this population and is driven by both traditional and non-traditional risk factors, including hyperhomocysteinemia and dyslipidemia (2).

Homocysteine, a sulfur-containing amino acid generated during methionine metabolism, is normally remethylated or transsulfu-

rated in a vitamin B-dependent pathway (3, 4). In patients with ESRD, homocysteine clearance is impaired due to reduced renal function, leading to elevated plasma levels – a condition known as hyperhomocysteinemia (4). Elevated homocysteine has been associated with endothelial dysfunction, oxidative stress, and vascular inflammation, all contributing to development of atherosclerosis (5).

Dyslipidemia in ESRD differs from that seen in the general population. It is often characterized by elevated triglycerides (TG), reduced high-density lipoprotein (HDL), and altered low-density lipoprotein (LDL) (6). The uremic milieu affects hepatic and lipoprotein metabolism, contributing to abnormal lipid profiles and increased cardiovascular risk (7).

Although homocysteine and dyslipidemia are independently linked to cardiovascular risk in ESRD, the relationship between them remains unclear. Some studies suggest homocysteine may influence lipid metabolism via oxidative mechanisms or inflammation, while others find no such association (7, 8). Exploring this relationship is crucial for understanding the interplay of modifiable risk factors in ESRD. Therefore, this study aimed to assess differences in homocysteine and lipid parameters between hemodialysis patients and healthy controls, and investigate the correlation between homocysteine levels and lipid profiles in examined groups.

MATERIALS AND METHODS

Patients and Study Design

This study was designed as a cross-sectional, clinical and descriptive-comparative study, including 83 subjects of both genders 34-78 years old. The subjects were divided into two groups: patients with end-stage renal disease on hemodialysis treatment (n=40) and a control group (apparently healthy subjects) (n=43). Inclusion criteria for study participants were: patients ≥ 18 years, patients with end-stage renal disease who were on hemodialysis treatment for more than six months; each patient included in the study had a comprehensive and systematically maintained medical history, and laboratory test results; healthy individuals of both genders who have no medical history or laboratory findings indicating kidney or liver disease; voluntary consent to participate in the study and willingness to cooperate; provided written informed consent to participate in the study. Exclusion criteria from the study were: patients who were on hemodialysis treatment for less than six months, individuals with severe malnutrition with albumin levels below 25 g/L, individuals with confirmed acute or chronic infections or malignant diseases, pregnant women, patients without complete medical documentation and individuals taking medications that affect blood lipid concentrations. All participants provided written informed consent to take part in the study, in accordance with the Declaration of Helsinki and with the approval of the Ethics Committee of the Clinical Center of the University of Sarajevo.

Methods

All biochemical analyses were conducted in the laboratories of the Clinic for Biochemistry and Laboratory Medicine,Clinical Center University of Sarajevo. For patients on hemodialysis, blood samples were taken before the hemodialysis procedure. Biochemical laboratory analyses, including measurements of total cholesterol, triglycerides, HDL, LDL and homocysteine, were conducted using an automated analyzer (Abbott Laboratories, Illinois, United States).

The following reference intervals were applied, as per standard clinical laboratory practice: total cholesterol: ≤ 5.0 mmol/L, triglycerides: ≤ 1.7 mmol/L, HDL: ≥ 1.2 mmol/L, LDL: ≤ 2.6 mmol/L, homocysteine: 5.0 – 15.0 μmol/L.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics software. Descriptive statistics were applied to monitor parameters. The Shapiro-Wilk test was utilized to evaluate data normality, and the Mann-Whitney U test was applied for comparisons between two independent

groups. Relationships between homocysteine and lipid parameters were tested using Spearman's correlation tests. A p-value<0.05 was considered statistically significant.

RESULTS

The study included 83 participants of both sexes, aged between 36 and 78. Testing the age differences between the two examined groups resulted in a p-value of 0.746, indicating that there is no difference in age between the two groups.

The values of total homocysteine, one of the key parameters in this study, were compared between the two predefined groups. The Mann-Whitney test results for homocysteine showed a p-value of $p < 0.001$, indicating a significant difference in homocysteine levels between the two groups of participants.

Table 1. Comparison of Total Homocysteine Levels Between Hemodialysis Patients and the Control Group

	HD (n=40)	CG (n=43)	p-value
Homocysteine (μmol/L)	25.60 (20.5-34.2)	11.43 (10.0-14.2)	**<0.001***

Median values with interquartile range, HD - Hemodialysis patients, CG - Control group, μmol/L – micromoles per liter

The results of the comparison of lipid parameter variables between hemodialysis patients and the control group are presented in Table 2. While comparing mean values of total cholesterol, LDH and HDL significant difference is observed between the two examined groups.

Table 2. Lipid Profile Comparison Between Hemodialysis Patients and the Control Group

Parameter	HD	CG	P Value
Cholesterol (mmol/L)	4.31 ± 1.07	6.06 ± 0.88	**<0.001***
LDL (mmol/L)	2.66 ± 0.99	4.12 ± 1.10	**<0.001***
HDL (mmol/L)	0.87 (0.73-1.03)	1.09 (0.93-1.32)	**<0.001***
TG (mmol/L)	1.62 (1.04-2.14)	1.785 (1.25-3.00)	0.082
VLDL (mmol/L)	0.74 (0.47-0.99)	0.94 (0.55-1.29)	0.035

HD - Hemodialysis, CG - Control group, LDL - Low-density lipoprotein, HDL - High-density lipoprotein, VLDL - Very low-density lipoprotein, TG - Triglycerides, mmol/L – millimoles per liter.

The correlation analysis of total homocysteine and lipid profile parameters in hemodialysis patients and the control group showed that none of the obtained values reached significance ($p > 0.05$).

Table 3. Relationship Between Total Homocysteine and Lipid Profile Parameters in Hemodialysis Patients and the Control Group

Parameter	HD ρ	P value	CD ρ	P value
HDL	-.001	0.993	-.152	0.349
Cholesterol	-.047	0.765	.134	0.410
LDL	.015	0.923	.161	0.321
VLDL	-.272	0.078	.077	0.635
TG	.016	0.083	.055	0.321

HD - Hemodialysis patients, CD - Control group, HDL - High-density lipoprotein, LDL - Low-density lipoprotein, VLDL - Very low-density lipoprotein, TG - Triglycerides.

DISCUSSION

Our results demonstrated a significantly higher level of homocysteine in hemodialysis patients compared to the control group (median 25.60 μmol/L vs. 11.43 μmol/L, $p < 0.001$), consistent with previous findings that identify hyperhomocysteinemia as a prevalent abnormality in ESRD (4, 5). Impaired renal function in these patients results in reduced clearance and metabolic processing of homocysteine, making its elevation a hallmark of uremia (18).

Despite markedly different homocysteine levels, no significant correlations were found between homocysteine and any lipid parameters, including total cholesterol, LDL, HDL, VLDL, or triglycerides. This is in line with studies that have failed to demonstrate a consistent relationship between homocysteine and lipid levels in patients with renal diseases (9, 10). The absence of significant correlation suggests that while both homocysteine and dyslipidemia are prevalent in ESRD, they may act through independent pathophysiological mechanisms in contributing to cardiovascular disease (19).

Interestingly, our study also identified significant differences in lipid parameters between groups. Hemodialysis patients showed

significantly lower levels of total cholesterol, LDL, and HDL (all $p < 0.001$), a pattern typical in ESRD, attributed to chronic inflammation, protein-energy wasting, and altered lipoprotein metabolism (6, 7). VLDL levels were modestly lower ($p = 0.035$), and triglycerides did not differ significantly, highlighting inter-individual variability influenced by factors such as dialysis adequacy, nutrition, and medication (11).

Although a weak negative correlation between homocysteine and VLDL (rho = -0.272, $p = 0.078$) was observed in the hemodialysis group, it did not reach statistical significance. The lack of association may also reflect confounding effects of inflammation or vitamin deficiencies, both of which are common in ESRD and can independently influence homocysteine and lipid profiles (12).

It is also worth noting that interventions aimed at lowering homocysteine, such as folic acid and vitamin B supplementation, have shown variable effects on cardiovascular outcomes, particularly in patients with CKD or ESRD. While these treatments can reduce homocysteine levels, large randomized trials have failed to demonstrate consistent cardiovascular benefit (13, 14). Therefore, the clinical relevance of homocysteine-lowering therapy in ESRD remains debated. Furthermore, our study confirms the high prevalence of hyperhomocysteinemia and dyslipidemia in ESRD patients. However, the absence of significant correlations between these parameters suggests that their roles in cardiovascular risk may be independent (15-19). Prevention strategies of cardiovascular disaeses in ESRD patients must be aggressive, individualized, and multidisciplinary due to the unique cardiovascular risks associated with kidney failure and dialysis.

Further large-scale and longitudinal studies are warranted to explore the mechanistic links and clinical implications of these findings.

CONCLUSION

Hemodialysis patients had significantly higher total homocysteine values compared to the control group. Control group patients had significantly higher total and LDL cholesterol values compared to hemodialysis patients. However, a negative correlation trend was observed between homocysteine and total, HDL, and LDL cholesterol in hemodialysis patients. Given the lack of a significant correlation between homocysteine levels and lipid parameters, it is not possible to definitively conclude that elevated homocysteine concentration holds substantial clinical importance in the evaluation of lipid profile values.

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ORIGINAL RESEARCH

Retrospective Analysis of Predictive Factors for Axillary Non-Sentinel Lymph Node Metastases in Sentinel Node-Positive Early-Stage Breast Cancer Patients

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Abstract

Introduction. Sentinel lymph node biopsy (SLNB) has significantly advanced axillary staging in clinically node-negative breast cancer, offering lower morbidity compared to traditional axillary lymph node dissection (ALND). Nonetheless, precise prediction of non-sentinel lymph node (non-SLN) involvement remains essential for optimizing surgical decisions and preventing unnecessary ALND.

Methods. A retrospective cohort analysis was performed on 176 patients with clinically node-negative breast cancer who underwent SLNB. Clinicopathological data were reviewed to evaluate associations between various predictive factors and non-SLN involvement. Variables analyzed included tumor size, histological grade, lymphovascular invasion (LVI), Ki-67 proliferation index, and sentinel lymph node characteristics.

Results. Multivariable logistic regression identified the type of SLN metastasis (OR=21.4; 95% CI 1.7–43.6; p=0.01), the number of positive SLNs (OR=5.66; 95% CI 1.18–36.6; p=0.03), and the number of negative SLNs (OR=0.04; 95% CI 0.006–0.27; p=0.001) as independent predictors of non-SLN metastases. The predictive model demonstrated excellent discriminatory power, with an area under the receiver operating characteristic curve (AUC) of 0.91.

Conclusion. Specific clinical and histopathological variables reliably predict non-SLN involvement in SLN-positive breast cancer patients. Incorporation of these predictors into clinical practice may enhance individualized axillary management and reduce unnecessary ALND procedures. Further validation through larger prospective studies is warranted.

Key words: Breast Neoplasms, Sentinel Lymph Node Biopsy, Axillary Lymph Nodes, Lymph Node Dissection, Neoplasm Staging.

INTRODUCTION

The sentinel lymph node biopsy (SLNB) has become the standard of care for axillary staging in clinically node-negative breast cancer patients, offering a less invasive alternative to axillary lymph node dissection (ALND) with reduced morbidity, particularly lymphedema (1-3). Patients who have negative SLNB could avoid ALND (4-7). Howe-

ver, when limited positive SLNs are observed, the optimal axillary treatment remains uncertain. Studies like American College of Surgeons Oncology Group Z0011 Trial (ACSOG Z0011), *After Mapping of the Axilla: Radiotherapy Or Surgery?* (AMAROS), and *Optimal Treatment of the Axilla – Surgery Or Radiotherapy (*OTOASOR), have shown no significant differences in axillary recurrence rates or overall survival between patients with 1-2 positive SLNs who underwent breast-conserving surgery with radiation, with or without ALND (8-10). This highlights the growing shift toward de-escalation in axillary surgery.

The OTOASOR trial directly compared ALND with regional nodal irradiation (RNI) in patients with SLN metastases (pN1) in early-stage breast cancer (stage I-II). Long-term follow-up data demonstrated that RNI without ALND does not increase the risk of axillary failure in selected patients with invasive breast cancer (cT ≤ 3 cm, cN0) and pN1 SLN (8). Similarly, the AMAROS trial, which included patients with T1-2 primary tumors and no palpable lymphadenopathy, confirmed the safety of RNI as an alternative to ALND. Both trials primarily enrolled patients undergoing breast-conserving surgery, with limited data on post-mastectomy cases and no reported differences in overall or progression-free survival (9).

Despite these findings, the role of ALND in early breast cancer patients with 1-3 positive SLNs following mastectomy remains debated. The practice in our center still heavily relies on ALND following positive SLNB. This underscores the need for predictive tools that can reliably estimate SLN and non-SLN status to guide surgical decision-making and reduce unnecessary interventions.

The primary objective of this study is to evaluate the potential of clinical, histopathological, and demographic variables to predict non-SLN status in patients with SLN metastases.

METHODS

Patients and Study Design

One hundred and seventy-six women aged over 18 with a pathohistologically confirmed diagnosis of early invasive breast cancer (BC) from core biopsy (T1-2) and clinically node negative disease who underwent surgery with SLNB between March 2019 and October 2024 were retrospectively included in the study. Exclusion criteria were as follows:1) completed neoadjuvant chemotherapy; 2) concurrent diagnosis of another primary tumor; 3) ductal carcinoma *in situ* (DCIS); 4) male breast cancer. Adjuvant treatments, including radiotherapy, chemotherapy, biological therapy, and hormonal therapy, were administered according to standard care protocols.

The study employed a retrospective-prospective design, with data collected for the period of March 2019 to October 2024.

Methods

Data related to patient demographics, including age and clinical presentation, were collected through the institutional electronic medical records system. Detailed tumor characteristics were extracted from the postoperative histopathology reports and included tumor stage, histological grade, hormone receptor status (ER, PR), HER2 status, Ki-67 proliferation index, lymphovascular invasion (LVI), perineural invasion (PNI), and SLN findings. SLN parameters comprised the total number of SLNs excised, number of positive and negative SLNs, and the type of metastatic involvement (macrometastasis vs micrometastasis). The primary endpoint of the study was the presence or absence of non SLN metastases.

Statistical Analysis

Continuous variables were summarized as means and standard deviations or medians with interquartile ranges (IQR) depending on the distribution, while categorical variables were presented as frequencies and per-

centages. Associations between SLN status and clinicopathological characteristics were evaluated using the chi-square test for categorical variables and the Mann–Whitney U test or Student's t-test for continuous variables. Statistical significance was defined as a p-value <0.05.

To identify predictors of SLN and non-SLN status, univariate logistic regression was initially performed. Variables with p-values <0.05 in the univariate analysis were subsequently included in a multivariate logistic regression model to adjust for potential confounders.

RESULTS

A total of 176 breast cancer patients were included in the analysis. Key demographic and tumor characteristics, including age distribution, tumor stage, histological grade, hormone receptor status, and other clinicopathological variables, are summarized in Table 1. The median age at diagnosis was 54.3 ± 14.3 years (24–83). The majority of patients (89.7%) had histological grade G2 or G3 carcinomas, with invasive ductal carcinoma NST being the predominant histological type (88.5%).

Table 1. Clinicopathological characteristics of patients

Characteristic	N (176)	%
Age Mean ± SD (range), years	54.3 ± 14.3 (24-83)	
Histological type		
NST	154	88.5
Lobular	10	5
Ductal + lobular	8	3.8
Mucinose	4	2.7
Tumor stage		
T1	96	54.5
T2	80	45.5
Hormone receptor status		
Positive	162	91.7
Negative	14	8.3
Her 2-receptor status		
Positive	12	7
Negative	164	93
Histopathological grade		
G1	18	10.2
G2	93	52.8
G3	65	37
Ki 67		
Median (range)	15.5 (3-80)	
SLN status		
Positive	71	40.35
Negative	105	59.65
Non SLN status (when SLN positive)		
Positive	29	39.63
Negative	42	60.37

SD – Standard deviation; SLN – sentinel lymph node; NST – no special type

Of the total cohort, 71 patients had positive SLNs. Associations between SLN positivity and clinicopathological factors were analyzed. SLN positivity was significantly associated with higher tumor stage ($p < 0.001$), the presence of LVI ($p < 0.05$), a high Ki-67 index ($p < 0.001$), and HER2-positive status ($p = 0.028$). No significant associations were observed between SLN status and age ($p = 0.302$), hormone receptor (HR) status ($p = 0.133$), perineural invasion (PNI; $p = 0.225$), tumor location ($p = 0.363$), or tumor type ($p = 0.469$).

Among patients with positive SLNs, 63% had no non-SLN involvement. Associations between non-SLN positivity and clinicopathological variables were also evaluated. Non-SLN positivity was significantly associated with the type of SLN metastasis, the number of positive SLNs, the number of negative SLNs, Tumor stage and LVI (Table 2).

Table 2. Association of characteristics with

Characteristic	Non SLN positive	Non SLN negative	P value
Tumor stage (T1/T2)	7/22	18/24	**0.01** [b]
Age			**0.678** [a]
Type of metastasis			**0.001** [b]
Micrometastasis	1	17	
Ene -	4	15	
Ene +	24	10	
Positive SLNs (1/2)	10/19	34/8	**0.02** [b]
Negative SLNs(0/1)	27/2	17/25	**0.001** [b]
Histological grade (G1-G2/G3)	15/14	29/13	**0.086** [b]
LVI (Pos/Neg)	26/3	33/9	**0.02** [b]

Non sentinel status
[a] Student's t-test; [b] Chi square test; ENE – extranodal extension; SLN – sentinel lymph node; LVI – lymphovascular invasion

Univariate logistic regression identified the following as significant predictors of non-SLN positivity:

- Tumor stage (OR = 2.35; 95% CI 1.82–6.71; p = 0.01),
- SLN metastasis with ENE (OR = 20.4; 95% CI 6.81–44.58; p < 0.001),
- SLN metastasis without ENE (OR = 4.53; 95% CI 1.79–16.27; p = 0.019),
- Number of positive SLNs (OR = 8.07; 95% CI 2.72–23.92; p < 0.001),
- Number of negative SLNs (OR = 0.07; 95% CI 0.01–0.24; p < 0.001), and
- Ki-67 index (OR = 2.3; 95% CI 1.08–6.11; p = 0.05).

Multivariate analysis revealed that the type of SLN metastasis, the number of negative SLNs, and the number of positive SLNs remained statistically significant predictors. In contrast, tumor stage, LVI, and Ki-67 index lost their statistical significance. These results are summarized in Table 3.

DISCUSSION

In this study, we evaluated the predictive abilities of clinicopathological and tumor characteristics in identifying SLN and non-SLN metastases in breast cancer patients. Our findings provide insight into key predictors that could guide axillary management decisions and reduce unnecessary surgical interventions. We found that SLN positivity was significantly associated with higher tumor stage, lymphovascular invasion (LVI), a high Ki-67 index, and HER2-positive status. These findings are consistent with previous studies that have identified tumor size, LVI, and proliferative activity as significant predictors of SLN metastasis (11). Interestingly, no significant associations were observed between SLN status and age, hormone receptor (HR) status, perineural invasion (PNI), tumor location, or tumor type. This aligns with findings from other studies, suggesting that while certain biological markers are critical, demographic factors and tumor location may have limited influence on SLN positivity (12, 13).

Among patients with positive SLNs, 63% had no non-SLN involvement, emphasizing the need for accurate predictors to avoid unnecessary ALND. Non-SLN positivity was significantly associated with the type of SLN metastasis, the number of positive SLNs, the number of negative SLNs, tumor stage, and LVI. These findings corroborate earlier studies that highlighted the importance of SLN tumor burden and nodal status in predicting non-SLN metastases (14, 15).

Univariate analysis identified several significant predictors of non-SLN positivity, including tumor stage, SLN metastasis with and without extranodal extension (ENE), the number of positive SLNs, the number of negative SLNs, and Ki-67 index. However, multivariate analysis revealed that only the type of SLN metastasis, the number of nega-

Table 3. Univariate and multivariate analysis of Non sentinel status

Factor		Univariate Analysis				Multivariate Analysis			
		OR	95% CI		P value	OR	95% CI		P value
			Lower	Upper			Lower	Upper	
Tumor stage	T2	2.35	1.82	6.71	0.01	3.05	0.61	17.44	0.02
LVI	Pos	2.36	1.58	9.62	0.02	1.78	0.6	7.58	0.83
Number of positive SLN	2	8.07	2.72	23.92	<0.001	5.66	1.18	36.6	0.03
Number of negative SLN	1	0.07	0.01	0.24	<0.001	0.04	0.006	0.27	0.001
Type of SLN metastasis									
	ENE +	20.4	6.81	44.58	<0.001	21.4	1.7	43.6	0.01
	ENE -	4.53	1.79	16.27	0.019	18.4	1.83	32.2	0.03
Ki status		2.3	1.08	6.11	0.05	1.95	0.44	8.57	0.311

OR – odds ratio; LVI – lymphovascular invasion; SLN – sentinel lymph node; ENE – extranodal extension

tive SLNs, and the number of positive SLNs remained statistically significant. These results are in line with studies indicating that SLN tumor characteristics and nodal count are the most reliable independent predictors of non-SLN involvement (15, 16).

Our finding that extranodal extension is a strong predictor aligns with some earlier evidence highlighting its role in predicting non-SLN involvement (15, 17). The inverse association between the number of negative SLNs and non-SLN positivity underscores the protective role of uninvolved nodes, as previously suggested (18, 19). However, the loss of statistical significance for tumor stage, LVI, and Ki-67 index in multivariate analysis suggests that these factors may not independently predict non-SLN involvement when accounting for SLN characteristics.

Patients with minimal SLN involvement, such as micrometastases or isolated tumor cells, and a high number of negative SLNs may safely forego ALND, consistent with recommendations from recent clinical trials such as ACOSOG Z0011 (10).

Some study limitations should be acknowledged. First, the sample size, while sufficient for robust statistical analysis, may limit the generalizability of the findings. Second, our study did not include imaging-based predictors, which could further enhance the predictive model. Future research should focus on integrating genomic data and radiological features to refine prediction and validate these findings in larger, diverse cohorts.

CONCLUSION

This study demonstrates that clinical and histopathological factors, such as the type and number of positive and negative sentinel lymph nodes, strongly predict non-sentinel lymph node metastasis in breast cancer patients. Incorporating these variables into clinical decision-making can help optimize axillary management and minimize unnecessary axillary lymph node dissection. These findings support a shift toward more personalized, less invasive surgical strategies, aligning with current trends in axillary treatment de-escalation. However, the relatively limited sample size and retrospective design of this study highlight the need for further validation in larger, prospective cohorts to confirm these results and strengthen their applicability in clinical practice.

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ORIGINAL RESEARCH

Exposure of Professional Pharmacists to Workplace Stress Factors in Bosnia and Herzegovina

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Abstract

Introduction. The term 'stress' represents experiences in which the demands of the environment outweigh an individual's perceived psychological and physiological ability to deal with them effectively. The aim of this study is to determine the level of stress to which a pharmacist is exposed to in their workplace, throughout Bosnia and Herzegovina. The main causes of stress in the workplaces of pharmacists were also investigated, as well as reactions to stress exposure.

Methods. The data were collected via an anonymous survey of 191 pharmacists across the country, over a period of two months. A previously modified and validated scale (of the Likert type) measured each of the variables.

Results. Respondents rated their perception of stress with an average score of 3.15±1.13, which corresponds to a 'very stressful' rating. The biggest source of stress was rated to be 'stress associated with unacceptable behavior in the workplace'. The lowest source of stress was deemed to be 'stress associated with unsafe or poor conditions at work'. All physiological responses to stress were rated by respondents as average (2.61±0.94). The overall rating of emotional responses to stress was prevalent (2.79±0.93). The overall score of behavioral change as a stress response corresponded to a score somewhere between what would be deemed as 'small' and what would be deemed as 'pronounced' (2.58±0.91).

Conclusion. This work may prompt further research towards creating a friendly and healthy working environment. This would improve the quality of services provided by pharmacists and raise current practice to an even higher level.

Keywords: work-related stress, pharmacist, pharmacy profession.

INTRODUCTION

The term 'stress' represents experiences in which the demands of the environment outweigh an individual's perceived psychological and physiological ability to deal with them effectively (1). Stress occurs when employees must deal with pressures that are not in line with their needs, skills, knowledge and expectations (1). Workplace stress is defined by the World Health Organization (WHO) as global epidemic. It is becoming even greater due to the recession, the global crisis and the fear of losing the job (2). Some occupations are considered more stressful than others. Healthcare workers, pilots, air traffic controllers and professional drivers are just some of the stressful occupations (2). One important distinction in studying stress is to differentiate between exposures to stressful events and the responses to these events (3). Stressful workplace events are described as discrete quantifiable circumstances that can have

severe negative impact. Reactions to stress can be physiological, psychological and behavioral (3).

There are four main areas that can lead to work-related stress if they are not managed properly. These are: workload, workplace, unclear distribution of tasks and responsibilities; long and inflexible working hours, working on weekends, the expectation of being constantly available via official phone or email; inadequate personal income, unsafe or poor physical working conditions, the lack of equipment and limited support from colleagues, conflicts, unacceptable behavior in the workplace, mobbing (3,4).

The aim of this study is to determine the level of stress to which a pharmacist is exposed to in their workplace, throughout Bosnia and Herzegovina. The main causes of stress in the workplaces of pharmacists were also investigated, as well as reactions to stress exposure.

MATERIALS AND METHODS

Patients and Study Design

The data were collected via an anonymous survey of 191 pharmacists across the country, including private, hospital and city pharmacies, over a period of two months. Each survey/questionnaire consisted of 3 topics.

Methods

The survey was conducted using questions created by the author based on a review of data and literature. The results of the analysis are presented tabularly and graphically in the number of cases, percentages, arithmetic mean, with standard deviation and range depending on the type of data.

The first part of the survey included basic sociodemographic data, including gender, age, years of work experience of the respondents, as well as the sector in which the respondents were employed. The second part contained questions about the causes of stress in the pharmacist's workplace. In the second part of the survey, respondents had the opportunity to express their individual levels of stress at the workplace using a previously modified and validated Likert scale (4). This corresponds to an average severity rating between 1= no stress and 5= extremely stressful for each stressor experienced (4). The third part of the survey/questionnaire was divided into three parts. It investigated the respondent's perception of reactions to stressors. The pattern, frequency, and duration of stressors are important determinants of the severity of the outcome, as is an individual's response to the stressors. The questionnaire was in accordance with medical ethics and duty of care. This questionnaire entailed no risk for the respondents. Respondents were assured of the questionnaire's confidentiality and of the fact that the results will be shared with them.

Statistical Methods

Descriptive and analytical statistical analyses were performed. Comparison of the influence of certain sociodemographic characteristics on the stress scale and the response to it were assessed using the Student's t-test and the One-way analysis of variance – ANOVA. Test results were considered statistically significant at 95% confidence level or with a value of $p<0.05$.The analysis was performed using the statistical package for sociological research – IBM Statistics SPSS v23.0.

RESULTS

191 respondents filled out the questionnaire. The time required to fill out the questionnaire was 1 minute and 43 seconds. The response rate was more than 50%.

The demographic characteristics of the respondents are shown in Table 1.

Table 1. Characteristics of the respondents

		N	%
Gender	Female	174	91.1
	Male	17	8.9
Age	24-35 years	95	49.7
	36-45 years	67	35.1
	>45 years	29	15.2
Years of work experience	0-10 years	111	58.1
	11-20 years	51	26.7
	>20 years	29	15.2
Sector in which the pharmacist works	Primary health care	130	68.1
	Hospital pharmacists	61	31.9
Total		**191**	**100.0**

Out of the total number of pharmacists, there were more women, 174 respondents or 91.1%, compared to 17 male respondents or 8.9%.

The largest number of respondents were aged 24-35 (49.7%). Next in order of frequency are respondents aged 36-45 (35%). The smallest number of respondents was over 45 years old – 29 respondents (15.2%).

According to the years of work experience, the largest number of respondents work from 0-10 years – 111 respondents (58.1%). The smallest number of respondents have work experience over 20 years – 29 respondents (15.2%).

In relation to the sector in which pharmacists work, it is evident that the majority of employees are employed in PHC – 130 respondents (68.1%) compared to pharmacists working in hospitals – 61 respondents (31.9%).

The second part of the survey/questionnaire included questions about the causes of stress in the pharmacist's workplace.

The respondents rated the perception of stress with an average rating of 3.15±1.13. This corresponds to the rating "very stressful". As the biggest source of stress, the respondents rated "Stress associated with unacceptable behavior at the workplace" with an average rating of 3.62±1.27. As the smallest source of stress, the respondents rated "Stress associated with unsafe or bad conditions at work" with an average rating of 3.25±1.28 (Table 2).

The third part of the survey/questionnaire investigated physiological, emotional responses and behavioral changes of respondents to stressors in practice. The respondents rated the physiological responses to the stress they felt on a scale from 1 to 4. 1 meant none, 2 – little, 3 – strong, and 4 meant extreme.

All physiological responses to stress were assessed by the respondents with an average score of 2.61±0.94. This is close to the rating strong. The most pronounced physi-

Table 2: Overview of respondents' exposure to stress at the workplace

	Mean	SD	Min.	Max.
What about the workload or workplace?	3.34	0.99	1	5
What about the unclear distribution of tasks and responsibilities?	3.38	1.05	1	5
What about the expectation of being constantly available via official phone or email?	3.30	1.35	1	5
What about inadequate personal income?	3.51	1.21	1	5
What about unsafe or poor physical working conditions	3.25	1.28	1	5
What about lack of equipment?	3.34	1.19	1	5
What about lack of control? Common areas beyond our control at work are: work processes decision - making performance targets?	3.43	1.13	1	5
What about changes within the organization?	3.42	1.27	1	5
What do you think about limited support from colleagues or overbearing supervision?	3.48	1.23	1	5
What about unacceptable behavior in the workplace?	3.62	1.27	1	5
What about underpromotion or overpromotion?	3.44	1.21	1	5
What do you think about the management's involvement in planning career opportunities?	3.57	1.24	1	5
Total	**3.15**	**1.13**	**1**	

ological response was assessed by the respondents as fatigue, with an average score of 2.58±0.84. The least pronounced physiological answer is dermatological problems with an average score of 1.94±1.12.

The total score of emotional responses to stress was 2.79±0.93. It corresponds to the score strong. The most pronounced emotional response to stress is irritability. Average grade 2.74±0.89. The least pronounced emotional response is depression. Average grade of 2.27±1.07.

A total rating of the change in behavior as a reaction to stress is 2.58±0.91. It corresponds to a rating between little and strong The respondents rated the most pronounced behavioral changes as lower tolerance for frustration and impatience with average score of 2.63±0.93. The least pronounced behavioral changes were increase in sick days and absence from work (Table 3).

DISCUSSION

Job satisfaction of pharmacists directly affects the safety of drug dispensing, which significantly affects the quality of patient care (5). Employees who are stressed, depressed or unhappy cannot produce the same quality of work as those who are satisfied and less stressed (5).

Table 3. Change in behavior due to exposure to stress

	Mean	SD	Min.	Max.
An increase in sick days or absenteeism	1.84	0.92	1	4
Aggression	1.92	0.91	1	4
Diminished creativity and initiative	2.53	0.97	1	4
A drop in work performance	2.22	0.90	1	4
Problems with interpersonal relationships	2.34	1.00	1	4
Mood swings and irritability	2.45	0.85	1	4
Lower tolerance of frustration and impatience	2.63	0.93	1	4
Disinterest	2.40	0.95	1	4
Isolation	2.32	0.98	1	4
Total	**2.58**	**0.91**	**1**	**4**

With this paper, the authors tried to determine the level of stress in the various roles of a pharmacist. The respondents' reaction to exposure to stress was also investigated. An individual's response to stress is sometimes more important than exposure to stress. Especially with respect to cumulative severity of the impact of stressors on the physical and mental health of the respondents.

A comparison of the influence of gender on individual stress scales shows no statistically significant influence (all $p>0.05$). It is noted that on all scales men show higher average scores compared to women. Total stress score: male 3.52±0.82 and female 3.12±1.00.

A significant influence of the age of respondents on the overall assessment of behavior change was recorded ($p<0.05$). Respondents aged 24-35 give the lowest rating for their change in behavior due to stress, 2.29±0.96. Respondents older than 45 rate their behavioral changes due to stress with the highest rating. 3.27±0.68.

Comparison of the influence of length of service on individual scales shows an average rating of $p>0.005$. In this section, respondents with 11-20 years of work experience gave the lowest rating 2.62±1.17. Respondents with 0-10 years of work experience gave the highest rating 3.35±1.09.

Comparative analysis of the influence of age on individual scales shows that there is a significant influence. Respondents in the group over 45 years of age show the highest score of 2.94±0.56 on stress. Of the scores of physiological responses to stress, all respondents show the score ($p>0.05$) in the section diarrhea/constipation (the respondents in the age group 24-35 years have the highest score) and in the section muscle tension (the respondents in the age group 45 years have the highest score).

The overall assessment of behavioral changes as a response to stress was achieved by all respondents in the categories: aggression, decline in work performance and lack of interest (p=0.0001). In general, respondents in the age group 36-45 have the highest score in the two sections, while the highest score in the isolation section was by respondents in the age group 45.

On all scales, respondents with 11-20 years of work experience gave the responses and behavioral changes the highest rating (3.16±0.63). Respondents with less than 10 years of work experience rated responses and behavioral changes with the lowest average rating (2.90±0.84).

All respondents have an overall assessment of behavioral change in response to stress in the sections: disinterest, aggression, a drop in work performance, problems with interpersonal relationships and increase in sick days and absences from work (p=0.0001). In general, respondents with 11-20 years of work experience have the highest score in the disinterest section.

The analysis of the influence of the sector in which the respondent works shows that its statistical impact was recorded on the overall assessment of physiological responses in the sense that respondents who are employed in hospitals rate physiological responses as more pronounced with a score of 3.30±0.68 compared to respondents who work in PHC and who evaluated the physiological responses with an average score of 2.35±0.90.

A significant influence of the sector in which the respondent works on behavioral change was also recorded. The respondents who work in hospitals evaluate their behavioral changes caused by stress with a higher average score of 3.23±0.85, compared to respondents who work in PHC.

Respondents in the PHC sector rate their behavioral changes as less pronounced with an average rating of 2.4±0.86.

In 2021, a study was conducted in Saudi Arabia among final year pharmacy students (6). The study was conducted with 437 students (6). It showed that pharmacy students consider working in a hospital pharmacy as the most desirable career and working in a community pharmacy as the least desirable (7). In contrast, a recent study conducted in Ethiopia among 232 pharmacists working in hospitals showed that job satisfaction among hospital pharmacy professionals was extremely low (7, 8).

In general, if we compare the impact of the sector and the evaluation of the change in behavior in Bosnia and Herzegovina, it is evident that the highest score is in the section increase in sick days and absenteeism for both sectors. Respondents who work in hospitals have a higher score in this section than respondents who work in PHC.
The aim of this paper was to determine the level of stress experienced by pharmacists at their workplace throughout Bosnia and Herzegovina. The main causes of stress at the workplace were also investigated, as well as the respondents' response to exposure to stress (9). The working environment and other variables were examined to see how they affect different dimensions of the quality of working life (10) (work setting, and other variables were examined for how they influence different dimensions of qua-lity of work life.) (11, 12). This work can sti-mulate further research to create a healthy work environment. In this way, the quality of services provided by pharmacists at their workplace would be improved (13). It would raise the level of current practice (14, 15).

CONCLUSION

Improving working conditions for pharmacists, clearly defining their roles, and investing in continuous education would lead to an improvement in their psychosomatic well-being, regardless of age.

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REVIEW ARTICLE

The Role Of Insular Cortex In Pathogenesis Of Anxiety Disorders, Major Depressive Disorder (Mdd), Schizophrenia And Autism Spectrum Disorders (Asd)

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Abstract

Insular cortex (i.e., insula; Latin for "island"), also known as the Island of Riel, represents a still poorly researched part of neural circuitry consisting of anterior and posterior areas divided by the insular central sulcus and surrounded by the peri-insular sulcus. Insula is involved in a variety of functions including gustatory and sensorimotor processing, somatic processing, as well as risk-reward behavior. Insula has been shown to play a major role in socio-emotional processes, such as emotional experience and introspection. Recent comprehensive meta-analysis studies suggest that lesion of the insular cortex can lead to significant psychiatric and neurological disorders as it plays a vital role in human motivation and emotional perception. Therefore, there is a growing interest in the medical community regarding this mostly unknown part of the human brain and the role of insular cortex in the pathogenesis of psychiatric disorders.

Keywords: insula, neocortex, mental disorders.

INTRODUCTION

Paralimbic cortex is a three-layered cortex that consists of piriform, entorhinal and parahippocampal cortex. It lies closely and is directly connected to the adjacent limbic system and its serves as a transitional area between paleocortex and neocortex, incorporating the region of prosiocortex. Being involved in complex cerebral interconnections, it integrates external sensory information with internal emotional and motivational states, among other functions. Insular cortex represents a part of the paralimbic structure situated between paleocortex and neocortex. Phylogenetically, it represents the most primordial part of telencephalon arising from anterior prosencephalon (1). The process of cortical development begins in the inferior cortical regions around the sixth week of fetal development and this particular region will later become, trough the act of folding, limen insulae. Through the disproportionate development of neocortex a horn-like structure is formed with the temporal lobe as its tip. The central window of this spiral opens into the insula. As the development of neurocortex continues, the rotation and compaction of the neural tissue buries the insula beneath the sylvian fissure (2).

The previously mentioned cortical folding does sever the insula's initial connections developed during the neural tube phase, which explains its intracerebral network with other parts of the brain. With the completion of neurological development, the insular cortex becomes fully formed (3). The insula is a highly heterogeneous region with regards to both its anatomical and functional features. Anatomically, the insula is comprised of at least three subregions defined by the presence or absence of granular cortical layer IV neurons, and each subregion has distinct structural connections. Functionally, the insula is implicated in a vast array of behaviors, ranging from experiencing saliency, to social and emotional processing, to interoception.

There is compelling evidence from basic and systems neuroscience research that the insula is altered in a host of psychiatric illnesses, and that the anterior insula in particular may represent a common substrate of psychopathology (4, 5). Yet, recent models have proposed that anterior insula and posterior insula alterations (both in structure and in function) are differentially implicated in multiple symptom profiles of depressive, psychotic, and substance use disorders, among others (6, 7, 8).

Recent research has shed light on the intricate connections between the insula and other brain regions, revealing the crucial role of this area in integrating sensory, emotional, and cognitive information. The unique anatomical position and extensive connectivity allow the insula to serve as a critical hub in the functional network of the brain and the insular involvement in emotional processes, highlighting its implications in psychiatric conditions (9). This paper presents new knowledge about the role of insular cortex in psychological processes, as well as the pathogenesis of psychiatric disorders.

THE INSULAR CORTEX

The insular cortex represents a triangular area of neocortex that forms the floor the Sylvian fissure. It is located deep to the insular operculum, formed by parietal, frontal and temporal lobes. Using axial magnetic resonance imaging (MRI) it can be observed lateral to the extreme capsule, claustrum, putamen and external capsule. Insular cortex primarily consists of two distinct areas: anterior and posterior area separated by the insular central and encompassed by the peri-insular sulcus. Anterior insula composes of anterior, middle and posterior, also known as short gyri, which are separated by two pre-central sulci.

The posterior insula consists of anterior and posterior gyri (short gyri) separated by singular post-central sulcus. Additionally, the insula contains other gyri (accessory gyrus and transverse gyrus), as well as lumen insulae in the anteroinferior apex (9). On the cytoarchitectural level, the insular cortex consists of an anterior dysgranular area and a posterior granular area. The anterior granular area contains pyramidal neurons in layer II and IV, while posterior granular area contains granular cells in layer II and IV. The research conducted by Kurth F. et al. showed that the posterior insula can on the cytoarchitectural level be separated in 3 distinct areas: two granular areas, referred to as Ig1 and Ig2 (insular lobe granular areas), found in dorsal posterior insula, and a dysgranular area (Id1) ("d" for dysgranular area) found in ventral posterior insula. The insular cortex has one unusual feature and that is the presence of large bipolar projection neurons called von Economo neurons (VENs), most common in the frontoinsular cortex (9, 10, 11).

Different parts of the insular cortex correlate with its different cerebral connections. The anterior portion of the insula is primarily connected with anterior cingulate, frontal, orbitofrontal and anterior temporal areas, while the posterior portion connects with posterior temporal, parietal and sensorimotor areas. The insular cortex is a true anatomical integration hub with heavy connectivity to an extensive network of cortical and subcortical brain regions serving sensory, emotional, motivational and cognitive func-

tions. It is not visible on an exterior view of the brain, as it is fully covered laterally by opercula of the parietal, frontal, and temporal lobes. Directly medial to the insula are the extreme capsule and the claustrum. The central sulcus of the insula is the most inferior extension of the Rolandic fissure (central sulcus) that separates the frontal and parietal lobes. It receives heavy sensory inputs from all modalities. Direct thalamic and horizontal cortical afferents carry information to the insula from outside the body (auditory, somatosensory, olfactory, gustatory and visual information) and from inside the body (interoceptive information).

Several of these inputs project to topographically organized insular sensory regions, giving rise to the 'visceral insular cortex', the 'gustatory cortex' (the primary taste cortex), and the insular auditory and somatosensory fields (11, 12).

FUNCTION OF THE INSULAR CORTEX

The insula's primary role is believed to be that of multimodal integration. With this increased research attention, knowledge regarding the insula's role in clinical syndromes has also increased. Among the first insights into the role of the human insula came from the seminal works and experiments by Penfield and Faulk in the mid-20th century, conducted through intraoperative electro-cortical stimulation on 36 patients with positive results at 82 stimulated points (13). The emergence of new diagnostic techniques, such as neuroimaging in the early 21st century, gave rise to interest and research possibilities regarding the insula's function.

Interest in the function of the insular cortex has increased drastically since the advent of functional neuroimaging techniques, which revealed insular activation in response to a wide variety of stimuli (14).

A meta-analysis of nearly 1,800 functional neuroimaging experiments by Kurth and colleagues suggested the existence of four functionally distinct regions in the human insula: 1) a sensorimotor region located in the mid-posterior insula; 2) a central-olfactogustatory region; 3) a socio-emotional region in the anterior-ventral insula; and 4) a cognitive anterior-dorsal region.

In recent years, tract-tracing studies have supported the view of a central viscero-somatosensory role for the insula, which is now known to receive visceral afferent projections conveying interoceptive information from all over the body (11).

The human insula has emerged as a core region affected across many psychiatric disorders including anxiety disorders, addiction, depression, schizophrenia and autism. Together with the dorsal anterior cingulate cortex, the insula cortex forms a hub that affects the brain's ability to switch between different functional networks according to internal and environmental demands, explaining why insula disturbances may be disproportionally disabling. Given this important role, the insula is one of the most promising targets for brain stimulation treatment of several psychiatric disorders (16, 17, 18).

THE ROLE OF THE INSULA AND PSYCHIATRIC DISORDERS

The insula exhibits altered structure and function across different forms of anxiety disorders, major depressive disorder, schizophrenia and autism spectrum disorders.

ANXIETY DISORDERS

They are the most prevalent mental health condition with a lifetime prevalence of 17%, resulting in significant individual and social impairment and a considerable overall burden of disease. Anxiety can be an adaptive response to unpredictable threats and pathological anxiety disorders occur when symptoms adversely impact daily life. Meta-analyses of functional neuroimaging studies of induced and pathological anxiety were therefore compared.

A systematic search was conducted in June 2019 on the PUBMED database for whole-brain functional magnetic resonance imaging articles. Eligible articles contrasted anxious patients to controls, or an unpredictable-threat condition to a safe condition in healthy participants. Five anxiety disorders were included: post-traumatic stress disorder, social anxiety disorder, generalized anxiety disorder, panic disorder, and specific phobia. 3433 records were identified, 181 met the criteria and the largest subset of task type was emotional (N=138).

Seed-based d-mapping software was used for all analyses. Induced anxiety (n=693 participants) and pathological anxiety (n=2554 patients and 2348 controls) both showed increased activation in the bilateral insula and cingulate cortex/medial prefrontal cortex.

When split by disorders, specific phobia appeared the most, and generalized anxiety disorder the least, similar to induced anxiety.

This meta-analysis indicates a consistent pattern of activation across induced and pathological anxiety, supporting the proposition that some neurobiological mechanisms overlap and that the former may be used as a model for the latter. Induced anxiety might, nevertheless, be a better model for some anxiety disorders than others (19).

Paulus and Stein have proposed that individuals who are more aware or focused on their bodily feelings may exhibit greater interoceptive prediction signals: that is, increased prediction of future aversive physical states may trigger anxiety, worry and avoidance behaviors. Measures of anxiety are correlated with the accuracy of heartbeat detection and activity in the right anterior insular cortex. Changes in insular-mediated anticipation and prediction of future events may lead to heighten anxiety. It may represent a unique opportunity to assess the precise neuronal mechanisms underlying the insula's role in healthy and pathological fear and anxiety. Increasing understanding of the neural correlates of anxiety symptoms in late-life depression (LLD) could inform the development of more targeted and effective treatments. A study that assessed grey matter volume (GMV) with volumetric magnetic resonance imaging in a sample of 113 adults ≥60 years with MDD using the following regions of interest: amygdala, anterior cingulate cortex (ACC), insula, orbitofrontal cortex (OFC), and temporal cortex.

Decreased OFC volumes may serve as a unique biomarker of anxiety symptoms in LLD. Future longitudinal and clinical studies with long-term follow up and more diverse samples will help further elucidate the biological, psychological, and social factors affecting associations between anxiety and brain morphology in LLD (20, 21).

Social anxiety disorder has been described as a persistent fear of one or more social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others. Neuroimaging of social anxiety disorder has revealed some important findings. However, there has not been enough study on patients with a social anxiety disorder to account for the exact reason for the occurrence of the disorder. The first study on structural brain imaging was done by Potts et al. nearly a quarter-century ago. In that study, it was reported that there was no statistically significant volume difference between patients with a social anxiety disorder and healthy control subjects (22). Kawaguchi et al. measured the insula volumes in patients with a social anxiety disorder in comparison with healthy control subjects and found that insula volumes were significantly reduced compared to those of healthy subjects (16). Atmaca et al. examined twenty-one patients with social anxiety disorder according to DSM-IV and twenty healthy controls. All patients and controls were subjected to magnetic resonance imaging (MRI). Insula volumes were measured by using the manual tracing method in accordance with the standard anatomical atlases and related previous studies on insula volumes. They found that the mean posterior and anterior insula volumes for both sides

of patients were statistically significantly reduced compared to those of healthy control subjects. In the present study, it was found that patients with a social anxiety disorder had reduced insula volumes compared to those of healthy control subjects. However, to build on this finding, novel studies with a larger sample size are required (23, 24).

MAJOR DEPRESSIVE DISORDER (MDD)

The insular cortex is part of a network of highly connected cerebral "rich club" regions and has been implicated in the pathophysiology of various psychiatric disorders, of which major depressive disease is one of the most prevalent. "Rich club" vulnerability can be a contributing factor in disease development. Depression is associated with systemic inflammation, and endotoxin administration, which causes systemic inflammation, elicits mild depressive symptoms, such as fatigue and reduced interest. The neural correlates of depressive symptoms that result from systemic inflammation are poorly defined. Major depressive disorder (MDD) is associated with emotional and cognitive impairments, including negative affect or loss of pleasure. Aberrant anatomy, connectivity and activation of the insula are found in human patients suffering from major depressive disorder. These alterations have been linked with the disease-characteristic anhedonia, the inability to experience pleasure (25, 26). The insula metabolism was altered in depressed patients and the direction of change indicated whether patients would respond better to either one or another of the two major treatment approaches for depression: cognitive behavioural therapy or drug-based therapies. Measuring insula metabolism could thus serve as one of the first neuroimaging biomarkers in the field of neuropsychiatric disorders to guide treatment selection. Hannestad et al. had conducted double-blind, randomized, placebo-controlled, crossover study where 9 healthy subjects received an intravenous dose of endotoxin (0.8 ng/kg body weight) on one day and placebo (saline) on another day, separated by one week. Brain glucose metabolism was measured with FDG-PET, with tracer injection 90 minutes after endotoxin/saline administration, when the systemic immune response peaks. Correlational analyses suggest that the insula may participate in the modulation of systemic levels of inflammatory cytokines, which is consistent with the known functions of the insula; this is a finding with important potential implications for depression that we plan to address in future studies (27).

High-resolution structural subfield analysis of insular volume in combination with cortical thickness measurements and psychological testing might elucidate the way in which the insula is changed in depression. High-resolution structural images of the brain were acquired using a 7T-MRI scanner. The mean grey matter volume and cortical thickness within the insular subfields were analyzed using voxel-based morphometry (VBM) and surface analysis techniques, respectively. The combination of differences in grey matter volume between healthy controls and patients with a positive correlation of cortical thickness with disease severity underscores the insula's role in the pathogeneses of MDD. The connectivity hub insular cortex seems vulnerable to disruption in the context of affective disease (19). Structural alterations of the insula in depression patients (28).

Interoception plays a crucial role in maintaining bodily homeostasis and promoting survival, and is considered the basis of human emotion, cognition, and self-formation. A malfunction of interoception is increasingly suggested to be a fundamental component of different mental health conditions, and depressive disorders have been especially closely associated. Interoceptive signaling and processing depends on a system called the "interoceptive pathway," with the insula, located in the deep part of the lateral fissure, being the most important brain structure in this pathway. Neuroimaging studies have revealed alterations in the structure and function of the insula in a large number of individuals with depression, yet the precise

relationship between these alterations and interoceptive dysfunction remains unclear. Interoceptive dysfunction has been linked to structural or functional impairments of the insula, which plays a central role in processing of interoceptive information. Three aspects of the potential relationship between interoceptive dysfunction and alterations in insular function in people with depression have been assessed, namely clinical symptoms, quantitative measures of interoceptive function and ability, and interoceptive modulation. Firstly, increased severity of somatic symptoms in people with depression was found to be associated with impaired insular function, and among the somatic symptoms, fatigue and pain are the most prominent and have been considered in greatest detail. Secondly, the insula has been demonstrated to be a brain region the function of which is most closely related to the interoceptive ability of an individual. Insular hypo-activation has been reported in people with MDD who were asked to focus on attending to their visceral sensations, indicating a weaker involvement of the insula in processing interoceptive information. Thirdly, interoceptive modulation, produced by various treatments which emphasize attending to bodily sensations and the body–mind connection, produces neuroplastic changes in the insula, restores impaired interoceptive function, rebuilds self-referential thinking and reduces the neural response to pain, and relieves symptoms of depression. Evidence from the existing studies has shown that the insula may be the central structure for the impaired interoceptive function as identified in people with depressive disorder. Future systematic assessments of interoceptive dysfunction and their association with insular function in those with depressive disorder are likely to be highly important in the treatment of MDD (29).

SCHIZOPHRENIA

In schizophrenia, it is evident how difficult the understanding of the specific neurobiological underpinnings of complex phenotypes can be. The positive, negative, and cognitive symptoms of psychosis span a wide variety of experiences and behaviors, and the insula's unique proposed role in integrating sensory information and providing awareness for higher-order social cognition make it a promising candidate for the study of schizophrenia. The anatomical and functional diversity of the insula is further recapitulated in the work investigating its relationship to different symptom profiles in schizophrenia (Stein et al., 2021), as it has been broadly associated with negative symptoms, positive symptoms, and cognitive impairment (30, 31, 32, 33).

Meta-analysis has shown that the bilateral insula is one of the top five most affected brain regions in schizophrenia with regards to reduced cortical thickness and it represents the only brain region whose cortical thickness is associated with earlier age of onset and longer duration of illness (34). Insula grey matter volume is also significantly reduced in chronic schizophrenia, at illness onset and in individuals at ultra-high risk for psychosis who ultimately develop schizophrenia (35, 36). There is additional evidence that insula volume progressively declines after first episode psychosis, particularly in those with non-affective (but not affective) psychotic disorders (37). Functional connectivity of the insula is also abnormal in schizophrenia, with evidence of both reduced and increased connectivity that deviates from normative patterns. Magnetic resonance imaging studies consistently find decreased grey matter volume and reduced cortical thickness in the insula of schizophrenic patients, which progress with increasing disease severity. Functional aberrations observed in schizophrenic patients, which are likely related to altered insula function, include pain insensitivity, deficits in sensory-emotional integration, such as poor recognition of emotions in facial expressions, emotionally blunt speech, impairments in distinguishing self from non-self, and the occurrence of hallucinations (38).

AUTISM SPECTRUM DISORDERS (ASD)

Autism spectrum disorders are complex neurodevelopmental disorders of unknown etiology. The insula has been consistently identified as a locus of hypoactivity and dysfunctional connectivity in ASD, and the pattern of functional connectivity of the insula can be used to discriminate individuals with ASD from typically developing children (39). The insula is essentially involved in multisensory and affective processing, as well as social functions like empathy, all of which are strongly affected in autistic patients and play a key role in the detection of behaviorally relevant stimuli and initiation of dynamic switching between an 'executive control network' of brain regions, which drives externally-oriented attention, and a 'default mode network', which is dedicated to internally oriented cognitive processing. Irregularities in salience-network connectivity are linked to autistic symptom severity. Together, these findings indicate that both functional changes within the insula, as well as in long-range connectivity between the insula and related brain regions, contribute to the behavioral and cognitive symptoms of ASD (40).

There is extensive evidence based on studies with preschoolers, school-age children, and adolescents with autism of altered communication between anterior insula (aINS) and other cortical nodes including those comprising the default mode (DMN) and the frontoparietal (FPN) networks, as well as the amygdala. These alterations include both patterns of hypo- and hyperconnectivity, depending on the region and age. Reduced functional connectivity (FC) between insula and other cortical nodes observed in several studies in children with autism may result in difficulties in transfer and integration of information across these networks, which then could contribute to the impaired processing of social signals (41).

However, given that autism onsets in early childhood and that most FC studies are conducted in older children, it is not clear to what extent the observed differences precede the emergence of symptoms of autism or emerge secondary to the way children with autism experience and interact with their social and nonsocial environment. The study suggests that the circuitry heavily involved in early development of social bonding and motivation may be hypo-connected in those with genetic predisposition for autism by four postnatal weeks and that this hypoconnectivity is linked with later emerging social vulnerabilities. These findings motivate future studies into the development of the networks involved in salience detection and allostatic regulation during the key transition from pre- to postnatal environment and their contribution to later behavioral outcomes relevant to autism (22). Hypoconnectivity between anterior insula and amygdala associates with future vulnerabilities in social development in a neurodiverse sample of neonates (42).

CONCLUSION

The insula is not an isolated 'island' but rather an integral brain hub connecting different functional systems underlying sensory, emotional, motivational, and cognitive processing. It is thus crucial in determining the valence of internal and external stimuli. Together, these features explain the important roles that the insula serves in several forms of reinforcement learning, emotion control, and decision-making. As a salience detector it has further been suggested that the insula marks the most relevant stimuli for further processing in other large-scale brain networks. In addition to these general roles, the insula contains multiple subregions, each characterized by different patterns of connectivity to the rest of the brain and at first sight distinct functional roles. How these different insular regions interact are open questions key to advance our understanding of the insula function. Through this paper, we hope to highlight the importance of the insula as an interface between sensation, emotion, and cognition, and to inspire further research into this fascinating brain

region. By recent human neuroimaging studies, the insula is re-emerging as an important brain area not only in the physiological understanding of the brain, but also in pathological contexts in clinical research. It is important to understand the anatomical and histological features of the human insula, summarize the physiological functions of the insula and underscore its pathological roles in psychiatric and neurological disorders that have long been underestimated. It is crucial to propose possible strategies through which the role of the insula may be further understood for both basic and clinical neuroscience.

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REVIEW ARTICLE

Applied Immunohistochemistry in Differential Diagnosis of Female Genital System PEComas

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Abstract

PEComa (Perivascular epithelioid cell tumors) are a rare type of tumor composed of cells exhibiting characteristics of smooth muscle cells and melanocytes. They most commonly occur in the female genital system. This study is a narrative review based on the differential diagnosis of tumors in the female genital system, focusing on PEComa. The aim of the research is to analyze the immunohistochemical markers characteristic of PEComa in the female genital system and compare them with markers of tumors that may appear in the differential diagnosis. Specifically, the study examines epithelioid smooth muscle tumor (STUMP), malignant melanoma, alveolar soft part sarcoma (ASPS), poorly differentiated endometrial carcinoma (EC) and trophoblastic tumors of the placenta (PSTT). Comparison of immunohistochemical markers of PEComa with markers of other tumors revealed that: PEComas show overlap in positive staining with STUMP, but are distinguished by markers such as HMB45, PNL2, MiTF, and MelanA/MART1; PEComas share some melanocytic markers with malignant melanoma, but differ in the expression of myogenic markers and hormone receptors; compared to ASPS, PEComas share some positive staining but differ in marker expression and negative staining; they differ from EC by the expression of specific markers such as MiTF and PAX8; PSTT show specificity for markers of trophoblastic differentiation and implantation, while PEComas emphasize melanocytic and myogenic differentiation. The general conclusion is that an accurate diagnosis of PEComa in the female genital system can only be achieved through a multidisciplinary approach. Immunohistochemical evaluation serves as a helpful tool, but standard morphological staining remains the gold standard. Also, the advanced diagnostic techniques, particularly next-generation sequencing, hold promise for enhancing the understanding and management of mPEComas. By uncovering the genomic landscape and facilitating targeted therapies, these methodologies may lead to more effective treatment and improved outcomes.

Keywords: female genital system, epithelioid smooth muscle tumor, malignant melanoma, endometrial carcinoma, trophoblastic tumor.

INTRODUCTION

Perivascular Epithelioid Cell Tumor (PECOM) is a general term for soft tissue neoplasms found in visceral locations. They are most commonly located in the female genital system (1) which accounts for just over a quarter of all PEComa cases described in the literature, with the most common uterine location. The vulva, cervix, vagina, ovaries, and broad ligament are less frequently affected (2). Other locations include

the stomach, intestines, lungs, and urogenital system, though they are less frequently found in bones. Thus, most PEComas are described in women, with a female-to-male ratio in some case studies reaching up to 9:1, suggesting a possible hormonal role in the pathogenesis because of increased expression of ER and PR demonstrated in patients with uterine PEComas (3-4). Most PEComas are benign and do not have the potential for recurrence following complete surgical excision (5-6). However, a subset of these tumors is best classified as having uncertain malignancy due to the possibility of recurrences years after the initial diagnosis. Criteria for evaluating the malignancy of gynecological PEComa are proposed and are based on the presence of more than four tumor characteristics that include tumor size ≥5 cm, high-grade nuclear features, necrosis, vascular invasion, or mitotic activity ≥1/50 HPF (7). This rare epithelioid, mesenchymal tumor originates from perivascular epithelioid cells (PECs) (1). Tumor cell growth, regardless of the pattern, is closely associated with an emphasized vascular component. This perivascular tumor distribution has led to the hypothesis of a possible origin near blood vessels (8). The presence of thin, delicate blood vessels, which may have thickened walls, is characteristic, often found in the peripheral areas of the tumor tissue (8, 9). PEComas are often sporadic and in 10% associated with tuberous sclerosis complex (10). Recent studies have shown that sporadic and tuberous sclerosis complex-associated PEComa may respond to mTOR inhibitors underscoring the importance of recognizing this tumor (7). Immunohistochemically, they express both smooth muscle and melanocytic markers, but also show positivity for the myogenic, with variable staining intensity and distribution. They display negative reactions for S100 protein, AE1/AE3, and PAX8, which assists in the differential diagnosis of this tumors (11-12). PEComas have often been confused with smooth muscle tumors as they show overlapping in morphological and immunohistochemical features (7).

Differential Diagnosis

The diagnosis mainly relies on pathological approach and should be differentiated from some other tumors. The differential diagnosis of PEComa, based on morphological and immunohistochemical overlapping includes: Epithelioid Smooth Muscle Tumor (STUMP), Malignant Melanoma (MM), Alveolar Soft Part Sarcoma (ASPS), Poorly Differentiated Endometrial Carcinoma (EC), and Placental Trophoblastic Tumor (PSTT) (10).

PEComas are morphologically well circumscribed or infiltrative with growth patterns in sheets and nests (9, 13). Noncohesive epithelioid cells are with clear to eosinophilic granular cytoplasm. PEComas may have a component of spindled cells (usually minor). Variable cytologic atypia and mitotic index of tumor cells could be present, as well as melanoma-like nucleoli, intranuclear pseudoinclusions, multinucleated cells, Touton's giant cells and melanin pigment (14). Tumor is characterized by thin and delicate vessels but may also have thick walled (generally peripherally located). Radial distribution of tumor cells identified in less than 25% (8). Stromal hyalinization is common. Immunohistochemically, positive on melanocytic markers (HMB45, PNL2, MiTF, Melan A/MART1) and myogenic markers (SMA, Desmin, Caldesmon, Cathepsin K, Estrogen, Progesterone, TFE3 and negative on S100 (focally positive in 20%), AE1/AE3 (focally positive in 11%) and PAX 8 (12).

Epithelioid Smooth Muscle Tumor – STUMP – of the uterus are rare and their prognostic factors are not well established. They have been described under various names, including leiomyoblastoma, epithelioid leiomyoma, clear-cell leiomyoma, and plexiform tumor (15). Most smooth muscle tumors of the uterus can be classified as benign or malignant based on their macroscopic and microscopic characteristics (16). STUMP cells are round, polygonal and spindled shaped. Immunohistochemically, they show positive expression for desmin, H-caldesmon, SMA, ER, PR, and WT1. Immuno-

histochemical STUMP shows negative staining for p16 (which is negative or patchy in STUMP), p53, and CD10 (17). They are usually HMB-45 negative, without characteristic capillarity network of blood vessels. PEComa is supplied by rich blood vessels and the tumor cells surround the blood vessels which are often HMB-45 positive (18).

Malignant Melanoma – MM – genitourinary melanomas represent 0.5% of all malignant melanomas (19-21). Immunohistochemical staining shows that MM exhibits positive expression for melanocytic markers, including MelanA (MART1), HMB45, SOX10, and PRAME. Additionally, positive staining is observed for S100 and nerve growth factor receptor (NGFR). Negative expression is noted for p16 (22).

Alveolar Soft Part Sarcoma – ASPS – is a rare and distinctive sarcoma that typically occurs in young patients. ASPS is characterized by uniform, organoid nests of polygonal tumor cells separated by fibrovascular septa and delicate capillary vascular channels (23-24). These nests exhibit pronounced cellular discohesion, leading to the distinctive pseudoalveolar pattern from which the tumor derived its name. The organoid appearance can be completely lost, and the tumor may be composed of sheets of epithelioid cells (23-27). ASPS shows positive expression in immunohistochemical staining for NSE, S100, TFE, reticulin, desmin, myoglobin, and HHF53. Negative staining for ASPS is observed with GFAP (glial fibrillary acidic protein) and S100 (29).

Poorly Differentiated Endometrial Carcinoma – EC – is the most common gynecological malignancy. Among endometrial cancers this one is, by far, the most prevalent (30). EC typically presents with marked and diffuse cytological atypia and various architectural patterns such as papillary, glandular, or solid growth. Key characteristics defining EC are often absent. Almost every case harbors a TP53 mutation, which is associated with abnormal p53 immunohistochemical expression (31). The immunohistochemical profile of EC includes positive expression for: CK7, CK8/18, CK19, Vimentin, CEA, CA-125, ER, PR, PTEN, CD10, IFITM1, D1, and Cyclin. In poorly differentiated endometrial carcinomas, the immunohistochemical profile of the undifferentiated component shows negative expression for CK7, PAX8, ER, WT1, Claudin4, p16, MLH1, PMS2, MSH2, MSH6, wild-type p53, and loss of expression of SWI/SNF complex proteins (BRG1, INI1, or co-loss of ARID1A and ARID1B) (32).

Placental Trophoblastic Tumor – PSTT – accounts for 0.2–3% of all gestational trophoblastic neoplasms, with an estimated incidence of 1 in 100,000 pregnancies (33-34). It most commonly occurs in women of reproductive age and can follow a normal pregnancy, miscarriage, or gestational trophoblastic disease (35-37). PSTT is characterized by a neoplastic monomorphic population of trophoblastic cells resembling implantation, often appearing as sheets of polygonal, rounded, or occasionally spindled cells that significantly infiltrate the myometrium (38). Tumor cells represent a monomorphic population of large polygonal cells with irregular hyperchromatic nuclei (39). The immunohistochemical profile of PSTT shows positive expression for: HPL, Cytokeratin, MUC4, HLA-G, MEL-CAM (CD146), CD10, GATA3, PDL1, and Ki67. Negative expression is observed with staining for p63, HCG, as well as Inhibin and PLAP (40).

DISCUSSION

In research regarding PEComa, Liu CH et al. detailed 114 cases in their report and found that the melanocytic marker HMB-45 exhibited nearly universal expression, being positive in 113 out of 114 cases. Additionally, among the smooth-muscle markers, desmin was the most frequently expressed, showing positivity in 50 out of 85 cases, which accounts for approximately 58.9%. This suggests a strong association between HMB-45 expression and the conditions studied, while desmin also plays a significant

role, albeit to a lesser extent (41). Bennett et al. reported that HMB-45 and cathepsin K were strongly expressed in all PEComas, with 83% and 93% showing high intensity, respectively. Melan-A and MiTF were found in 77% and 79% of tumors with variable expression. All PEComas exhibited at least one smooth muscle marker, with smooth muscle actin (90%) being the most frequent, followed by desmin (76%) and h-caldesmon (75%). These results highlight the unique immunophenotype of PEComas and their consistent marker profiles (9).

In a recent meta-analysis, Travaglino et al. analyzed immunohistochemical patterns in gynecological STUMPs, classifying p53 as "abnormal" or "wild-type", p16 as "diffuse" or "focal/negative", and Ki-67 levels as ≥ 10% or < 10%. While p53 and p16 aid in risk assessment, they are not standalone prognostic markers (42). Additionally, studies by O'Neill et al. and Ünver NU et al. showed that CD10 negativity and H-Caldesmon positivity can help differentiate endometrial stromal nodules, whereas p16, p53, and Ki-67 are valuable for diagnosing STUMP (43-44). Additionally, several studies prove that PR and ER are commonly expressed in STUMP and leiomyoma but are less frequent in leiomyosarcoma. One study found that high PR and low p53 expression could effectively rule out leiomyosarcoma (44-45).

Comparing information about PEComas and Epithelioid tumors of smooth muscle origin of unknown malignant potential, there is an overlap in certain markers such as Desmin, ER, and PR when observing positive staining, while specific markers for PEComas, such as HMB45, PNL2, MiTF, and MelanA/MART1, are significantly different. Furthermore, when observing negative staining, there is a difference in negative staining between PE-

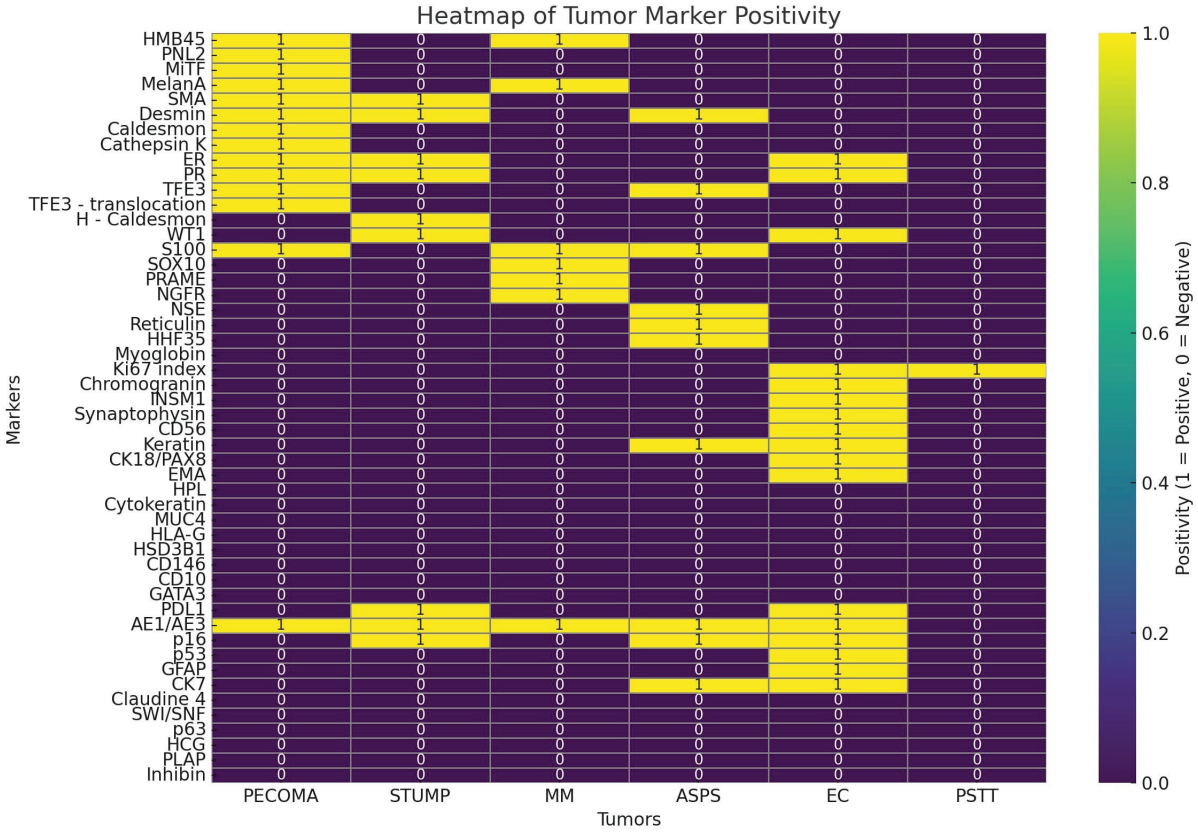

Table 1. The heatmap table lists the positive and negative staining patterns for all differential diagnoses of PEComas, showing the expression of each stain in: smooth muscle cell tumors of unknown malignant potential, malignant melanoma, alveolar soft part sarcoma, poorly differentiated endometrial carcinoma, and placental trophoblastic tumor. Positive staining is indicated by (1) and is colored in yellow. Negative staining is indicated by (0) and in color purple.

PECOMA - Perivascular epithelioid cell tumors; STUMP - study examines epithelioid smooth muscle tumor; MM - Malignant Melanoma; ASPS - Alveolar Soft Part Sarcoma; EC - Poorly Differentiated Endometrial Carcinoma; PSTT - Placental Trophoblastic Tumor.

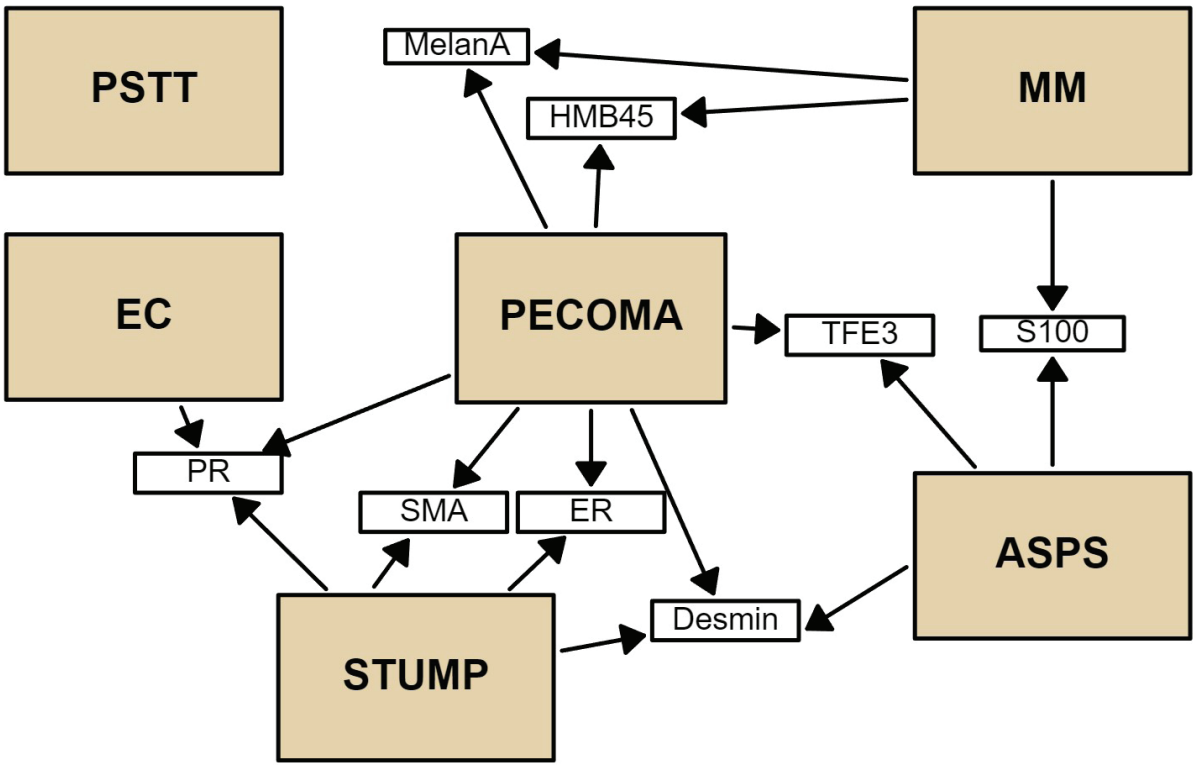

Figure 1. The diagram highlights the overlapping in immunohistochemical markers used to differentiate PEComa from other tumors – some demonstrate diagnostic challenges, while others help narrow the differential diagnosis.
PECOMA - Perivascular epithelioid cell tumors; STUMP - study examines epithelioid smooth muscle tumor; MM - Malignant Melanoma; ASPS - Alveolar Soft Part Sarcoma; EC - Poorly Differentiated Endometrial Carcinoma; PSTT - Placental Trophoblastic Tumor.

Comas and tumors of smooth muscle origin of unknown malignant potential. PEComas are negative for staining with S100, CK18/PAX8, AE1/AE3, while tumors of smooth muscle origin of unknown malignant potential are negative for staining with CD10, p16, p53. Additionally, specificities such as S100 and AE1/AE3, which can be focally positive in PEComas, further highlight the different characteristics of these two entities.

HMB-45 immunostaining revealed positive expression confined to MM and actively proliferating melanocytes, such as junctional nevus cells and cells in Spitz nevus, with no positivity in other skin components. This underscores HMB-45's high specificity for MM diagnosis (46). The results of the study conducted by Xia et al. show that S100 protein is highly sensitive for diagnosing melanoma (MM) and pigmented nevi, with positive rates of 96.8% and 100%, respectively, which is consistent with previous studies. However, S100 is also expressed in normal tissues, including glial cells, Schwann cells, muscle cells, and fibroblasts, which limits its specificity for MM and pigmented skin diseases. In one study, S100 expression was observed in hair follicle myoepithelial cells and some fibrocytes, indicating high sensitivity but low specificity in clinical practice (47-48). Regarding MITF, a higher copy number is associated with lower survival rates and poorer prognosis. However, MITF expression in various pigment diseases and MM has shown inconsistency across studies. It is not considered sensitive or specific for diagnosing desmoplastic and spindle cell melanomas, though it does have advantages in identifying these variants and metastatic melanomas (49-52).

The comparison between PEComas and Malignant Melanoma can be divided into several points based on the type of marker. Thus, we can observe that for melanocytic markers, PEComas show positive expression for staining with HMB45, PNL2, MiTF, MelanA/MART1, while malignant melanoma shows positive expression for HMB45, MelanA/MART1, and PRAME. Both entities share the expression of HMB45 and MelanA/MART1.

PRAME is specific to malignant melanoma, while PNL2 and MiTF are specific to PEComas. Myogenic markers showing immunohistochemical positive expression in PEComas include SMA, Desmin, Caldesmon, Cathepsin K, while malignant melanoma does not show specific myogenic markers with positive expression, leading to the conclusion that PEComas exhibit variable expression of myogenic markers, while malignant melanoma does not have specific markers in this context. Regarding hormonal receptors, PEComas show positive expression for ER, PR, and TFE3, while malignant melanoma does not have specific hormonal receptors, thus concluding that hormonal receptors are specific to PEComas, which may be important in differential diagnosis. Among other markers with positive staining, PEComas show focal positive expression for S100 in 20% of cases, while malignant melanoma shows strong positive expression for S100 staining, as well as SOX10, NGFR, and PRAME. Thus, PEComas and malignant melanoma have similarities in the expression of certain melanocytic markers but differ in the expression of myogenic markers and hormonal receptors. The highest sensitivity for visualizing invasive melanoma is shared between both tumors through S100, Sox10, and NGFR. Negative staining contributes to the differentiation between PEComas and malignant melanoma. While PEComas may show focal positivity for S100, specific negative staining such as CK18/PAX8 and AE1/AE3 can be helpful in distinguishing them from malignant melanoma, which is characterized by a complete loss of p16 expression.

ASPS are proven negative for epithelial markers like cytokeratins and epithelial membrane antigen, as well as for neuroendocrine markers such as chromogranin A and synaptophysin. They also lack specific melanocytic markers like HMB-45 and Melan-A. Non-specific markers, including neuron-specific enolase and vimentin, may be present in about 30–50% of cases. Interest in muscle-related protein expression in ASPS arises from the belief that it represents an unusual form of myogenic tumor. Antibodies to pan, smooth, and skeletal muscle actins have shown positivity in nearly 50% of cases, though actin expression is not specific for myogenic differentiation. Desmin is expressed in around 50% of ASPS cases but usually in only a small subset of neoplastic cells. It is important to recognize that desmin is not exclusive to myogenic tumors and can also be found in lesions such as melanoma, tenosynovial giant-cell tumor, Ewing's sarcoma, and angiomatoid "malignant" fibrous histiocytoma (28).

Comparing positive and negative markers between PEComas and Alveolar Soft Part Sarcoma, we conclude that in terms of positive staining, PEComas and alveolar soft part sarcoma share positivity for Desmin, but PEComas also show variable expression of other markers. Myogenic markers expressed by PEComas are SMA, Desmin, and Caldesmon, while alveolar soft part sarcomas emphasize Reticulin, Desmin, HHF53, and Myoglobin, which indicates overlaps in differential diagnosis, as both diagnoses show positive expression for desmin. In comparing hormonal receptors between PEComas and alveolar soft part sarcoma, only PEComas show expression for ER and PR, while alveolar soft part sarcoma does not. However, in some cases, TFE3 may be specific to both tumors under certain conditions. Comparing negative staining, we conclude that PEComas may show focal positivity for S100 and are negative for AE1/AE3, while alveolar soft part sarcoma is positive for S100, which may help in differential diagnosis. Additionally, alveolar soft part sarcoma shows negative expression for GFAP. Although PEComas and alveolar soft part sarcoma share some positive staining, differences in the expression of melanocytic, myogenic markers, hormonal receptors, and negative staining enable their differentiation in pathological analyses.

The extent of p16 expression helps distinguish between uterine serous and grade 3 endometrioid carcinomas. Serous carcinomas show p16 in 90–100% of cells, while grade 3

endometrioid tumors show 10–90%. A study found that serous carcinomas are typically ER/PR-negative, PTEN-positive, diffusely p16-positive, and show aberrant p53 staining. In contrast, grade 3 endometrioid tumors are often ER/PR-positive, PTEN-negative, focally p16-positive, and exhibit wild-type p53 (53). An immunohistochemical analysis of 180 cases (34 grade 3 endometrioid, 15 serous) showed p53, p16, and PTEN were more frequently expressed in serous tumors (69%, 90%, 100%) than in grade 3 endometrioid tumors (39%, 19%, 61%) (54). While WT1 is not routinely used for differential diagnosis, its diffuse expression suggests serous carcinoma (55). Han et al.'s study of 12 markers found that TFF3, ARID1A loss and beta-catenin were highly specific but had low sensitivity for endometrioid carcinoma. p53 (94%), p16 (80%), and IMP3 (63%) were strongly associated with serous carcinomas, compared to lower rates in grade 3 endometrioid tumors (26%, 11%, 11%) (56).

In differentiating PEComas and poorly differentiated endometrial carcinoma, PEComas show positive expression for melanocytic markers – HMB45, PNL2, MiTF, MelanA/MART1 – and myogenic markers – SMA, Desmin, Caldesmon, Cathepsin K, while poorly differentiated endometrial carcinoma shows positive expression for marker Ki67, which has an elevated index in this case, EMA, Keratin, PR, Chromogranin, INSM1, synaptophysin, and CD56. PEComas show negativity for S100, AE1/AE3, PAX8, while poorly differentiated endometrial carcinoma shows negativity for p53, p16, CK7, PAX8, ER, WT1, Claudin4, SWI/SNF complex, and mismatch repair deficiencies (MLH1, PMS2, MSH2, MSH6). PEComas are recognized by the expression of markers indicating melanocytic and myogenic differentiation, while poorly differentiated endometrial carcinoma shows specificity in the expression of markers associated with high proliferative index (Ki67), epithelial and neuroendocrine characteristics, which is crucial for establishing the correct diagnosis and for choosing treatment and prognosis for the patient.

Human placental lactogen (hPL) is typically highly expressed in histological sections and serum, with upregulation of β1-glycoprotein and CA-125 also common (57).

Comparing information on PEComas and Placental Trophoblastic Tumor, it can be concluded that PEComas show positive staining for markers related to melanocytic and myogenic differentiation, while placental trophoblastic tumors show specificity in the expression of markers related to trophoblastic differentiation and implantation – HPL, Cytokeratin, MUC4, HLA-G, HSD3B1, MEL-CAM, CD10, GATA3, PDL1. Also, PEComas are negative for S100, AE1/AE3, PAX8, while placental trophoblastic tumors are negative for p63, HCG (which is focally expressed in some cases), PLAP, and Inhibin (focally expressed). PEComas and placental trophoblastic tumors show different markers, reflecting different differentiation lines of these tumors. PEComas are characterized by the expression of melanocytic and myogenic differentiation markers, while placental trophoblastic tumor shows specificity for markers of trophoblastic differentiation and implantation.

PEComas, or perivascular epithelioid cell tumors, are a rare form of soft-tissue sarcoma characterized by their origin from perivascular epithelioid cells. Malignant PEComas (mPEComas) are particularly aggressive, often resulting in local and distant recurrences. In diagnosing and treating mPEComas, a significant advancement emerged from the AMPECT trial, which tested nab-sirolimus, an mTOR inhibitor, and demonstrated a response rate of 39% with a median progression-free survival of 8.9 months. In patients with TSC2 mutations, the response rate was even higher at 89% (58). In this issue, Akumalla et al. conduct a detailed analysis of the genomic landscape of malignant PEComas using next-generation sequencing (NGS). Their findings enhance our understanding of the pathogenesis of mPEComas and clarify why mTOR inhibitors are effective. Previous studies established that TSC1/2 gene inactivation is common in PEComas, leading to mTOR pathway activation. However, this study re-

veals that the genomic landscape of mPEComas is diverse, but they predominantly operate through the mTOR pathway (59).

Another study conducted by Groisberg et al. explores advanced gene inactivation mechanisms in PEComas, particularly loss of heterozygosity (LOH). It reveals that TSC1/2 is often bi-allelically knocked down via LOH, even in patients with "wild-type" TSC1/2, who still show mTOR pathway inactivation. Notably, FLCN mutations and unique TFE3 fusion partners also contribute to this pathway. While 31 cases were analyzed, only 20 could be explained, suggesting that unexplained mPEComas may still activate the mTOR pathway through alternative mechanisms. Future studies should adopt more comprehensive sequencing methods, like single-cell RNA sequencing. The findings stress the utility of next-generation sequencing (NGS) in understanding sarcoma subtypes and highlight the need for detailed genomic profiling beyond common mutations. Additionally, they advocate for targeted therapies focused on specific aberrations in ultra-rare tumors like mPEComas. Overall, the evolution of NGS demonstrates its increasing practicality in cancer research, signaling the importance of new technologies for future discoveries (60).

CONCLUSION

In comparison to smooth muscle tumors of unknown malignant potential, PEComas exhibit overlap in positive staining but are distinguished by specific markers such as HMB45, PNL2, MiTF, and MelanA/MART1. Relative to Malignant Melanoma, PEComas share some melanocytic markers, while differing in the expression of myogenic markers and hormonal receptors, which is crucial for differentiating these two entities. In comparison to Alveolar Soft Part Sarcoma, PEComas and this sarcoma share some positive staining (e.g., Desmin) but differ in the expression of markers and in negative staining, allowing for precise pathological diagnosis. Compared to poorly differentiated endometrial carcinoma, PEComas are distinguished by the expression of specific markers such as MiTF and by negative expression of CK18/PAX8, while endometrial carcinoma is characterized by positive expression of CK18/PAX8 and by the expression of markers associated with a high proliferative index and epithelial characteristics. Comparison with placental trophoblastic tumor highlights the diversity of markers reflecting different differentiation lines, with PEComas emphasizing melanocytic and myogenic differentiation, while placental trophoblastic tumor shows specificity for markers of trophoblastic differentiation and implantation.

After conducting the study, the general conclusion is that the definitive diagnosis of PEComa originating from the female genital system, can only be achieved through a multidisciplinary approach. Immunohistochemical evaluation of tumor cells is a good "helper", but for a definitive diagnosis, standard staining and morphological evaluation remain the "gold standard".

Also, the advanced diagnostic techniques, particularly next-generation sequencing, hold promise for enhancing the understanding and management of mPEComas in the future. By uncovering the complex genomic landscape and facilitating targeted therapies, these methodologies may lead to more effective treatment strategies and improved patient outcomes.

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REVIEW ARTICLE

Rediscovering Hormone Replacement Therapy in Menopause: Understanding the Balance of Benefits and Risks through Landmark Studies

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Abstract

Menopause represents an inevitable transition in a woman's life, presenting with vasomotor symptoms, mood disorders, sleep difficulties, and prolonged risks such as osteoporosis and cardiovascular diseases. Hormone replacement therapy emerged as the cornerstone of menopausal management, particularly for alleviating symptoms and preventing postmenopausal osteoporosis.

However, findings from the Women's Health Initiative (WHI) study in 2002 highlighted increased risks of breast cancer, cardiovascular disease, and stroke associated with hormonal replacement treatment, leading to a significant global decline in its usage. Consequently, numerous women were deprived of essential therapy, endangering their health and quality of life.

This review presents the findings of the WHI study, discusses its methodological errors, and evaluates its benefits and harms. We explore landmark studies that have reestablished the benefits and risks of hormone replacement therapy over the past two decades. Guidelines supported by these findings are presented in this review.

Despite advancements, public perception of hormone replacement treatment remains influenced by outdated findings, limiting its utilization in many regions, especially in developing countries. Our objective is to provide evidence that misconceptions about hormone replacement therapy significantly impact women's general health and quality of life, as well as to clarify the short-term and long-term impacts of hormone replacement therapy.

We conclude that hormonal replacement treatment is effective and safe when administered according to established guidelines. Access to information, coupled with knowledgeable physicians who consistently interact with women, is as vital as the contributions of menopause healthcare specialists. Conflicting information from outdated professionals can likely lead to treatment failure in patients.

Keywords: menopause, women's health, estrogens, progestins, quality of life

INTRODUCTION

Menopause represents a transitional phase in a woman's life, marked by the cessation of ovarian function and the consequent decrease in estrogen levels. The most common symptoms associated with menopause are vasomotor symptoms (VMS), mood disorders, anxiety, depression, and sleep difficulties. Prolonged risks of menopause are most often osteoporosis and cardiovascular diseases. Vasomotor symptoms affect 30 to 80% of women (1). They are closely related to depression (2, 3) and reduced quality of life (4).

Throughout history, menopause has been inadequately researched and often seen as a natural phase of aging. This led to a lack of adequate medical and psychosocial support for most women. A key milestone in the field was the WHO Report on Menopause Research in the 1990s, which underscored menopause as a public health problem (5). This report emphasized the correlation between osteoporosis and cardiovascular risks during menopause, indicating that the prevalence of osteoporosis and heart disease nearly doubles within ten years post-menopause (5).

Hormone replacement therapy (HRT) has since then been considered as a gold standard for alleviating menopausal symptoms and mitigating postmenopausal osteoporosis. In the United States, roughly 38%–40% of postmenopausal women utilized hormone replacement therapy, equating to almost 15 million women annually in the late 1990s. However, everything changed when the results of the Women's Health Initiative study were released in early 2000, and for the last two decades, HRT has been rediscovered.

Our objective is to provide evidence that misconceptions about hormone replacement therapy significantly impact women's general health and quality of life, as well as to clarify the short-term and long-term impacts of hormone replacement therapy.

RESULTS OF THE WOMEN'S HEALTH INITIATIVE STUDY

The Women's Health Initiative (WHI) study included over 161,000 postmenopausal women. The primary goal of the study was to examine the impact of hormone therapy on cardiovascular health and bone density, and the incidence of breast cancer due to the fear of a connection between estrogen and breast cancer. Two interventions were used: combined estrogen-progestin therapy for women with a uterus and estrogen-only therapy for women without a uterus. The results, published in 2002, had significant impact at menopause management.

The WHI found that women on combined HRT had a 26% increased risk of breast cancer Furthermore, there was a twofold increase in the incidence of venous thromboembolism and an increased risk of coronary heart disease and stroke (7).

Women on estrogen-only therapy (who did not have a uterus) did not show a significant increase in the risk of breast cancer but did show increased risk of stroke and venous thromboembolism (8).

These findings contradicted earlier observational studies that suggested a cardioprotective effect of hormone replacement treatment (7). The study confirmed the efficacy of hormone therapy in reducing the risk of osteoporosis-related fractures by addressing fracture frequency and improving bone density (7).

WOMEN'S HEALTH INITIATIVE STUDY IMPACT

The negative findings of the study attracted significant attention and raised concerns within both medical and public domains. The media indicated that hormone replacement therapy presents more hazards than advantages for all women. Following such an impact, there was a swift decline in the prescription of hormone therapy. In the United States, the utilization of HRT diminished by around 50% over the subsequent two years (9).

Subsequent years saw the emergence of skepticism over the findings of the WHI study. The study was considered to have methodological and statistical flaws. The selection of subjects was unacceptable, as the study involved elderly women who experienced menopause over a decade ago, putting the implications of cardiovascular illnesses and breast cancer inapplicable to healthy younger women. Subsequently, only one type of conjugated estrogen (CEE) and progestin (medroxyprogesterone acetate) were evaluated. The risks associated with these formulations cannot be generalized to other formulations, including transdermal estrogen and micronized progesterone. Regardless, the results of this study caused considerable harm prior to being reevaluated. A large number of physicians stopped prescribing hormone replacement therapy, which resulted in a concomitant decrease in the number of women utilizing this treatment.

KEY PITFALLS OF THE WOMEN'S HEALTH INITIATIVE STUDY

Although the Women's Health Initiative (WHI) study was groundbreaking, it has met with significant criticism for its methodology, population selection, and interpretation of results. The main pitfalls are as follows:

1. Inclusion of Older Participants

The subjects in the study were women between the ages of 50 and 79, with an average age of 63. Therefore, its results cannot be generalized to the risk of cardiovascular disease and breast cancer in younger women (10).

2. Neglected Time Hypothesis

Most of the women were 10 or 20 years after menopause. Which means that the study did not take into account the "temporal hypothesis", according to which the impact of HRT on cardiovascular status depends on the time of initiation of therapy. HRT was introduced to participants even a decade or more after menopause; therefore, it resulted in increased cardiovascular risk. The applicability of these results to women who started therapy closer to menopause is limited (11).

3. Use of Conjugated Equine Estrogens (CEE) and Medroxyprogesterone Acetate (MPA)

The study used CEE and MPA, which do not cover all HRT formulations. Modern formulations, such as transdermal estradiol and micronized progesterone, show a reduced risk. Therefore, these results are not applicable to newer and safer regimens of hormone replacement therapy (12).

4. Overestimated Breast Cancer Risks

In this study, the risks associated with HRT and the risk of breast cancer are overemphasized. The association of these risks did not take into account the type of replacement therapy, duration, or time of introduction. On the other hand, therapy with estrogen alone did not lead to an increase in the risk of cancer, but these results were not sufficiently presented (13). Furthermore, the reported 26% increase in breast cancer cases corresponds to a total of 8 additional cases per 10,000 patients. This translates to an increase in absolute risk of 0.08% for breast cancer (14).

5. Ethnic and Demographic Limitations

The subjects were predominantly white postmenopausal women, which limits the applicability of the findings to other ethnic groups. The results do not represent a different population of menopausal women worldwide (15).

Short Follow-Up for Primary Outcomes

Postmenopausal women treated with combined therapy were observed for an average of 5.2 years, whereas those receiving estrogen therapy alone were observed for 7.1 years, which is insufficient for a thorough evaluation of long-term risk. The brief follow-up period may have compromised the evaluation of long-term cardiovascular risks, as well as those related to osteoporosis and dementia (16).

Media Misinterpretation of Results

Media reports after the release of the WHI study results outlined the risks of hormone therapy for all women in general, lacking appropriate context. This resulted in increased fear, and many women then denied therapy, resulting in increased risks of osteoporosis, cardiovascular disease, and diminished quality of life (6).

LANDMARK STUDIES BEHIND THE CURRENT STANCE

Over the past two decades, research on menopause has focused on challenging the findings of the WHI study. The new studies provided important insights into the true risks and benefits. Their findings outline the significance of initiating hormone replacement therapy in younger women, ideally within 10 years post-menopause—while considering individual risk assessments. This approach has minimized risks and optimized benefits. Our review outlines significant studies that have influenced the current understanding of the safety and efficacy of hormone replacement therapy.

CURRENT GUIDELINES ON HORMONE REPLACEMENT THERAPY: COMBINED NICE AND NAMS PERSPECTIVES

The National Institute for Health and Clinical Excellence (NICE) guidelines highlight the importance of an individual approach to hormone replacement therapy taking into account woman's age, symptoms, and associated risk factors. Estrogen-only therapy is safer for women lacking uterus, whereas combined estrogen-progestogen therapy is essential for those with a uterus to avert endometrial hyperplasia. NICE confirms the effectiveness of HRT in alleviating vasomotor symptoms and preventing osteoporosis. However, it is recommending against its use

Table 1. Landmark post-WHI studies on menopause overview

Trial	Sample	Findings
KEEPS (Kronos Early Estrogen Prevention Study) (2005-2010) (17)	727 healthy women in early postmenopausal aged 48 to 58	low doses of oral and transdermal estrogen improve vasomotor menopausal symptoms and quality of life; no negative cardiovascular risks were observed; based on this research, low-dose hormone replacement therapy is safe and effective for younger, healthy postmenopausal women
Danish Osteoporosis Prevention Study (DOPS) (1990-2010) (18)	2016 healthy women aged 45-58	overall fracture risk and the risk of forearm fractures were significantly reduced in woman using HRT
WHI Follow-Up Studies (2013, 2017, 2020) (19)	27000 women aged over 13	HRT in women younger than 60 or within ten years after menopause does not significantly increase cardiovascular risk while providing relief of symptoms and improving quality of life
ELITE (Early versus Late Intervention Trial with Estradiol) Study (2005-2011) (20)	643 postmenopausal women divided into two groups: early and late postmenopausal	progression of atherosclerosis was significantly reduced in women who started HRT 6 years before the onset of menopause: this protective effect was not found in women who started therapy six years after the onset of menopause
Collaborative Group on Hormonal Factors in Breast Cancer (CGHFBC) (2019) (13)	meta-analysis of 54 studies in 26 countries included 53,297 women with breast cancer and 100,239 women without breast cancer	small increase in breast cancer risk in current users of combined oral contraceptives (COC) and in women who had stopped use in the past 10 years, without evidence of an increased risk in more than 10 years after stopping use.
The ESTHER Study (1998-2018) (21)	271 women over 20 years evaluated transdermal estrogen treatment compared to oral HRT	transdermal estrogen was associated with a lower risk of venous thromboembolism compared to oral formulations
WHI Estrogen-Alone Trial (2004, Follow-Up 2020) (11)	10,739 postmenopausal women over seven years	20% reduction in breast cancer incidence on conjugated equine estrogen monotherapy
SWAN Study (1996-present) (22)	3302 ethnically diverse women aged 42–52 investigated physiological changes related to menopause	–5% annual decline in bone mineral density (BMD) during early postmenopause and significant associations between hormonal changes and increased risks of osteoporosis, cardiovascular disease, anxiety, depression, and metabolic syndrome
Cognitive Health and Dementia Studies (2021) (23)	more than 8,000 postmenopausal women	HRT may contribute to cognitive improvement and reduce the risk of dementia when started early

for the primary prevention of cardiovascular disease or dementia (24).

The Menopause Society, formerly the North American Menopause Society (NAMS), guidelines are consistent with NICE in endorsing hormone replacement therapy for symptomatic women, especially those under 60 or within a decade of menopause onset. They recommend utilizing low doses for the minimal duration required while also taking into account newer hormone replacement therapy formulations, such as transdermal estradiol and micronized progesterone, because of their advantageous safety profiles (25). Both organizations highlight the significance of shared decision-making grounded in individual benefits and risks.

Key Recommendations of NAMS Hormone Therapy Position Statement

- Hormone replacement treatment is the most efficacious treatment for vasomotor and genitourinary symptoms of menopause and mitigates bone loss and fractures.
- The risk associated with hormone therapy is dependent upon the type, dosage, duration, mode of administration, and the time of therapy initiation. A personalized strategy yields the optimal benefit-risk ratio.
- The advantages of the therapy surpass the possible hazards when administered to women under 60 years of age or within 10 years of starting menopause.
- For genitourinary symptoms that do not respond to systemic hormone therapy, recommend using low-dose vaginal estrogen or alternative approved treatments like vaginal DHEA or oral ospemifene (25).

DISCUSSION

The Women's Health Initiative study showed a strong association between the risk of breast cancer, cardiovascular events and stroke, and hormone replacement therapy. These results were followed by a significant reduction in the HRT use.

Over the past two decades, there has been a renewed interest in hormone replacement therapy. Subsequent research showed numerous flaws in WHI study and pointed out to the effectiveness and safety of HRT if the approach is individualized, especially if it takes into account age, time of introduction of therapy, associated diseases, and HRT formulation. Today, we possess robust evidence regarding the risks and benefits of HRT, as well as evidence-based guidelines for its use.

Studies such as DOPS, WHI Follow-Up Studies, and the Global Consensus Statement on Menopausal Hormone Therapy have not observed a significant increase in breast cancer risk (18, 19). The results from the Collaborative Group on Hormonal Factors in Breast Cancer (CGHFBC) show that combined estrogen-progestogen therapy in combiner oral contraceptives is linked to a slightly higher risk, but the absolute risk is still very low (13). The estrogen and progestin used in combined oral contraceptives are different from the estrogen and progesterone used in hormone replacement therapy. COCs contain conjugated hormones, while HRT contain regulated bioidentical hormones. The doses in HRT are also much lower because it is not aimed at stopping ovulation, which is the main goal of COCs. On the other hand, the WHI estrogen-alone trial found a 20% reduction in breast cancer incidence in estrogen treatment only, and long-term follow-up confirms continued safety (8). Several studies, such as KEEPS, ELITE, and DOPS, supported the "timing hypothesis" (18), suggesting that starting hormone therapy nearer to menopause may provide cardiovascular advantages and lower risks (17, 18, 20).

When considering the type of HRT formulation, findings from KEEPS, ESTHER, and WHI follow-up studies may be beneficial. Findings stated that transdermal estrogen improved vasomotor menopausal symptoms and quality of life with no increase in cardiovascular risk. The findings revealed a lower associa-

tion between transdermal estrogen and venous thromboembolism when compared to oral formulation. These findings also provide reassurance regarding the risk of breast cancer (18, 25). Hormone therapy clearly benefits bone health and reduces fracture risk (16, 19). Furthermore, research such as SWAN and Cognitive Health and Dementia Studies (2021) indicates that the early commencement of hormone therapy may help maintain cognitive function (19, 23).

A key takeaway from post-WHI research is the necessity of customized HRT approaches. This approach leads us to a personalized, evidence-based practice that balances risks and benefits.

Bosnia and Herzegovina is a developing country. Despite the medical profession's efforts to align with contemporary medicine on critical topics, several matters remain unaddressed – menopause being one of them. In our society, women's health during menopause is often viewed as a luxury rather than a fundamental necessity. The addition of prejudice-based treatment to this fact creates a vicious cycle that affects menopausal women. For medical professionals, breaking this cycle with knowledge is essential to paving the way for a medically evidence-based approach that ensures a fulfilling and balanced midlife experience for women. This review is significant as it represents an initial step in this direction, setting a starting point for further progress and research in this field in our country.

CONCLUSION

Regardless of numerous studies and results obtained in the last twenty years, the public's opinion about HRT is mostly unchanged, especially in developing countries. This outdated view has adversely impacted the health and quality of life of women in many regions globally.

Menopausal hormone therapy is the most effective intervention for vasomotor symptoms. There is no alternative that is as effective as hormone replacement therapy in treating menopause symptoms and preventing diseases such as cardiovascular disease, cognitive decline, and osteoporosis. However, currently, hormone replacement therapy is only approved for VMS and osteoporosis prevention.

Unless there are contraindications, clinicians should thoroughly evaluate the short- and long-term benefits and risks before providing treatment to women seeking HRT. Research indicates that hormone therapy is both safe and effective for women under 60 years of age, with menopause onset within 10 years. The risk is further minimized with treatment duration of less than 5 years, along with the use of micronized progesterone (for women with uterus) and transdermal preparations of estrogen.

Enhanced access to information in less developed countries, coupled with knowledgeable physicians who consistently interact with women, is as vital as the contributions of menopause healthcare specialists. Conflicting information from outsourced medical professionals can likely lead to a treatment failure in patients.

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REVIEW ARTICLE

Telmisartan – A Potent Antihypertensive With Proven Cardio-Renal-Metabolic Beneficial Effects

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Abstract

Due to an epidemic of risk factors, such as hypertension, and an increase in life expectancy, cardiovascular disease (CVD) has an overwhelming morbidity and mortality burden worldwide.

Various treatment options are available to disrupt pathophysiological processes along the cardiovascular continuum by focusing on distinct regions of the renin-angiotensin-aldosterone system (RAAS). As a RAAS inhibition, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are recommended first-line treatments for hypertension and CVD. Both ACE inhibitors and ARBs prevent CVD by lowering blood pressure (BP). Furthermore, a number of studies have shown that RAAS blockade can lower cardiovascular risk in ways that go beyond what could be predicted from lowering blood pressure alone.

However, the ARBs are not all equally effective. Telmisartan is a long-lasting ARB that effectively controls BP over the full 24-hour period. In high cardiovascular risk patients, telmisartan reduces cardiovascular events in a manner comparable to that of the ACE inhibitor ramipril beyond lowering blood pressure alone, but with better tolerability.

Research points to possible benefits for adipose tissue activity, neurovascular function, and enhancements in glucose and lipid metabolism. According to several studies, telmisartan has partial peroxisome proliferator-activated receptor gamma (PPARγ) agonist activity, which improves insulin resistance in diabetic patients by modifying adipokine levels.

The combination of telmisartan and indapamide as metabolically neutral diuretic has an additional positive antihypertensive as well as cardioprotective effects.

In addition to reviewing current CVD management guidelines, this article will examine important clinical trial and clinical practice data that assess the role of telmisartan/indapamide in CVD.

Keywords: arterial hypertension, angiotensin II receptor blocker, telmisartan, cardiovascular risk.

INTRODUCTION

Arterial hypertension is a leading factor in the global disease burden yet control of the condition remains inadequate (1). The initiation of antihypertensive therapy should be guided by blood pressure measurements and the presence of elevated atherosclerotic cardiovascular disease (CVD) risk. First-line pharmacologic management for hypertension typically includes an angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB), combined with thiazide or thiazide-like diuretic and/or a calcium channel blocker. Dosages of these medications should be titrated based on both office and home systolic and diastolic blood pressure readings to achieve a target systolic blood pressure (SBP) and diastolic blood pressure (DBP) of <130/80 mm Hg for treated individuals with the use of fixed-dose single-pill combination treatment (1, 2).

The choice of therapy should be in accordance with the systolic and diastolic function of the left ventricular heart, renal and liver function, the presence of comorbidities, primarily diabetes mellitus, all with the goal of impacting primary and secondary prevention of cardiovascular events (3). The selection of pharmacological agents should be based on the pharmacokinetic and pharmacodynamic properties of the drug, with careful consideration of the atherosclerotic process throughout the entire body, including its effects on cerebral and renal circulation (4).

The most recent international guidelines recommend using ACEIs or ARBs in patients with coexisting microalbuminuria, renal dysfunction, and chronic kidney disease (CKD), as well as those with metabolic syndrome, diabetes mellitus (DM), atherosclerosis, chronic stable angina, a history of myocardial infarction (MI), atrial fibrillation (AF), and heart failure (HF) (1, 5, 6).

Angiotensin Receptor Blockers

Guidelines recommend both angiotensin receptor blockers (ARBs) and ACE (angiotensin-converting enzyme) inhibitors as first-line treatments for hypertension. ACE inhibitors and ARBs did not differ statistically significantly in their effects on AMI, HF, stroke, or composite CVEs; however, ARBs exhibited a better safety profile with reduced risks for cough, GI bleeding, angioedema, and acute pancreatitis (3). ACE-Is are less likely to be tolerated than ARBs, besides that ARBs were shown to reduce withdrawal due to adverse effects. For patients unable to tolerate ACEI therapy due to side effects such as a cough or angioedema, ARBs are recommended as an alternative. The currently available angiotensin receptor blockers include azilsartan, candesartan, eprosartan, irbesartan, valsartan, losartan, telmisartan, and olmesartan (3).

Telmisartan

Telmisartan is indicated for treatment of arterial hypertension and for reducing cardiovascular mortality in adults with risk factors for serious cardiovascular events, especially in those who do not tolerate angiotensin-converting enzyme inhibitors (ACEIs) (3). Additionally, it is utilized for the prevention of stroke and myocardial infarction (3). Telmisartan acts by selectively blocking the binding of angiotensin II to AT1 receptors, resulting in vasodilation, decreased aldosterone production, and reduced sodium and water retention (7). Its long biological half-life allows for sustained 24-hour blood pressure control with a once-daily dosing regimen (8). At an 80 mg dose, telmisartan's antihypertensive efficacy is comparable to amlodipine and significantly superior to enalapril and ramipril (9).

Unlike ACE inhibitors, telmisartan does not inhibit angiotensin-converting enzyme (kinase II), which is responsible for the degradation of bradykinin. As a result, it does not induce bradykinin-mediated side effects, such as persistent cough (10). Beyond its antihypertensive effects, telmisartan offers additional cardiovascular benefits, including a reduction in the risk of myocardial infarcti-

on and a decrease in left ventricular hypertrophy (11). In terms of renal function, it effectively lowers blood pressure and reduces proteinuria, particularly in patients with diabetic nephropathy (12).

Several studies have indicated that telmisartan possesses partial peroxisome proliferator-activated receptor gamma (PPARγ) agonist activity, which modulates adipokine levels to improve insulin resistance in diabetic patients. Furthermore, there is a growing body of evidence that activators of PPARγ-gamma exert anti-inflammatory, anti-oxidative and anti-proliferative effects on vascular wall cells, thus decreasing the risks for atherosclerosis. This may provide additional therapeutic benefits for conditions such as diabetes mellitus, obesity, and hyperlipidemia, without the safety concerns typically associated with full PPARγ agonists (13).

Additionally, indapamide that comes in a single-pill combination with telmisartan has positive metabolic effects. Because it has less of an effect on glucose tolerance and lipid metabolism than some other diuretics, it is a good choice for patients with metabolic issues like diabetes or dyslipidemia (14).

Consequently, a fixed-dose combination of telmisartan and indapamide may be a viable alternative for managing hypertension and all components of metabolic syndrome (13).

These pleiotropic effects position the telmisartan and indapamide combination as a promising therapeutic approach, offering comprehensive cardiovascular management beyond blood pressure control. This combination has been shown to decrease fasting plasma glucose (FBG), fasting plasma insulin, homeostasis model assessment (HOMA) index, and triglyceride (TG) levels (14).

Among angiotensin receptor blockers (ARBs), telmisartan has the longest duration of action, providing 24-hour blood pressure control. This extended effect is crucial, as the incidence of acute myocardial infarction and other cardiovascular events exhibits circadian variation, with higher incidence observed during the morning (15).

With multiple benefits for all elements of the metabolic syndrome, such as obesity, diabetes mellitus, hypertension, and hyperlipidemia, telmisartan may therefore be regarded as a multi-useful therapeutic option.

INDICATIONS

The indications based on clinical studies that evaluated the use of telmisartan are presented in Table 1.

TELMISARTAN AND INDAPAMIDE IN METABOLIC SYNDROME

Telmisartan, a partial peroxisome proliferation-activator receptor gamma (PPARγ) agonist, is useful in prevention and treatment of type 2 diabetes mellitus because it can improve insulin sensitivity and decrease ectopic fat deposition (14). Data from numerous recent studies suggest that the AT-1 agonist telmisartan, either as a result of its AT-1 antagonistic and/or PPARγ agonistic properties, may also have beneficial metabolic effects including improvements in glucose and lipid profiles, adipokine levels and/or fat distribution and body weight (16-19).

Peng et al. demonstrated in a study involving 221 patients that the combination of telmisartan and indapamide has an effect on the incidence rate of metabolic syndrome (14). Weidmann showed that indapamide did not significantly impact glucose and lipid metabolism (16). Imenshahidi et al. suggest that telmisartan is effective in preventing and improving metabolic syndrome, and this well-tolerated drug can be strongly recommended for treating various components of metabolic syndrome (16-19).

Telmisartan's ability to lower blood sugar has been attributed to a variety of mechanisms. One of these thought-to-be mechanisms is antioxidant activity. Another way telmisartan helps to restore insulin resistance is by reducing the inflammatory response in adipose tissue (13).

Table 1. Telmisartan in clinical trials

Study	Sample	Methodology	Findings
The Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET) (15)	25,620 pts HTN with coronary, peripheral, or cerebrovascular disease or diabetes with end-organ damage	Prospective, randomized 4.7 years Subgroup: Telmisartan 80 mg/day vs ramipril 10 mg/day	Mean blood pressure was lower in telmisartan group than in ramipril group. Telmisartan 80 mg was as effective as ramipril 10 mg in preventing new-onset diabetes in patients at high vascular risk. Telmisartan was equivalent to ramipril in patients with vascular disease or high-risk diabetes and was associated with less angioedema.
The Telmisartan Randomised AssessmeNt Study in ACE iNtolerant subjects with cardiovascular Disease (TRANSCEND) (15)	5926 pts Intolerance to ACE inhibitors + coronary, peripheral, or cerebrovascular disease or diabetes with end-organ damage	Prospective, randomized 4.7 years Telmisartan 80 mg/day vs placebo	Patients who could not tolerate ACE inhibitors responded well to telmisartan. Telmisartan 80 mg was more effective than placebo in LVH reduction. The group taking telmisartan experienced a 37% decrease in new-onset LVH. Telmisartan modestly reduces the risk of the composite outcome of cardiovascular death, myocardial infarction, or stroke.
Telmisartan to Prevent Recurrent Stroke (PRoFESS) (17)	20,332 pts Age 50 years + recent ischemic stroke (120 days)	Prospective, randomized 2.5 years 80 mg/day vs placebo	Significant advantage for stroke and cardiovascular events was seen after 6 months (considerable time may be necessary to modify the atherosclerotic process).
The prospective, randomized investigation of the safety and efficacy of telmisartan versus ramipril using ambulatory blood pressure monitoring (PRISMA I) (18)	801 patients with mild-to-moderate hypertension	Prospective, randomized telmisartan 80 mg for 14 weeks or ramipril 5 mg for 8 weeks and then force titrated to ramipril 10 mg for the last 6 weeks.	Telmisartan is much more effective than ramipril at lowering blood pressure over the course of the 24-hour dosage period, especially in the final six hours, when there is a higher chance of cerebro- and cardiovascular events. Both drugs were well tolerated, although ramipril was associated with a higher incidence of cough.
Sanchez Muñoz-Torrero JFS et al. (12)	42 patients Homeostatic model assessment–insulin resistance (HOMA-IR) in hypertensive subjects with MS	Cytokines and metabolic parameters were analyzed before and after treatment with telmisartan	Telmisartan treatment reduced HOMA-IR by 35%. In MS patients with low serum cytokine levels, telmisartan had more positive effects on glucose homeostasis.
Jie Peng et al. (14)	221 subjects with high-normal blood pressure were randomly assigned to telmisartan, 213 to indapamide and 230 to placebo	Prospective 3 years Metabolism changes especially in abdominally obese individuals	The intervention for high-normal blood pressure with telmisartan and indapamide reduced the risk of metabolic syndrome Telmisartan was more effective, whereas indapamide had better pharmacoeconomic benefits.
Telmisartan vs. Ramipril in Renal Endothelial Dysfunction (TRENDY) (19)	87 patients HTN with type 2 diabetes	Prospective Randomised Telmisartan vs ramipril adiponectin levels at baseline and following 9 weeks treatment	There was a significant increase in adiponectin levels in the telmisartan but not in the ramipril group.
Dragos Vinereanu et al. (30)	56 patients (57 ± 9 years, 52% men) with mild-to-moderate hypertension and type 2 diabetes	Prospective, randomized compare the effects of 2 types of diuretics, indapamide and hydrochlorothiazide, on ventricular and arterial functions in patients with hypertension and diabetes	Indapamide improved measures of endothelial and arterial functions and increased longitudinal left ventricular function compared with hydrochlorothiazide.

ACEI, angiotensin-converting enzyme inhibitor; HTN, hypertension; LVH, left ventricular hypertrophy; HOMA-IR, homeostatic model assessment–insulin resistance; MS, metabolic syndrome.

In a double-blind, randomized study, hypertensive patients with metabolic syndrome showed improvements in fasting glucose, plasma insulin, insulin resistance, glycosylated hemoglobin insulin, and homeostasis after administration 80 mg/day of telmisartan for three months; however, administration of 50 mg/day of losartan did not demonstrate any appreciable positive effects in this study (16-19).

Telmisartan was found to significantly lower serum insulin levels in 36 patients with metabolic syndrome and hypertension. The HOMA-IR model (homeostasis model assessment of insulin resistance) was also used in this study to measure insulin resistance, and the results demonstrated that telmisartan significantly reduces insulin resistance (18, 19).

Apart from the mechanisms common to other ARBs, telmisartan also uses other mechanisms to exert its anti-obesity effects. In addition to its anti-hypertensive effects, telmisartan exhibits remarkable effects on the structure and function of adipose tissue. By partially activating the PPARγ receptors, telmisartan reduces the buildup of visceral adipose tissues (20).

According to a meta-analysis of telmisartan's effects on body fat distribution in 651 participants who were overweight or obese, telmisartan improves fat distribution and lowers visceral fat, which may make it particularly helpful for obese hypertensive patients (21).

In dyslipidemic conditions, ARBs and statins may also have some additive effects that result in further effects on lipid metabolism. One of the likely mechanisms of telmisartan's anti-dyslipidemic effects is its hepatic partial PPARγ agonist activity, which raises lipoprotein lipase expression through a PPARγ-dependent pathway (22).

TELMISARTAN AND POST-CEREBROVASCULAR ACCIDENT

Telmisartan demonstrated effective antihypertensive activity in hypertensive patients with chronic-stage stroke, without altering hemispheric blood flow. In fact, it even enhanced regional cerebral blood flow in most regions studied (23). Kono et al. found that telmisartan significantly reduced inflammation and protected the neurovascular unit through its pleiotropic effects in hypertensive rats after ischemic stroke (24). Iwanami et al. suggested that the beneficial effects of telmisartan on stroke are partly due to the activation of PPARγ-gamma and the blockade of the angiotensin 1 receptor (25). Additionally, the post hoc analysis of the PRoFESS trial, as well as the ONTARGET and TRANSCEND studies, indicated that telmisartan notably reduced the incidence of strokes compared to a placebo (26). Furthermore, it was demonstrated that telmisartan was superior to ramipril in lowering the incidence and severity of AF in hypertensive patients with metabolic syndrome, as well as in preventing recurrence of AF in hypertensive patients (46).

COMBINATION OF TELMISARTAN AND INDAPAMIDE – EFFECT ON DIASTOLIC DYSFUNCTION

The risk of cardiovascular morbidity and death is raised by left ventricular hypertrophy (LVH) and diastolic disfunction, a common type of target organ damage linked to hypertension. Anomalies in neurohormonal systems, including the renin-angiotensin-aldosterone system, as well as metabolic disorders, can also lead to LVH. Regression of left ventricular mass (LVM) significantly lowers this risk, and LVM reduction is linked to antihypertensive medication. Telmisartan is a long-acting ARB that has shown regression of LVH and improved diastolic function in several clinical trials.

Chang et al. reported that telmisartan reversed left ventricular hypertrophy and the E/A ratio, suggesting that telmisartan can enhance LV remodeling and diastolic function in cardiorenal HF with preserved ejection fraction (HFpEF) (27). Cardiac fibrosis plays a key role in the pathophysiology of cardiorenal HFpEF, and modulation of the RAAS with telmisartan effectively reduces cardiac fibrosis and helps maintain diastolic function in this rat model (27). Given the evidence of telmisartan's effect on LV mass itself, this effect could lead to improvements in both systolic and diastolic function.

In the TRANSCEND trial, after 5 years of therapy LVH was reduced to 9.9% in the tel-

misartan group compared to 12.8% in the placebo group. Furthermore, telmisartan reduced new-onset LVH by 37% when compared with placebo. In the ONTARGET trial, telmisartan was also slightly more effective than ramipril in reducing LVH (28).

The diuretic's effect on diastolic function suggests that combining telmisartan with indapamide in patients with HFpEF, especially in those with metabolic syndrome who are likely to have undiagnosed HFpEF in a large percentage, could be beneficial. After 6 months of treatment, indapamide was shown to enhance endothelial and arterial function, as well as improve longitudinal left ventricular function, compared to hydrochlorothiazide in patients with hypertension and diabetes (29).

TELMISARTAN AND KIDNEY FUNCTION

Telmisartan has been tested in many different studies, in diverse patient cohorts, in comparison with placebo or other commonly used antihypertensives as presented in detail in Table 2.

It has been demonstrated that telmisartan in standard and especially higher doses safely and effectively lowers blood pressure (SBP and DBP, daytime and night-time) and reduces proteinuria, even independent of BP, in diabetic and nondiabetic hypertensive patients, with proteinuria and CKD of various degrees, preserves renal function, and potentially ameliorates atherosclerosis (24-26, 33-36).

Being a RAAS inhibitor and due to its aforementioned properties and effects, telmisartan should be one of the first logical choices for modern therapy of hypertension in wide spectrum of CKD patients, especially, and in accordance with recent guidelines (ESC 24) – in single-pill combinations. Such combination with indapamide is a very useful tool in nephrology practice for indapamide is a proven better choice of antihypertensive and diuretic than hydrochlorothiazide for its better preservation of renal function and reduction of morbidity and mortality (28, 37). It is even more pronounced in patients with metabolic disturbances because of its metabolic effects (14, 16, 17), and also preservation of renal function through reducing the often neglected but growing problem of renal calculosis (25,39).

Maximal tolerable dosage of ARB/ACEi in patients with elevated UACR or proteinuria irrespective of eGFR or even BP is used in the widest spectrum of renal patients as a recommended practice for lowering proteinuria as the most relevant renal prognostic factor, and also decreasing hyperfiltration (12, 41, 42). Telmisartan alone and in combination with indapamide might be very useful in this common scenario.

Regardless of its cause, CKD as a continuum and through all its stages, starting from subclinical kidney injury, endothelial dysfunction, from microalbuminuria to macroalbuminuria, from hyperfiltration to declining GFR and finally end-stage renal disease, represents independent and progressively increasing CV risk factor. Hence, contemporary treatment that warrants not only renal but CV and other benefits in a holistic manner should encompass lowering of blood pressure and proteinuria together with many pleiotropic renal, metabolic and CV protective effects described earlier in this manuscript (12). Telmisartan, and its combination with indapamide offers it all.

TELMISARTAN AFFECTS ENDOTHELIAL DYSFUNCTION, INFLAMMATION AND PROMOTES RISK REDUCTION IN CHRONIC CORONARY SYNDROME PATIENTS

Multiple randomized controlled trials demonstrated reduction of mortality and hospital admissions in high-risk patients with cardiovascular disease when treated with an ARB (43).

In the 2011 study by Akhras et al., it is reported that blocking the RAAS with telmisartan provides optimal cardio protection in high-risk patients and is well-tolerated (44).

Vascular homeostasis is primarily regulated by the endothelium, and hypertensi-

Table 2. Telmisartan and CKD (24-26, 33-36)

Study	Sample	Methodology	Findings
Rysava et al. 2005 (32)	92 (60 with DM) hypertensive proteinuric pts with CKD (including mild-to-moderate)	Prospective telmisartan 40 mg for 3 mo, followed by telmisartan 80 mg subsequent 3 mo (target SBP/DBP of <130/85 mmHg) antihypertensive and antiproteinuric efficacy and safety	-reduced BP (office and ABPM- SBP and DBP, daytime and night-time) -regression of proteinuria in DM and nonDM
Aranda et al. 2005 (35)	78 non-DM, hypertensive pts with biopsy-proven chronic proteinuric nephropathies	Prospective randomized telmisartan 80 mg once daily (40 pts) or 80 mg twice daily (38 pts) after 4-week wash-out mean follow-up 24.6 +/- 2.2 mo long-term renoprotective effects of "standard" vs "high" doses of telmisartan	No difference in BP, potassium and lipid profile Cr increased and eCrCl declined with standard but no change with high dose proteinuria decreased more with high dose
Nakamura et al. 2008 (31)	30 untreated hypertensive pts with moderate renal insufficiency	Prospective randomized 40 mg telmisartan (15 pts) vs 5 mg amlodipine (15 pts) 12 months follow-up Comparison of renal and vascular protective effects	-BP decreased equally -serum Cr, proteinuria, baPWV, IMT, IL-6 and MMP-9, Chol decreased and 24-h Ccr increased better with telmisartan
Bakris et al. 2008 (33)	860 hypertensive DM pts with UACR 700 or more	Prospective, randomized telmisartan or losartan difference in UACR at 52 weeks	Telmisartan more effective in reducing proteinuria despite no difference in reduction in BP
Mann et al. 2009 (37)	5927 pts with CV or DM with end-organ damage without macroalbuminuria or HF intolerant of ACEi	Prospective, randomized From 2001 to 2004, follow up until 2008 Telmisartan, 80 mg/d (n = 2954 placebo (n = 2972) plus standard treatment for a mean of 56 months long-term renal effects	No important difference in composite renal outcomes but with only 17 pts starting dialysis Albuminuria increased less with telmisartan than with placebo (32% [CI, 23% to 41%] vs. 63% [CI, 52% to 76%]; P < 0.001)
Agrawal et al. 2016 (36)	55 adult pts, mean 48.23 years (96.36% hypertensive; 63.61% diabetic)	Prospective, observational effects of 40 mg/day telmisartan on CKD in real-life setting	24-h urinary protein, spot urine protein-to-creatinine, Cr and BP significantly reduced at the end of 3 month treatment
Kitamura et al. 2020 (33)	61 non-nephrotic stage 3–4 CKD	Prospective, randomized from 2009 to 2014 (follow-up up to 104 weeks) telmisartan 40 mg (32 pts) or 80 mg (29) dose-dependent renoprotective effects	No significant difference in primary renal outcomes and eGFR after 24 weeks and in adverse events urinary protein level significantly lower with 80 mg

CKD – chronic kidney disease; DM – diabetes mellitus; Pts – patients; BP – blood pressure; SBP – systolic BP; DBP – diastolic BP; Mo – months; Cr – serum creatinine; HF – heart failure; ABPM – Ambulatory blood pressure monitoring; 24-h Ccr – 24-h creatinine clearance; eGFR – estimated Glomerular Filtration Rate; baPWV – brachial-ankle pulse wave velocity; IMT – intima-media thickness; IL-6 – plasma interleukin-6; MMP – plasma matrix metalloproteinase-9; Chol – total cholesterol.

on-associated vascular change is believed to be significantly influenced by endothelial dysfunction (45). Despite having comparable effects on lowering blood pressure, telmisartan had more positive effects on endothelial function-related functional parameters in hypertensive patients than valsartan. Furthermore, in hypertensive patients with type 2 diabetes, telmisartan decreased arterial stiffness more than a placebo (46).

Telmisartan has been demonstrated to lower inflammatory markers like C-reactive protein and interleukin-6 in patients with hypertension or diabetes at least as well as ACE inhibitors like ramipril or other ARBs like valsartan and olmesartan (47, 48).

Finally, based on the ONTARGET and TRANSCEND studies, RAAS blockade with telmisartan is shown to provide optimal cardio protection and significant reduction of cardiovascular morbidity and mortality in high-risk patients with CVD, diabetes mellitus and end-organ damage, along with a good tolerance profile (27, 37).

CONCLUSION

When choosing an antihypertensive treatment to prevent CVD, consideration should be given to agents whose actions extend beyond optimal BP lowering. Telmisartan is recommended for the prevention of cardi-

ovascular disease in addition to the potent treatment of hypertension.

Compared to other ARBs, telmisartan has distinct pharmacological characteristics that may have clinical implications. Telmisartan stands out among RAAS inhibitor medications due to its long duration of action, which allows for 24-hour blood pressure control, and its appropriate tolerability. Telmisartan has several therapeutic advantages, such as anti-diabetic, anti-inflammatory, and protective cardiovascular effects, due to its partial agonistic activity on PPARγ receptors and antagonistic activity on AT1 receptors. The benefits of telmisartan's effects on endothelial dysfunction, metabolic factors, and inflammatory markers may contribute to its heart and kidney prevention effects in addition to its antihypertensive action.

Together, these characteristics make telmisartan a perfect multipurpose medication for patients with metabolic syndrome, diabetes mellitus, hypertension, CKD and other cardiovascular conditions who represent the majority of patients seen in primary and secondary care practices worldwide.

Through numerous studies indapamide has also demonstrated cardiovascular protective effects and its metabolic neutrality and no or minimal effect on potassium, creatinine, and lipid and glucose profiles. The use of telmisartan with indapamide in a fixed dose combination has an additional positive effect on the metabolic profile of the patient, as well as on the diastolic function of the left ventricle, making it also a recommended option for hypertensive patients with left ventricular diastolic dysfunction.

The administration of telmisartan/indapamide combination to patients may also improve medication adherence by lowering the number of pills and polytherapy side effects.

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CASE REPORT

Unmasking an Incidental Tenosynovial Giant Cell Tumor on Fluorodeoxyglucose Positron Emission Tomography / Computed Tomography in a Melanoma Patient

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Abstract

Introduction: Tenosynovial giant cell tumor (TGCT) is a benign, yet metabolically active tumor affecting the synovium, bursa, or tendon sheath.

Aim: The purpose of the current case report is to evaluate the importance of fluorodeoxyglucose positron emission tomography / computed tomography in diagnosis of extra osseous soft tissue lesion.

Case report: We present a 48-year-old male with malignant melanoma undergoing fluorodeoxyglucose positron emission tomography / computed tomography surveillance. A highly fluorodeoxyglucose-avid mass in the right foot raised concern for melanoma metastasis. However, biopsy revealed an unexpected diagnosis of TGCT.

Conclusion: This case highlights the importance of fluorodeoxyglucose positron emission tomography / computed tomography in diagnosis of extra osseous lesions, particularly in cancer patients. In such scenarios, considering alternative diagnosis and pathohistological diagnosis confirmation become crucial to avoid misdiagnosis of metastases.

Keywords: positron emission tomography / computed tomography, giant cell tumor, melanoma.

Learning objectives

- Highlight the difficulties in diagnosing Tenosynovial giant cell tumor particularly in cancer patients.
- Underline a very high level of metabolic activity in Tenosynovial giant cell tumor mimicking metastases.
- Realize the importance of fluorodeoxyglucose positron emission tomography / computed tomography in diagnosis of extra osseous soft tissue lesion.

INTRODUCTION

The diagnosis of Tenosynovial giant cell tumor (TGCT) presents a significant challenge due to its lack of specific clinical and imaging features. This often leads to confusion with other benign and malignant processes affecting the synovial lining, particularly in cancer patients. Especially, TGCT can mimic bone metastasis, further complicating its identification. While typically involving the appendicular skeleton, it can also be found incidentally in imaging studies without obvious symptoms. This case report highli-

ghts the difficulties in diagnosing TGCT and underlines the importance of considering it in the differential diagnosis, especially for metabolically active soft tissue lesions detected on fluorodeoxyglucose positron emission tomography / computed tomography (FDG PET/CT) in cancer patients. The aim of article was to evaluate the importance of FDG PET/CT in diagnosis of extra osseous soft tissue lesion and to underline very high level of metabolic activity in TGCT mimicking metastases.

CASE PRESENTATION

We present a case of a 48-year-old man with a history of malignant melanoma of temporal region. The patient underwent a PET/CT examination for surveillance, which demonstrated no suspicious abnormality except for very high-level metabolic activity localized to the mass of the right foot. Maximum standardized uptake value (SUV) in this area was 28.8 (Figure 1).

On the corresponding CT, soft tissue mass was seen in tarsal sinus, affecting talocrural joint and talar corpus, correlating with this abnormal focal metabolic activity (Figure 2).

This lesion was considered suspicious for metastatic disease, due to the history of operated melanoma.

A contrast-enhanced MRI of the joint was performed to better assess the anatomy of the underlying lesion, and it revealed a tumoral mass measuring 65x57mm intraarticular in tarsal sinus and in subtalar joint, destroying talus bone subcortically.

Given the suspicion for metastatic melanoma, this mass was biopsied. Histopathology revealed a giant cell tumor of tendon sheath, most probably localized type. The described cells were Melan A negative, CD 163 CD 68 positive and osteoclast-like cells TRAP positive.

On orthopedic surgery evaluation, the decision was made to provide surgical management.

In a patient with cancer, the discovery of a metabolically active lesion within or adjacent to skeletal muscle, but not clearly involving bone, should raise suspicion of a coexistent process. It should not be conclusively diagnosed as a metastatic lesion solely by PET imaging. Distant metastatic lesions to skeletal muscle as the sole suspicious abnormality would be unusual in melanoma. The differential diagnosis must include soft tissue sarcoma or malignant nerve sheath tumor, among other entities. Additional imaging and tissue diagnosis should be pursued in such a situation, as they were in our case. If equivocal, excisional biopsy of the lesion should be considered.

If this lesion had been misdiagnosed on PET/CT as metastatic malignant melanoma, the patient could have received unindicated therapy.

Discussion

TGCT was originally described as an inflammatory lesion involving the tendon sheath rather than as a malignant lesion. According to the 2013 World Health Organization Classification of Soft tissue Tumors, TGCT can be subdivided into localized (L-TGCT) and diffuse forms (D-TGCT). L-TGCT is primarily located in the digits of the hand and feet, while D-TGCT is more involved in the large joints, especially the knee. TGCT-D is often associated with bone erosions, cartilage loss and osteophyte formation and typically involves the appendicular skeleton, rarely the axial skeleton. No clear histological distinction can be made between the two main subtypes, so L-TGCT and D-TGCT are mainly differentiated by a radiological distribution of tumor within the joint.

The common symptoms of patients with TGCT include pain, limitation of motion, and minimal to mild joint swelling, heat, and tenderness, but TGCT can also be found incidentally in imaging studies of patients without any of these symptoms and manifests as a nonspecific well-defined soft-tissue mass.

This case study highlights the occurrence of PET/CT incidental discovery of TGCTs, in

particular, and metabolically active soft tissue lesions, in general. Although our lesion was conspicuous and suspicious, the foamy macrophages on biopsy were suggestive of inflammation and consistent with TGCT pathology. It has been suggested that the increased presence of monocytes and macrophages, due to TGCT tumor cell t(1:2)(p13;q37) translocation and resulting in increased colony stimulating factor 1 (CSF1) expression, is directly responsible for the increased FDG uptake. The increased expression of GLUT-1 and hexokinase II within macrophage-containing lesions has been reported in literature to correlate with SUV max and is thought to explain the mechanism of the increased FDG uptake. There are no known case reports of non–PET-avid TGCT in literature.

Conclusion

This case highlights the importance of FDG PET/CT in diagnosis of extraosseous lesions, particularly in cancer patients. In such scenarios, considering alternative diagnosis and pathohistological diagnosis confirmation become crucial to avoid misdiagnosis of metastases.

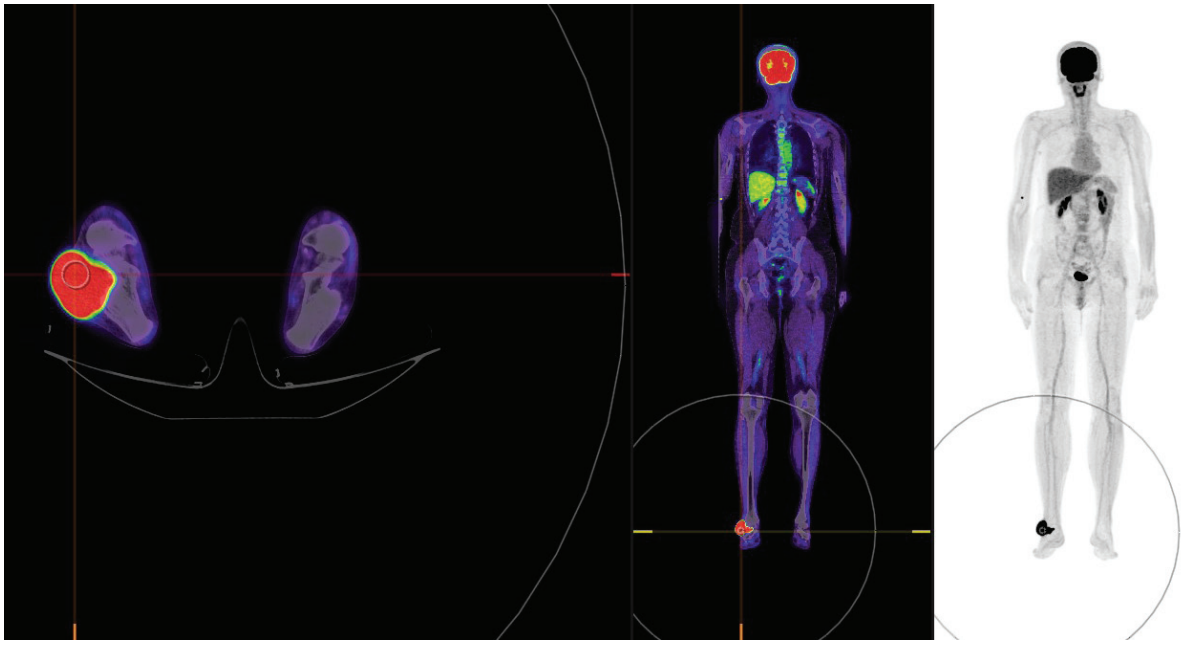

Figure 1. High-level metabolic activity localized to the mass of the right foot

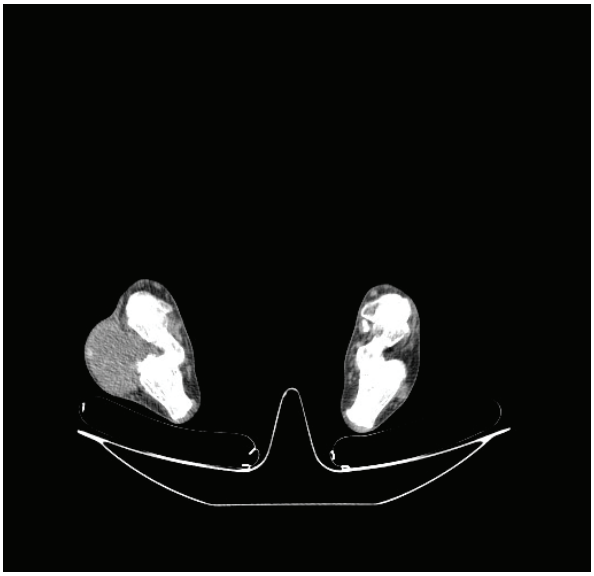

Figure 2. Soft tissue mass was seen in tarsal sinus

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Authors' Contributions: Conceptualization: AS. Project administration: AS. Resources: AS, SC. Software: AS, SC. Supervision: AS, SC, TC. Visualization: AS, SC, TC. Writing – original draft: AS, SC, TC. Writing – review & editing: AS, SC, TC.

Declaration of patient consent: The patient gave consent after receiving information about writing the case report.

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IMAGES IN MEDICINE

Severe functional mitral stenosis due to a left atrial myxoma

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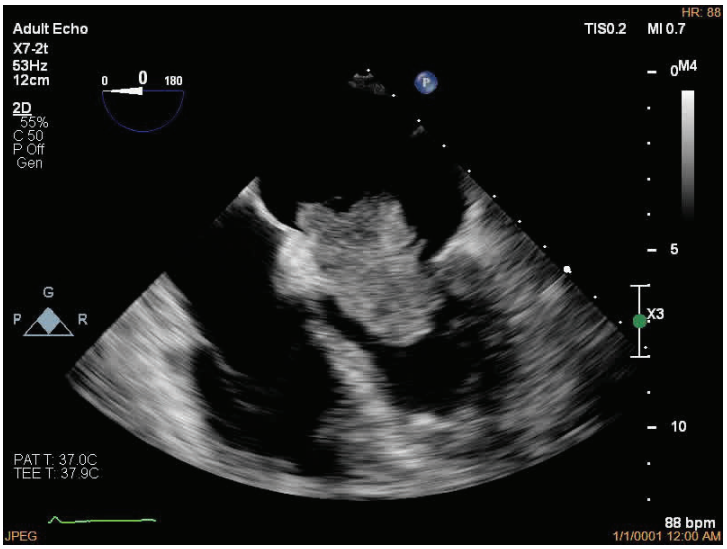

Figure 1. Obstruction of the mitral valve due to the presence of a tumor mass

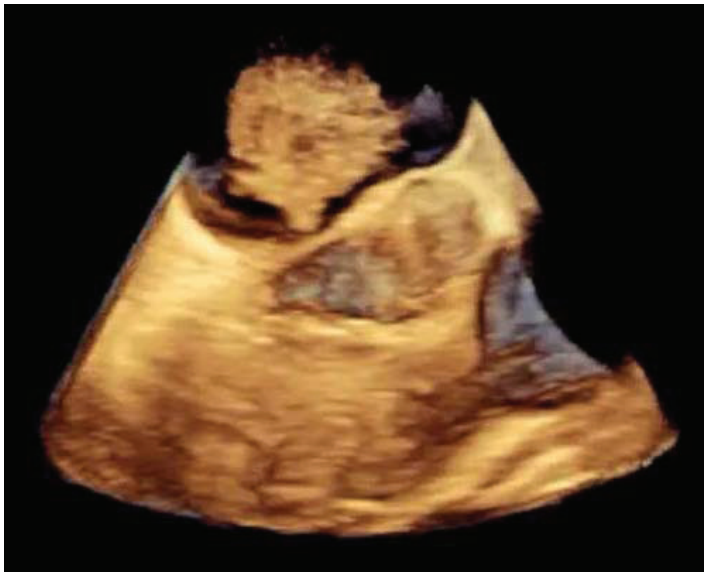

Figure 2. 3D transesophageal presentation of the tumor mass with a clear stalk which arises from the interatrial septum

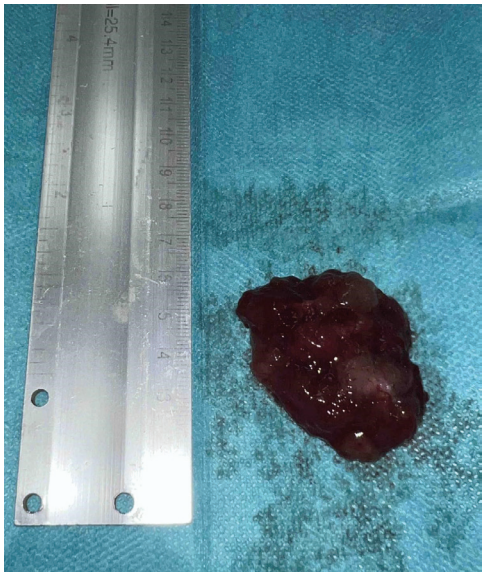

Figure 3. Surgical excision of the mass

The 71-year-old female patient was admitted for examination due to exertional intolerance. Patient's medical history included arterial hypertension and hypothyroidism. During the physical examination low-pitched sound during mid-diastole was noted, otherwise there were no remarkable findings. Laboratory findings included elevated C-reactive protein and erythrocyte sedimentation rate. There were no specific findings on ECG and chest X-ray. Two-dimensional transthoracic echocardiography (TTE) revealed a left atrial pedunculated mass, which arises from interatrial septum close to the mitral annulus, and prolapses into the mitral orifice in the diastolic phase mimicking severe mitral valve stenosis (MeanG 13 mmHg). 3D transoesophageal echocardiography (TOE) confirmed TTE findings with better assessment of tumor size (4.6 x 2.6 x 2.0 cm) and the site of tumor attachment, providing information that is even more accurate in the planning of surgical treatment. Surgical excision was performed after preoperative preparation in the ICU. Intraoperative finding included gelatinous, pedunculated left atrial mass arising from the interatrial septum (size 4,5 x 3,5 x 2.0 cm). Pathological and immunohistochemistry analysis described gelatinous structure consisting of myxoma cells embedded in a stroma, positive for calretinin, and negative for S100 protein and actin. A diagnosis of cardiac myxoma was confirmed. Myxomas are the most common type of primary cardiac tumor, with over 75% originating in the left atrium, typically at the mitral annulus or the fossa ovalis border of the interatrial septum (1-3). Patient was discharged after successful recovery (functionally without signs of mitral valve stenosis).

Consent: The author have obtained written consent from the patient to submit and publish this case report, including images and accompanying text, in accordance with COPE guidelines.

Authors' Contribution: MK, KR and AD were responsible for the conceptualization, methodology, formal analysis, visualization, writing of the original draft, and the review and editing of the paper.

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