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Predrag Furtula (1920–1995), Bust of Academician Milivoje Sarvan, bronze, 1979. Courtesy of the Pediatric Clinic of Sarajevo University Clinical Center. Photo: Dženet Dreković

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Prevention of Oral Injuries during Endotracheal Intubation: Patients' and Anesthesiologists' Perspective

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Abstract

Objective. The aim was to design accessible, simple, inexpensive protection for teeth and soft tissues during ETI, compare damage occurrence with and without protection, and investigate post-ETI orofacial pain symptoms. **Materials and Methods.** The selection procedure for adequate protection was carried out after which a reduced elastomer mouthguard was selected. Fifty patients were divided into 2 groups. In the first group, ETI was carried out using a mouthguard, while in the second group it was performed without it. The mouthguard was fabricated by anesthesiologists. After the ETI procedure, the patients and anesthesiologists were asked to complete a survey. **Results.** No difference in intubation severity and time required for intubation between the two groups was present. Seven patients from the non-mouthguard group suffered injuries during the ETI procedure. No injuries were present in the mouthguard group. In 92% of cases anesthesiologists agreed that mouthguards should be used during ETI. However, most of them (96% of cases) agree that the mouthguard should be used only when there is an increased risk of tooth loss and/or tooth damage. There was a significant ETI effect on the emergence of new orofacial pain cases. **Conclusion.** The mouthguard adequately protected dental and soft tissues and did not affect the work of the anesthesiologist. A significantly higher number of patients experiencing temporomandibular joint and masticatory muscles pain after surgery indicates that ETI might be a risk factor for orofacial pain.

Key Words: Mouthguard ▪ Endotracheal Intubation ▪ Orofacial Pain.

Introduction

Endotracheal intubation (ETI) is a medical procedure in which a tube is placed directly into the trachea. During ETI, complications might occur. Injuries are common, including dental trauma, oropharyngeal laceration, perforation, and other soft tissue injuries (1). The guidelines of the European Resuscitation Council from 2021 state that endotracheal intubation is not considered a priority in the initial phase, but the use of basic ventilation as the first line of airway control if it is effective. Only expert operators with a high success rate of intubation should perform it, weighing the benefits and risks of the procedure (2). Tooth trauma can range from simple fracture to avulsion. Tooth avulsion is the complete dislocation of the tooth from the alveolus. If this happens, desiccation, ischemia and bacterial contamination of the dental pulp and periodontal ligament begin (3).

Injuries are caused by a laryngoscope, a metal device used to establish the airway. The occurrence of dental injuries is estimated to be between

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0.17 and 12.1% (1). The main causes of damage of dental tissues are the poor condition of the teeth before the ETI procedure, aggressive laryngoscopy, emergency interventions and lack of experience of the doctor (4, 5). Additional causes of damage are difficult airway and reduced visibility (6). Although emergency surgery is not associated with a higher risk of dental trauma compared to elective surgery, the inability to prepare a mouthguard could be associated with the frequency of perioperative injuries (7).

Dental damage is increasingly common as people become older, with the majority of injuries affecting the periodontal ligament. Crown fractures are more prevalent in younger individuals, with the maxillary front teeth being the most commonly impacted (8). To reduce the risk of complications caused by the ETI procedure, various individually made or commercial protective appliances have been used to protect dental tissues (9). The literature states that all preoperative patients who are scheduled to have an ETI procedure should have a preventive dental examination and risk assessment, and those at risk of tooth loss should have a mouthguard made (5).

However, the production of adequate individual mouthguards would require multidisciplinary work between anesthesiologists and dentists and is time-consuming. Also, such mouthguards are extremely expensive (9). Croatia is a country where mouthguards during endotracheal intubation are not a standard practice. Nevertheless, specialists are raising concerns about the number of teeth and soft tissue damage that is happening during the procedure. Dental injuries are the most common reason for complaints against specialists in anesthesiology, resuscitation and intensive care (10). Luxation and avulsion of multiple anterior teeth during elective surgery are risk factors for complaints. Also, the lack of informing the patient about possible postoperative complications is a risk factor for conviction (11). The experience of the anesthesiologist is no guarantee that injuries to the patient's oral cavity will not occur, mainly because injuries most commonly occur due to pathologically weakened teeth and rarely as a consequence of manual manipulation (12). Another unpleasant complication associated with ETI is postoperative orofacial pain. Strong forces, applied with a laryngoscope or manual manipulation during this procedure, can cause damage to the masticatory system and the appearance of pain. It can result in postoperative symptoms such as difficulty in mouth opening, pain in the temporomandibular joints (TMJ), masticatory muscles, and surrounding structures (13).

This study aimed to assess and compare the occurrence of damage to dental structures and soft tissues during the endotracheal intubation (ETI) procedure among two patient groups: those wearing mouthguards and those without, while also examining the overall occurrence of orofacial pain symptoms following ETI.

Materials and Methods

This was a two-centre interventional study conducted at the Department of Removable Prosthodontics, School of Dental Medicine University of Zagreb and The Clinic for Anesthesiology, Resuscitation and Intensive Care of the Sveti Duh Clinical Hospital.

Mouthguard Selection Procedure

A comparative evaluation of four distinct mouthguard designs was conducted at the Department of Removable Prosthodontics, utilizing two test participants. The aim was to identify a mouthguard design that could be easily communicated to anesthesiologists and utilized in Croatian hospitals. Additionally, the assessment sought to determine if a custom-made silicone splint could meet the necessary retention and stabilization requirements (Figure 1).

1) ***Commercial thermoplastic tray***
   The thermoplastic tray (*Mammoth XT*®, *HealthCentre*, *Berlin*, *WI*, *USA*) was placed in hot water and after one minute adapted on the teeth with fingers. Once the material had been set, the tray was returned to the mouth to check retention. All steps were by the manufacturer's instructions.

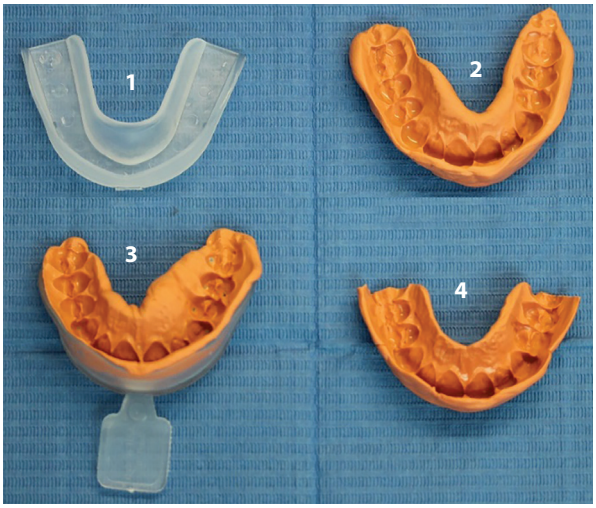

Figure 1. Four types of tested mouthguards (1- commercial thermoplastic tray, 2- custom-made elastomer mouthguard that covered all the teeth of the upper jaw, 3- individualized commercial thermoplastic tray (custom-made elastomer mouthguard placed in a commercial thermoplastic tray), 4- reduced custom-made elastomer mouthguard that covered the frontal teeth up to the second premolar of the upper jaw).

2) ***Custom-made elastomer mouthguard that covered all the teeth of the upper jaw***
The manufacturing process consisted of mixing the base and catalyst vinyl-polysiloxane (*3M™ Express™ STD Putty*, *St. Paul*, *MN*, *USA*) according to the manufacturer's instructions. The material was adapted to the teeth and surrounding soft tissues. Once the material had been set, the mouthguard was removed from the mouth and reduced with a scalpel as needed. After reduction of the mouthguard outside the mouth, it was returned to the mouth to check retention.
3) ***Individualized commercial thermoplastic tray (custom-made elastomer mouthguard placed in a commercial thermoplastic tray)***
Mixed base and catalyst vinyl-polysiloxane (*3M™ Express™ STD Putty*) were put in the commercial tray (*Mammoth XT®*, *HealthCentre*). Both mixed material and commercial tray were put in the mouth to adapt to the teeth. After the material had been set, the tray was returned to the mouth to check retention.
4) ***Reduced custom-made elastomer mouthguard that covered the frontal teeth up to the second premolar of the upper jaw***
The manufacturing process was the same as in 2); however, the material was adapted to cover the frontal teeth up to the second premolar of the maxilla. All splints had thickness between 3 and 4 mm.

The assessment was carried out in two steps. First, feedback from the patients was collected by the examiners (DA, MA) using a short survey in which retention, comfort, urge to vomit and overall satisfaction with mouthguards were examined on a 5-point Likert scale. The mouthguard that was rated the highest in all categories was considered the best option. Second, an experienced clinician (EV) made a clinical assessment of each mouthguard in the mouth. The mouthguard of choice had to meet the following criteria: i) retention and stability – it had to stand still on the teeth, ii) visibility – its size should not interfere with throat visibility, and iii) comfort – it should not induce the urge to vomit or be causing any discomfort. After both steps were carried out a reduced custom-made elastomer mouthguard was selected as the best option for teeth protection during an ETI procedure. Moreover, the elastomer mouthguard was deemed to be a financially viable solution, presenting no significant challenges for anesthesiologists in its handling.

One study found that the optimal thickness of a mouthguard for proper protection falls within the range of 3-4 mm. It was observed that wearing a mouthguard with a thickness exceeding 4 mm, although potentially providing enhanced protection, was less comfortable for participants (14). Therefore, the mouth guard thickness was determined to be 3 mm.

Sample Size Calculation

The sample size calculation was derived from study, aiming to compare intubation times between patients without a mouthguard and those with one. To detect a difference of 7 seconds with a margin of error of ±5 seconds, and an allocation ratio of 1:1 between cases and controls, we determined the need to include 26 participants (13 in each group), ensuring a power of 95% at an alpha

level of 0.05. Also, in the same study authors calculated that a sample size of 40 patients in each group would provide a 99% power to detect a 5-second difference in intubation period with and without a mouthguard. If these calculations can be generalized and applied to our sample and design, the size of our sample (N=25 in each group) provided acceptable power to identify tested differences.

Eligibility Criteria

Study participants were patients of the Sveti Duh Clinical Hospital scheduled for a planned procedure under general anesthesia that requires an ETI procedure. To account for potential errors in interpreting the effects of intubation, it's important to note that loosened or previously damaged teeth could inadvertently influence the outcomes. Additionally, adjustments made to the mouthguard itself might pose a risk of injury to teeth that are not in optimal condition. In our study, we specifically excluded patients with compromised dental health to avoid these confounding factors and ensure a more accurate assessment of the impact of ETI. Therefore, only patients with natural teeth in the upper anterior segment were included in the study with at most one prosthetic work in the anterior segment of the upper jaw and the lower anterior teeth present. Excluding criteria were age <18 years, body mass index (BMI)>35 kg/m^2, Mallampati modified classification>3, interincisal distance <4 cm, complete edentulousness, upper jaw defects, extensive prosthetic works in the area of the upper front teeth, implant-prosthetic works in the anterior segments of the upper jaw, tooth mobility >2mm, lack of teeth in the anterior lower segment (due to disabled measurement of the interincisal opening of the mouth), tumor or carcinoma of the oral cavity, difficult/impossible intubation. The patients were assessed by anesthesiologist and two researchers of the School of Dental Medicine, University of Zagreb (MA, DA). After deciding to include a patient in the study based on inclusion and exclusion criteria, the anesthesiologist responsible for recruiting participants would assign a code to the subjects, ensuring the anonymity of the participants during data processing. Finally, 50 patients were included in the intervention study and were randomly assigned into 2 groups. The investigator, blinded to the patient statuses, conducted simple randomization using the RAND function within the Excel program.

The first group consisted of 25 patients that underwent the procedure with mouthguard adjusted to their frontal maxillary teeth (mouthguard group), whereas the second group consisted of 25 patients that underwent the procedure without any protection on their teeth (non-mouthguard group) (Figure 2).

Study Protocol

Demographic data (age, sex, height, weight, BMI) were collected for both groups of patients. Two student examiners (DA, MA) educated anesthesiologists on how to assess the dental status of the patients. The condition of the oral cavity was marked as either treated or non-treated. Non-treated implied the presence of large amounts of soft dental plaque, calculus and/or dental caries. After the assessment of the dental status, each patient in the mouthguard group received a mouthguard.

The mouthguard was fabricated by anesthesiologists who were trained by two researchers of the School of Dental Medicine, University of Zagreb (DA, MA). It was adapted to the teeth and surrounding soft tissues as explained previously (Figure 3). After adjusting and checking the mouthguard, the anesthesiologists continued the further usual procedure of preparation for surgery. The mouthguard was removed from the mouth by anesthesiologists after the ETI procedure was completed.

Additional Data Collected

1) ***Oral aperture size at the maximal possible opening of the mouth***
   To assess the oral aperture size, patients were asked to open their mouths as wide as possible. Aperture size was measured as the interincisal

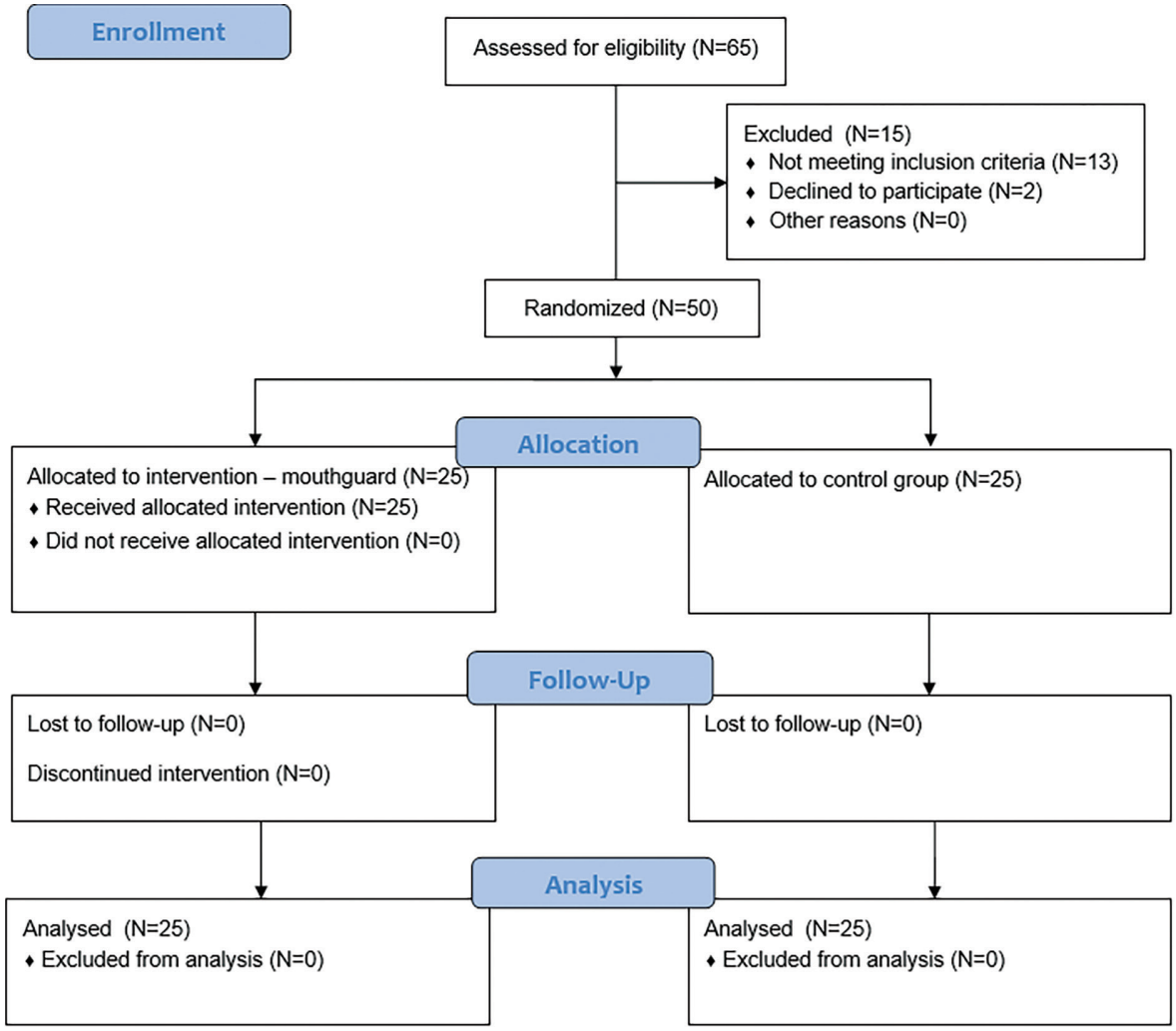

Figure 2. Flow diagram of selection of the participants.

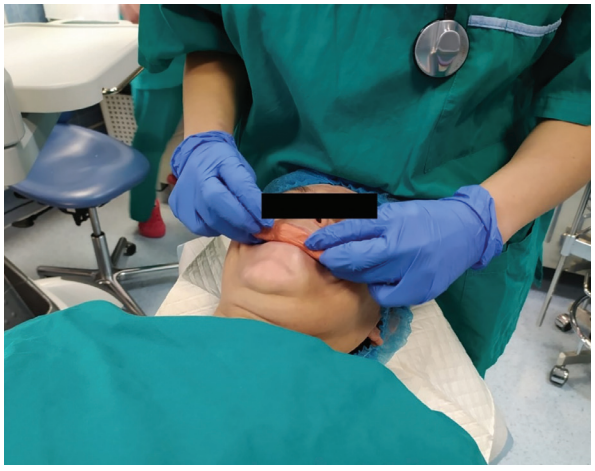
Figure 3. Adaptation of mouthguard before the ETI.

distance between the upper and lower central incisors (in the non-mouthguard and the mouthguard group before the placement of the mouthguard) or as the distance between the lower edge of the mouthguard and the incisal edge of the lower central incisors (in the mouthguard group after the mouthguard placement) (15).

2) ***Mallampati modified classification***
Mallampati modified classification was assessed in both groups. Determination of the Mallampati modified classification is a routine part of preoperative anesthesia preparation. Mallampati modified classification is

used to assess the severity of intubation and is determined by the visibility of structures in the oropharynx (16). There are four levels of Mallampati classification which are determined in a sitting position with the head in a neutral position, open mouth, maximally protruding tongue without phonation. In the first degree, the tonsils, palatine arches, soft palate and uvula are visible. In the second stage, the palatine arches, uvula and the upper arch of the pharynx are visible. In the third degree, part of the soft palate and part of the uvula are visible, while in the fourth degree only the hard palate is visible. Grade three and four predictors are for difficult intubation (17).

3) ***Required time for intubation***
During the ETI procedure, the time required to perform intubation was monitored (T0 - entry into the oral cavity with a laryngoscope to T1 - inflated balloon on the endotracheal tube), and the time required for intubation was calculated as the difference between T1 and T0 times.

Outcome Assessment

To assess the effectiveness of the mouthguard and the occurrence of orofacial pain after the ETI procedure, the patients were asked to complete a survey within 24 hours following the procedure. The survey consisted of 23 questions, examining awareness of the need to use mouthguards and the occurrence of damage within the oral cavity after the ETI procedure (roughness of teeth, lack of part or all of the tooth, tooth mobility, lip and soft tissue injury and palate injury). In the mouthguard group, the urge to vomit, the existence of discomfort during the mouthguard adjustment procedure and the feeling of security with the mouthguard, were additionally examined.

The patients' survey also examined the occurrence of new symptoms of orofacial pain (primarily symptoms related to temporomandibular disorders - difficulty in mouth opening, pain in the masticatory muscles and temporomandibular joint). Given our expectation that the mouthguard wouldn't impact orofacial pain, we pooled and analyzed orofacial pain data from both groups, regardless of whether a mouthguard was worn. The intensity of pain before and after the surgery patients recorded on a numerical pain rating scale (NPRS). The NPRS is a subjective measure in which individuals rate their pain on a numerical scale from zero to ten, where zero indicates a pain-free condition and ten the strongest pain possible. Anesthesiologists who carried out the procedure completed a survey to assess the extent to which the mouthguard potentially interfered with their work and/or airway visibility. Also, doctors used the Likert scale (1-completely agree, 2-partially agree, 3-neither agree nor disagree, 4-disagree, 5-least disagree) to assess the difficulty of making the mouthguard, its mobility and whether the mouthguard should become a standard part of the preoperative preparation.

Ethics Statement

All patients were informed in detail about the objectives and course of the research and voluntarily signed informed consent. The Ethics Committee of the Faculty of Dentistry (05-PA-30-XXII-12/2020) and the Ethics Committee of the Sveti Duh Clinical Hospital (01-03-4148/1) approved this research.

Statistical Analyses

Collected data were organized into a database (Excel spreadsheets) and processed using the statistical program IBM SPSS Statistics, 27.0 (Armonk, NY: IBM Corp). Statistical data analysis consisted of descriptive statistics. Also, the Shapiro-Wilk test was used to test the normality of the distribution and appropriate statistical tests were used to test differences between groups. To test for differences in age, height, weight, BMI and oral aperture size between the mouthguard and non-mouthguard groups, a t-test for independent samples was used. The Mann – Whitney U-test was used to examine the differences in time required to perform the ETI procedure between the mouthguard and non-mouthguard groups. A t-test for dependent

samples was used to test the difference in the oral aperture size in the mouthguard group before and after mouthguard adaptation. Due to the simplicity of interpretation and analysis of the results of the Likert scale, the categories "strongly agree" and "partially agree" were interpreted as affirmative answers to the questions asked, while the categories "disagree" and "strongly disagree" were interpreted as negation. The McNamar test was used to determine whether there is a procedure (ETI) effect on the emergence of muscle and joint pain. A value of $P<0.05$ was considered statistically significant.

The statistician was blinded to group assignment. We hypothesized that there will be differences in the presence of damage to dental structures and soft tissues between participants who underwent an ETI with protection and subjects without any protection to their teeth. Another hypothesis was that there will be more orofacial pain symptoms after the ETI than before.

Results

The demographic data of the patients are shown in Table 1.

A significant difference between the groups was present for the variable "age" ($t=-2.534$, $P=0.015$). According to the data on the weight of the patients, there were no significant differences between the groups. Also, there was no difference in BMI ($P>0.05$). Dental status was described as treated in 94% (N=47) of patients and untreated in 6% (N=3) of patients. There was no significant difference in the dental status between the two groups of patients ($P>0.05$). When the data of the Mallampati classification were analyzed, of the total number of patients, the first degree of Mallampati classification was present in 48% of patients (N=24), the second degree in 40% (N=20) and the third in 6% (N=6). There was no difference in intubation severity between the mouthguard and non-mouthguard group since the distribution of patients in both groups was identical (12 patients in first degree, 10 patients in second degree and 3 patients per group in third degree of Mallampati classification).

A significant reduction in the oral aperture size before (38.52±4.83 mm) and after (36.04±4.68 mm) mouthguard adaptation was present in the mouthguard group ($t=3.82$, $P<0.001$). However, there were no differences in the size of the oral aperture between the two groups (non-mouthguard group: 36 ±10.89, mouthguard group: 36.04±4.68; $t=-0.02$, $P=0.98$).

Of the total number of patients, 38 (76%) were not aware of the possibility of damage of dental tissues during ETI, while 12 (24%) were. A total of 46 (92%) patients did not know that there is protection for teeth during ETI, while four (8%) knew that there is a way to protect dental tissues during ETI. There was no difference between the mouthguard and non-mouthguard groups

Table 1. Comparison of Data between Groups

Variable	All participants N=50 ($\bar{x}$ ± SD)	Mouthguard group N=25 ($\bar{x}$ ± SD)	Non-mouthguard group N=25 ($\bar{x}$ ± SD)	P*, †
Age (years)	46.34 (11.12)	42.56 (10.37)	50.12 (10.73)	0.015
Height (m)	171.46 (8.75)	172.6 (9.06)	170.32 (8.45)	0.36
Weight (kg)	77.38 (15.97)	79.6 (14.00)	75.16 (17.73)	0.33
BMI (kg/m^2)	26.18 (4.24)	26.68 (4.09)	25.67 (4.41)	0.41
Aperture size (mm)	37.28 (4.92)	36.04 (4.68)	36 (10.89)	0.98
Time required for ETI (s)	72.10 (70.34)	77.20 (62.55)	67.0 (78.33)	0.25
Gender: male/female (N; %)	17: 34 / 33:66	10: 40 / 15:60	7:28 / 18:72	0.37
Mallampati score 1(%)/2 (%)/3 (%)	24/40/6	48/ 40/12	48/40/12	>0.05

N=Number of respondents; SD=Standard deviation; BMI=Body mass index; ETI=Endotracheal intubation; *Differences present between mouthguard and non-mouthguard group, a value of $P<0.05$ was considered statistically significant; †T-test for independent samples was used.

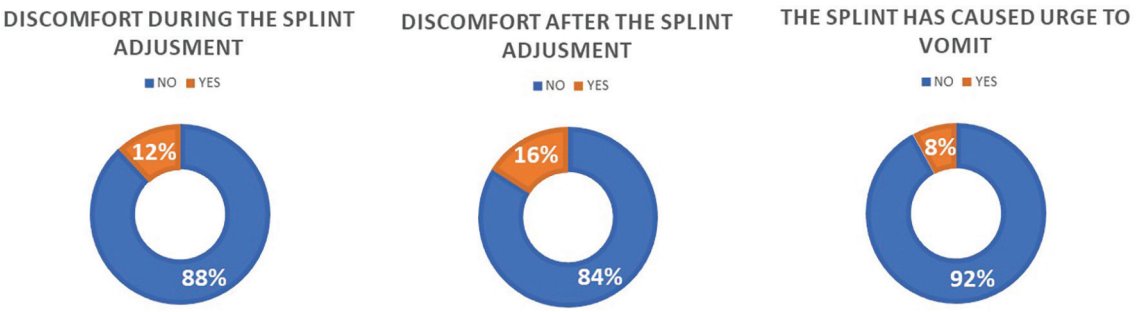

Figure 4. Percentage of discomfort during and after splint adjustment. Orange - YES; Blue - NO.

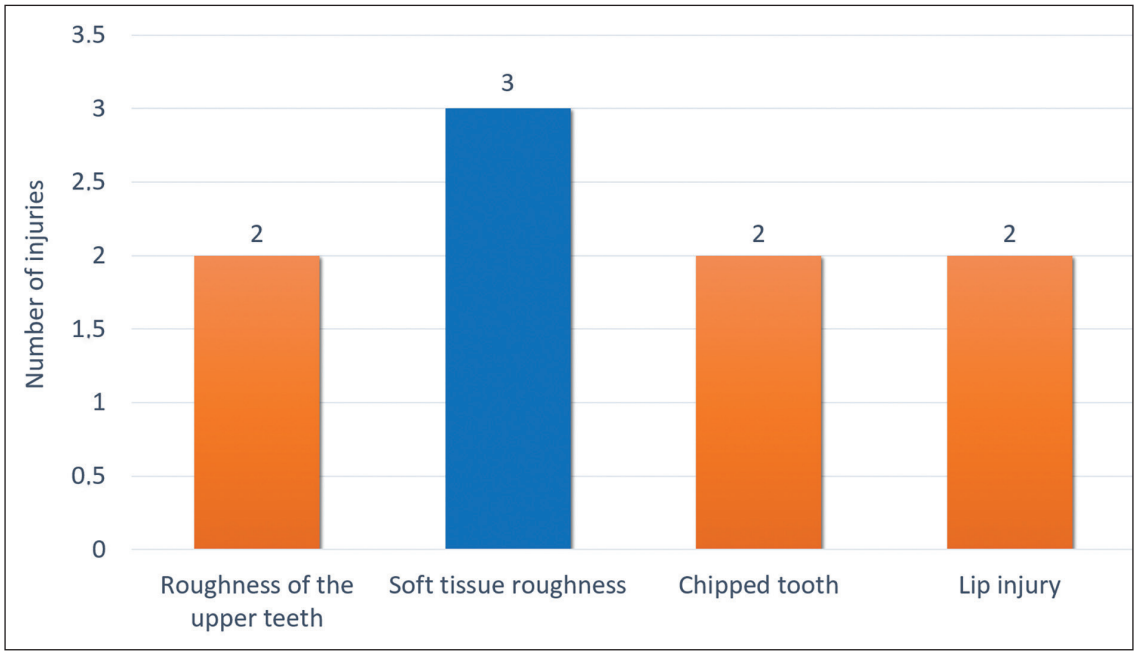

Figure 5. Number of individual injuries present in a total of 7 non-mouthguard patients that experienced injuries.

concerning prior knowledge of damage and protection (P>0.05). In the mouthguard group, 22 patients did not feel discomfort while adjusting the mouthguard, while three felt. After adjusting the mouthguard, 21 patients said that the mouthguard did not bother them, while four said it did. Also, after adjustment, 23 patients did not feel the urge to vomit, while two did (Figure 4).

Outcome Data Obtained from Patients

Only two patients reported roughness of the upper teeth after the ETI procedure. They stated that it was a milder roughness – one respondent reported roughness of all incisors of the upper jaw, while the other felt the roughness of both maxillary central incisors. Both belonged to the non-mouthguard group. The lack of a part of the tooth (chipped tooth) was reported by two patients from the non-mouthguard group. Both patients reported a minor chipping – one subject lacked part of the right central maxillary incisor and the other of the left lower central incisor. Three patients who reported soft tissue damage were from the non-mouthguard group, two with a minor injury, while one stated that he only feels a roughness he doesn't see. Lip injury was present in two patients from the non-mouthguard group. Both patients reported minor

injuries. None of the patients reported tooth mobility or lost a tooth after the ETI procedure. The number of individual injuries in the non-mouthguard group is shown in Figure 5. When the total number of injuries was observed and compared between groups, 7 patients from the non-mouthguard group (28% of the total N=25) suffered injuries during the ETI procedure, of which two patients had a combination of soft tissue and tooth damage. No injuries were present in the mouthguard group.

The cumulative incidence of injury in study population during ETI was 18%. When asked about the fear of injury, after participating in the study, 44 patients (88%) answered that they agree that they fear of tooth damage during ETI (15 patients fully agree, while 29 patients agree to some extent). Forty-eight patients (96%) answered affirmatively to the question "Would you feel more comfortable with the mouthguard during the ETI procedure?" (19 patients fully agree while 29 patients agree to some extent). Both responses did not differ depending on whether the patients had a mouthguard or not (P>0.05).

Outcome Data Obtained from Anesthesiologists

The mean time required for ETI was 72.10±70.34 seconds. It took 77.20±62.55 seconds in the mouthguard group and 67.0±78.33 seconds in the non-mouthguard group with no significant difference in the time required to perform the ETI procedure between the groups (Mann – Whitney U-test, P=0.25). In 74% (N=21) cases, anesthesiologists stated that they did not agree with the statement that the mouthguard made it difficult to see through the ETI procedure, and in 80% (N=20) cases they stated that the mouthguard did not complicate the ETI procedure. In 88% of cases, when asked about the mobility of the mouthguard, anesthesiologists answered that the mouthguard did not move or was negligibly movable. In 96% of cases, the procedure of making an elastomer mouthguard was not demanding for anesthesiologists. When asked if they thought mouthguards should be used during ETI, anesthesiologists answered affirmatively in 92% of cases. However, in 96% of cases, they agree that the mouthguard should be used only with indication (increased risk of tooth damage and/or tooth loss).

Outcome Data on Post-operative Orofacial Pain

Seven patients (14%) reported having pain in the muscles before the surgery, whereas five new cases emerged after the surgery. There was a significant ETI effect on the emergence of new muscle pain cases (P<0.001). Five patients (10%) reported having joint pain before the surgery, whereas 11 new cases emerged after the surgery). There was a significant ETI effect on the emergence of new joint pain cases (P<0.001). Muscle and joint pain did not exceed NPRS=2 which means that minor discomfort was present. None of the patients reported a feeling of a reduced mouth opening.

Discussion

One of the goals of this research was to find appropriate dental protection that anesthesiologists, without the presence of a dentist, could make and use without interference with the ETI. Previous research has shown that the use of individual and commercial mouthguards in ETI reduces the occurrence of dental trauma (18). Although the initial idea was that an individual mouthguard made of thermoplastic material, precisely tailored to the patient's teeth and made in a dental laboratory, would be the best option for protecting dental tissues, such a variant was not acceptable to the Clinical Hospital Sveti Duh's anesthesiologists as they suggested a simpler method without problems such as difficulties in coordinating with dentists and time-consummation. Mentioned problems are often cited in the literature as aggravating factors (5). When considering commercial mouthguards some disadvantages, such as impracticality and bad retention, were observed during this study's mouthguard selection procedure. The impracticality and uneconomical nature of commercial mouthguards despite good protection and the greatest reduction of the forces were addressed

by Monaca et al. (19). Individualized protection made out of high-viscosity elastomer adapted to the most endangered teeth, proved to be an option that meets all the set conditions. It is also a cost-effective option that, with relatively simple training of non-dental staff, can be introduced into the routine procedure of preoperative preparation of patients scheduled for ETI.

Although the oral aperture size significantly decreased in patients after mouthguard adaptation, data analysis showed that there was no difference between the mouthguard group and the non-mouthguard group in the size of the oral aperture. Also, the mouthguard did not lead to a decrease in the visibility of the structures nor to significant increase in the execution time of the ETI Although the time required for ETI was generally higher in mouthguard group, anesthesiologists did not consider the ETI procedure to be more difficult when the mouthguard was in place. These results prove that a carefully adjusted elastomer mouthguard did not affect the performance of the procedure and in a large percentage did not bother patients (Figure 4). Therefore, we can say that the ETI was successful in both groups demonstrating that our mouthguards were safe to use.

Contrary to our findings, Brosnan et al. noticed a significant difference in the time required for ETI in patients with and without a mouthguard. However, authors distance themselves from the clinical significance of such results, given that the period of adjustment of the mouthguard itself was included in the measurement of the time required for the procedure (20). The protective efficacy of an elastomer mouthguard was demonstrated in this study by comparing the damage of teeth and soft tissues between patients who underwent the procedure with a mouthguard and a group without it. Given that all injuries to teeth and soft tissues happened in the non-mouthguard group we managed to confirm our hypothesis. Interestingly, a total of nine reported injuries of tooth and soft tissue injuries occurred in this study and all injuries occurred in the non-mouthguard group, even the lower central incisor injury. This result can be explained by the fact that the mouthguard was carefully adapted to the most endangered teeth in the jaw. Also, it may have influenced the work of the anesthesiologist, who was aware of the presence of the mouthguard, to be more careful during the procedure. A more careful work of the anesthesiologist in the mouthguard group would explain the lack of damage to unprotected, mandibular teeth because, as we can see, they can also be damaged.

As far as we know, no study has compared the efficiency of dental protection in two groups during ETI, one with and one without a mouthguard. Lee et al. made individual thermoplastic mouthguards for all patients categorized according to risk factors and the evaluation was performed based on a notation on the patient's complaint after surgery in their medical charts. Also, they did not examine soft tissue injuries or compare the group with the control, so their results cannot be a representation of the real mouthguard efficacy in comparison to a situation without a mouthguard (18).

Despite a relatively high cumulative incidence of 18%, it is important to note that all of the injuries were characterized as minor or mild, and there were no losses of a substantial portion of the tooth or complete tooth loss during the ETI procedure in this study. This may be attributed to the criteria patients had to meet for inclusion in the study. They had their own, mostly rehabilitated teeth, without mobility, without major prosthetic works, and belonged to the low-risk group for dental injuries during ETI (5, 18). Additionally, asking patients to notice potential new injuries due to participation in the study may have led to noticing more than they would have otherwise (such as tooth roughness and lip damage). Both could be injuries they may not have noticed or linked to the event without being prompted.

Because anesthesiologists responded in a high percentage that mouthguard should be used with an indication rather than in all patients, risk assessment for hard dental and soft tissue injuries should become a routine part of the preoperative examination. However, for anesthesiologists to be able to recognize risky situations such as advanced periodontitis, impaired prosthetic work,

or caries-destroyed teeth, they would need to undergo some training. Another option would be to include a dentist in the team which is a potential financial and logistical problem (5, 18, 21). In addition to the poor condition of the oral cavity, the risk of tooth damage is also difficult intubation (5). Risk factors such as reduced mouth opening range and impaired visibility should also become a factor in the decision to use a mouthguard. Patients should always be informed of the risk that exists and be able to decide whether they want a mouthguard or not.

The second hypothesis of our study was that after ETI, a significant number of new symptoms of orofacial pain will appear, which was confirmed by the results. A greater number of patients reported muscle and joint pain after the procedure which showed that ETI affected the emergence of new orofacial pain symptoms. The pain was of low intensity, and this coincides with claims in the literature that say the pain is of lower intensity and mostly short-lived (13). In assessing orofacial pain, categorizing patients based on their groups was unnecessary since the study did not anticipate the mouthguard having any impact on post-operative pain. The sole influencing factor was endotracheal intubation itself. Therefore, it wasn't significant to mask or blind participants regarding the presence or absence of a mouthguard in relation to symptoms of temporomandibular disorder (TMD). It is important to note that for an appropriate assessment, it would be good to measure the extent of postoperative maximal interincisal opening to be able to objectively assess possible functional limitations resulting from the ETI procedure. Given the results obtained, endotracheal intubation could be considered a risk factor for acute orofacial pain. A greater sample size and a medical history that would examine the previous existence of temporomandibular disorders symptoms in more detail, would provide a better insight into this issue. Identifying the risk of subsequent orofacial pain is important to minimize such consequences of ETI.

The clinical implications of our findings are twofold. Firstly, our study emphasizes the critical importance of implementing dental protection protocols during ETI procedures to mitigate the risk of dental trauma. Dental injuries during ETI can have significant implications for patients, leading to discomfort, pain, and potentially long-term dental issues. By utilizing effective dental protection measures, such as mouthguards, healthcare providers can substantially reduce the likelihood of such injuries, thereby enhancing patient safety and well-being during medical procedures. Secondly, the observation of postoperative orofacial pain following ETI underscores the necessity for comprehensive pain management strategies in patients undergoing such procedures. The occurrence of muscle and joint pain post-ETI, albeit of low intensity, indicates a need for healthcare practitioners to be vigilant in assessing and addressing potential pain symptoms. Comprehensive pain management approaches may include pharmacological interventions, physical therapy modalities, and patient education on pain management techniques. By proactively addressing postoperative pain, healthcare providers can optimize patient comfort, promote faster recovery, and improve overall patient satisfaction with their medical care.

Limitations of the Study

A limiting factor of the study could be the significant difference in age between groups, which is a consequence of random selection and distribution of patients into groups. Additionally, a significant limitation stems from the subjective nature of the results, as both injuries and orofacial pain rely on patients' subjective reports. Having a dentist and an expert in orofacial pain evaluate post-operative outcomes would enhance the validity of the findings. Due to the specific situation caused by the COVID-19 pandemic, it is a great achievement that we managed to educate the staff of the Sveti Duh Clinical Hospital and train them for basic assessment of dental status and making an elastomer mouthguard that proved effective for protecting dental and soft tissues. Last, in this paper, we proposed an effective model of patient protection during ETI.

Conclusion

The placement of a mouthguard can effectively mitigate the side effects associated with endotracheal intubation (ETI) without adding complexity to the anesthesiologists' procedure. We firmly advocate for the incorporation of mouthguards as a standard part of preoperative preparation, particularly in patients with an elevated risk of tooth and soft tissue injuries. A thorough preoperative assessment is essential to evaluate the potential for oral tissue injuries and orofacial pain. The observed increase in postoperative orofacial pain compared to preoperative levels underscores the ETI procedure as a significant risk factor for such symptoms. This highlights the imperative need for proactive measures such as the use of mouthguards to mitigate associated risks and enhance patient comfort and safety.

What Is Already Known on This Topic:
Endotracheal intubation (ETI) is a medical procedure in which a tube is placed directly into the trachea. During ETI, complications might occur. The literature states that all preoperative patients who are scheduled to have an ETI procedure should have a preventive dental examination and risk assessment, and those at risk of tooth loss should have a mouthguard made.

What This Study Adds:
To create adequate protection for teeth and soft tissue and to investigate the occurrence of oral injuries during endotracheal intubation (ETI). Another aim was to assess the occurrence of orofacial pain following ETI.

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Development of Acute Kidney Injury Predictor Score in Intensive Care Unit Patients in Padang, Indonesia

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Abstract

Objective. This study aims to develop and create a specialized acute kidney injury (AKI) predictor score for the intensive care unit (ICU) patients in Padang, Indonesia. **Patients and Methods.** This study was a prospective observational study on 352 ICU patients at three specialized hospitals in Padang City; Dr. M. Djamil General Hospital, Dr. Rasidin General Hospital, and Siti Rahmah Islamic Hospital. Data regarding demographics, clinical characteristics, laboratory results, and outcomes related to AKI were gathered. The factors that predict AKI were identified using multivariate logistic regression analysis to determine independent factors. The predictor scores were created using regression coefficients and then internally confirmed. **Results.** Out of a total of 352 patients, 128 individuals (36.4%) suffered from AKI. Factors that independently predict the occurrence of AKI include age over 60 years old, having a history of chronic kidney disease, having sepsis, need for vasopressors, and having creatinine level 1.3 mg/dL (IQR 1.0-1.8) upon admission to ICU. An area under the curve (AUC) of 0.85 (95% CI 0.80-0.90) indicated the strong performance of the constructed predictor score. **Conclusion.** The constructed AKI predictor score a scale factor of 10, resulting in a range of 0–10 for the AKI predictor score. It demonstrates a good level of accuracy in predicting AKI in ICU patients in Padang. This score can be used by healthcare professionals to quickly identify and categorize individuals based on their risk level, facilitating timely intervention and personalized treatment.

Key Words: Acute Kidney Injury ▪ Creatinine ▪ Hospitals ▪ Intensive Care Units ▪ Sepsis.

Introduction

Acute kidney injury (AKI), also known as acute renal failure, is a medical condition characterized by rapid deterioration in kidney function (1, 2). It is a significant concern in clinical practice due to its significant impact on patient morbidity and mortality. AKI can worsen a critically ill patient's condition, carry a significant risk of complications, longer hospital stays, higher healthcare costs, and mortality (3). In general, the occurrence of AKI in the intensive care units (ICU) varies between 20% and 50% (4). A multinational study reported the occurrence of AKI in ICU patients with rates ranging from 35% to 40% (5). The most severe consequence of AKI is increased mortality, with patients in stage 3 AKI facing the greatest risk of death (6, 7). Timely detection of AKI is crucial for healthcare providers to initiate appropriate treatment, such as optimizing fluid balance, avoiding nephrotoxic medications, and closely monitoring kidney function. Early intervention can effectively prevent or mitigate the severity of AKI, while decreasing the likelihood of complications and mortality (8, 9). Additionally, timely detection allows clinicians to tailor therapy and monitoring for each patient based on their individual risk of AKI, improving ICU resource utilization and care efficiency.

Several AKI diagnostic tools to assist doctors in identifying individuals at increased risk for AKI has been established. The scores utilize a range of clinical and laboratory factors, including

age, comorbidities, administration of nephrotoxic medications, and fluctuations in blood creatinine levels, to determine the likelihood of AKI for individual patients. Three frequently utilized AKI diagnostic tools include RIFLE (Risk, Injury, Failure; and Loss; and End-stage kidney disease) (10), AKIN (Acute Kidney Injury Network) (11), and KDIGO (Kidney Disease: Improving Global Outcomes) (12). The RIFLE score utilizes alterations in serum creatinine levels and urine output to categorize the extent of acute kidney injury (AKI). AKIN score is a revised version of the RIFLE score that offers a more streamlined and user-friendly approach. KDIGO score is the most recent consensus for defining and categorizing AKI, and it provides guidelines for managing it.

While current AKI prediction scores have demonstrated utility in clinical practice, they possess various limitations. A significant constraint is the absence of external verification for particular demographics, such as ICU patients in Indonesia. The scores were derived from data collected from Western populations; hence, they may not entirely align with the features of Indonesian patients. Furthermore, these ratings are limited in their ability to accurately predict AKI in patients with specific circumstances, including chronic kidney disease, multiple organ failure, and those receiving renal replacement therapy. Hence, it is imperative to formulate a more precise and reliable AKI prediction score tailored to the ICU patients in Indonesia.

The objective of this study is to create a specialized AKI predictor score for the ICU patients in Padang, Indonesia. This predictor score is expected to assist doctors in identifying patients who are at a significant risk of developing AKI and enable them to promptly intervene, thereby minimizing the negative effects of AKI on the health outcomes and death rates of ICU patients in Indonesia.

Methods

Study Design and Participants

This study employed a prospective observational research approach. The selection of this design was based on its ability to enable immediate and ongoing monitoring of the progression of AKI in ICU patients, as well as the identification of related risk factors. The study was carried out at three specialized hospitals in Padang City; Dr. M. Djamil General Hospital, Dr. Rasidin General Hospital, and Siti Rahmah Islamic Hospital, which have sufficient intensive care unit (ICU) resources. The three hospitals were chosen based on the criteria of AKI case representation in the ICU and their willingness to participate in the research.

Data collection spanned a duration of one year, commencing in January 2023 and concluding in December 2023. This duration was deemed acceptable to achieve a sufficient and representative sample size. Throughout the study period, all adult patients (>18 years) who received treatment in the ICU at the three hospitals were included. Sample homogeneity and bias were minimized by applying inclusion and exclusion criteria. The inclusion criteria encompass adult patients who are over 18 years old, have been treated in the intensive care unit (ICU) for a minimum of 24 hours, and possess comprehensive data pertaining to the research variables. Patients who have stage 5 chronic kidney disease, a history of kidney transplantation, or a history of kidney replacement therapy (dialysis or hemofiltration) are excluded from the study based on specific criteria. Figure 1 illustrates the study flow and design.

Data Collection

Prospective data collection was conducted using patient medical records. Data collection was conducted by a skilled research team utilizing a structured data form. The data form contains demographic information, including age, gender, and history of comorbidities such as hypertension, diabetes mellitus, heart disease, chronic lung disease, and chronic liver disease. It also includes clinical data, such as the primary diagnosis that led to ICU admission and the use of vasopressors in patients. Vasopressors use is administration of vasopressor drugs to increase blood pressure in emergent hypotensive situations and maintain adequate perfusion when patients being treated in ICU. The

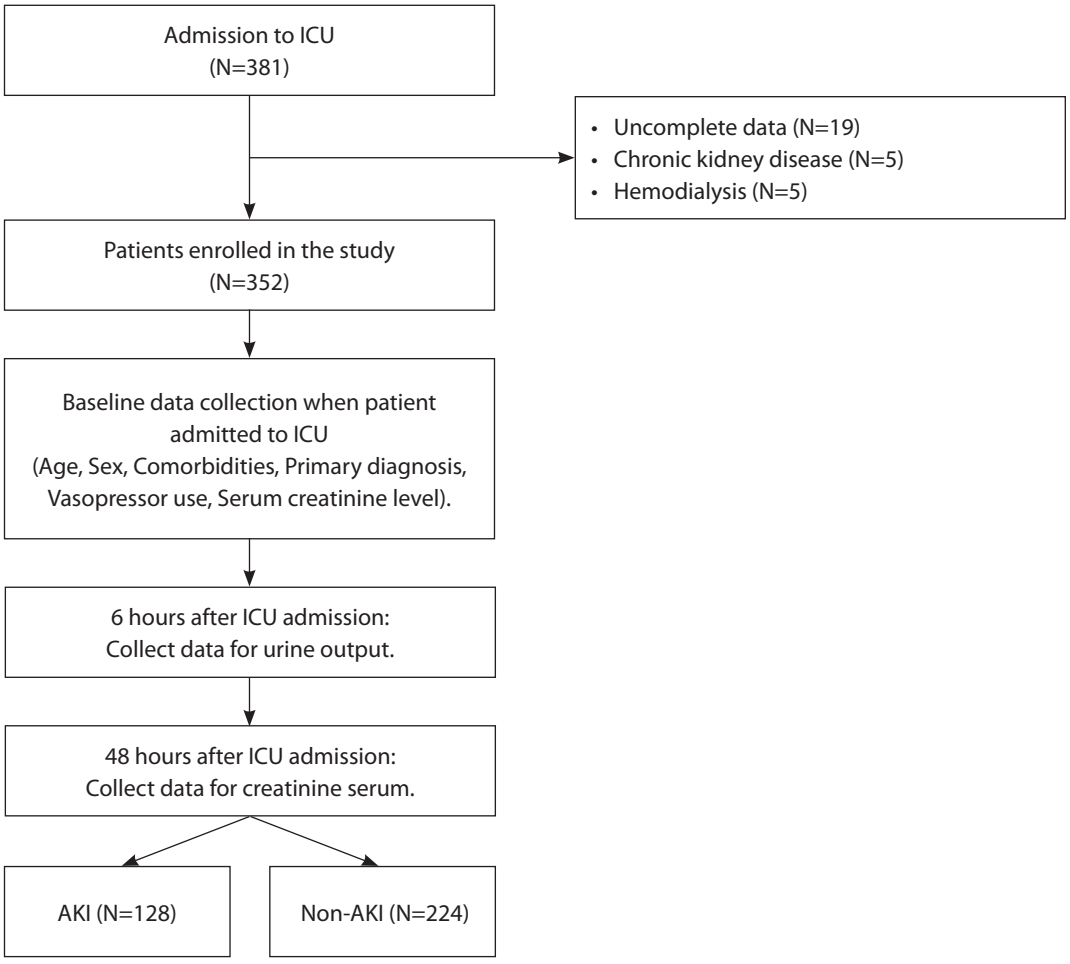

Figure 1. Study flow diagram.

presence of sepsis was determined by Sepsis-3 criteria (suspected infection plus two out of four systemic inflammatory response syndrome (SIRS) criteria; axilla temperature more than 38.3°C or less than 36°C, heart rate more than 90 bpm, respiratory rate more than 20 or $PaCO_2$ less than 32 mmHg, white blood cell count more than 12,000 or less than 4,000). The laboratory data were also evaluated, which includes serum creatinine levels at the intensive care unit (ICU) and during therapy, serum urea levels, serum electrolyte levels (sodium, potassium, chloride), and other important parameters like hemoglobin, leukocyte count, and lactate.

The definition of AKI was established according to the KDIGO criteria, which serves as a globally recognized standard for diagnosing and categorizing AKI. The diagnosis of AKI based on alterations in serum creatinine levels or urine output occurring within a 48-hour timeframe. The KDIGO criteria for AKI in this study was AKI stage 1 which include a rise in serum creatinine of at least 0.3 mg/dL within 48 hours a rise in serum creatinine of at least 1.5 times the baseline value within 7 days, and a urine output of less than 0.5 mL/kg/hour for 6 hours (12).

Ethical Approval

The study has received ethical approval from Health Research Ethics Committee of Faculty of Medicine, Andalas University, Padang, Indonesia

(LB.01.03/5/8/501/2023). The study techniques adhered to the principles outlined in the Declaration of Helsinki and relevant ethical guidelines.

Statistical Analysis

The data were analyzed using the statistical program SPSS version 26 (IBM, Jakarta, Indonesia). The study employed descriptive analysis to provide a detailed description of the demographic and clinical characteristics of the patients, as well as the occurrence rate of AKI. The AKI and non-AKI groups were compared using bivariate analysis, employing either the chi-square test or an independent t test to examine their features. The study employed multivariate logistic regression analysis to determine the factors that independently predict the occurrence of AKI. The regression model included only the variables that showed statistical significance (P<0.05) in the bivariate analysis. Only factors that maintained their significance after accounting for other variables made up the final model. AKI predictor scores were derived using the regression coefficients from the final model. The score for each variable is obtained by multiplying its regression coefficient with the corresponding scale factor. The cumulative score is determined by summing the scores of all predictor factors. The performance of the AKI prediction score was assessed by calculating the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The AUC is a metric that quantifies the discriminatory power of a prediction model. A value of 0.5 suggests that the model performs no better than random guessing, while a value of 1.0 indicates a flawless prediction model.

Results

Table 1 displays the demographic and clinical characteristics of patients who encountered AKI and those who did not (non-AKI) while receiving care in the intensive care unit (ICU). There was a significant difference in age between the AKI and non-AKI groups, with a p-value of less than 0.001. The median age of AKI patients was 65 years (IQR 55–72), which was higher than the median age of non-AKI patients at 52 years (IQR 40–60). The gender distribution between the two groups did not show any statistically significant difference (P=0.985), suggesting that gender is not a risk factor for AKI in this population. The prevalence of hypertension was significantly greater in AKI patients (64.1%) compared to non-AKI patients (45.5%) (P<0.001). While there was a difference in the incidence rate between the two groups, this difference did not reach statistical significance. There was a notable disparity in the primary diagnosis between the two groups, with a P-value of less than 0.001. AKI patients had a higher prevalence of sepsis (43.0%) compared to non-AKI patients (14.3%). The utilization of vasopressors was significantly higher in AKI patients (53.1%) compared to non-AKI patients (20.1%) (P<0.001). There was a notable disparity in serum creatinine levels upon admission to the intensive care unit (ICU) and in 48 hours between the two groups (P<0.001). Patients with acute kidney injury (AKI) exhibited a higher median serum creatinine concentration of 1.3 mg/dL in comparison to non-AKI patients. Table 1 demonstrates that being over 60 years old, having hypertension, sepsis, using vasopressors, and having a serum creatinine level greater than 1.0 mg/dL at admission to the ICU are significant factors that increase the risk of AKI in ICU patients.

Table 2 displays the results of a multivariate logistic regression analysis that identifies autonomous risk factors for the incidence of AKI in patients in the intensive care unit. The predictors examined in this study were age over 60 years, a previous diagnosis of chronic kidney disease, sepsis, the use of vasopressors, and a serum creatinine level exceeding 1.0 mg/dL upon admission to the intensive care unit. This study included these variables in logistic regression model to develop a predictive score for the likelihood of acute kidney injury in ICU patients.

Patients aged 60 and above had a 3.21-fold greater probability of suffering AKI compared to younger patients (P<0.001). Patients who have previously had chronic kidney disease are at a

Table 1. Demographical and Clinical Characteristics of Patients

Characteristics	AKI (N=128)	Non-AKI (N=224)	P-value
Age (years), median (IQR	65 (55-72)	52 (40-60)	<0.001*
Gender, N (%)			
Male	78 (60.9)	136 (60.7)	0.985†
Female	50 (39.1)	88 (39.3)	
Comorbid disease, N (%)			
Hypertension	82 (64.1)	102 (45.5)	<0.001†
Diabetes mellitus	45 (35.2)	58 (25.9)	0.085†
Heart disease	38 (29.7)	42 (18.8)	0.032†
Chronic lung disease	25 (19.5)	30 (13.4)	0.128†
Chronic liver disease	12 (9.4)	18 (8.0)	0.679†
Main diagnosis, N (%)			
Sepsis	55 (43.0)	32 (14.3)	<0.001†
Heart failure	28 (21.9)	40 (17.9)	0.315†
Pneumonia	20 (15.6)	35 (15.6)	0.999†
Trauma/injury	15 (11.7)	22 (9.8)	0.603†
Others	10 (7.8)	95 (42.4)	<0.001†
Vasopressor use, N (%)	68 (53.1)	45 (20.1)	<0.001†
Serum creatinine levels upon admission to ICU (mg/dL), median (IQR)	1.3 (1.0-1.8)	0.8 (0.6-1.0)	<0.001*
Serum creatinine levels in 48 hours after admission (mg/dL), median (IQR)	2.0 (1.5-2.8)	0.9 (0.7-1.2)	<0.001*
Urine output (mL/kg/hour), median (IQR)	0.3 (0.2-0.4)	0.8 (0.6-1.0)	<0.001*

*Independent t-test; †Chi-square test.

Table 2. Independent Predictors of Acute Kidney Injury (AKI) Determined by a Multivariate Logistic Regression Analysis

Predictors	Regression coefficient	Odds ratio (OR)	95% confidence interval (CI)	P-value*
Age >60 years old	1.17	3.21	1.85 - 5.56	<0.001
History of chronic kidney disease	1.15	2.87	1.54 - 5.34	<0.001
Sepsis	2.43	4.72	2.58 - 8.65	<0.001
Vasopressor use	1.38	3.98	2.25 - 7.04	<0.001
Serum creatinine levels upon admission to ICU >1.0 mg/dL	0.92	2.53	1.42 - 4.51	<0.001

*Multivariate logistic regression analysis.

significantly increased risk, 2.87 times greater, of developing acute kidney injury (P<0.001). Patients diagnosed with sepsis had a risk of having acute kidney injury (AKI) that was 4.72 times greater than that of those without sepsis (P<0.001). Patients who were administered vasopressors had a 3.98-fold increased chance of developing AKI (P<0.001). Patients admitted to the ICU with serum creatinine levels exceeding 1.0 mg/dL had a 2.53-fold increased chance of developing AKI (P<0.001) with median value for AKI group is 1.3 (IQR 1.0-1.8) and non AKI group is 0.8 (IQR 0.6-1.0). All predictors in Table 2 have a P value less than 0.001, indicating that the results are highly statistically significant. Therefore, all these predictors can be deduced as significant independent risk factors for the onset of AKI in patients in the intensive care unit.

This study's multivariate logistic regression analysis yielded the AKI predictor score, which Table 3 displays. The regression coefficient quantifies the extent to which a predictor variable affects the likelihood of AKI, while also taking into account the impact of other predictor factors. In logistic regression analysis, the regression coefficient were computed using a natural logarithm (ln) scale. The regression coefficient was transformed into an OR to facilitate understanding, comparing the likelihood (odds) of AKI between two patient groups with a one unit difference in the predictor variable. For example, the regression coefficient for people over the age of 60 is 1.17. Each additional year of age above 60 raises the likelihood of AKI by a factor of 3.21, after accounting for the impact of other predictor variables. In order to create an AKI predictor score, the regression coefficient of each predictor variable is multiplied by a uniform scale factor. This results in a rounded score that is straightforward to read. The study utilized a scale factor of 10, resulting in a range of 0–10 for the AKI predictor score. The AKI predictor score is determined by summing the scores of all predictor variables. As the total score increases, so does the patient's risk of developing AKI.

Table 3. Acute Kidney Injury Predictor Score

Predictors	Score*
Age > 60 years old	2
History of chronic kidney disease	2
Sepsis	3
Vasopressor use	2
Serum creatinine levels upon admission to ICU >1.0 mg/dL	1

*Range scale from 0-10. Low risk score: 0-3, high risk score: 8-10.

The ROC curve depicted in Figure 2 demonstrates the AKI prediction score's efficacy in distinguishing between patients who develop AKI and those who do not. AUC=0.85 implies that the predictor score has high accuracy in predicting AKI. A model's performance improves when the ROC curve deviates more from the diagonal line, which represents a random prediction model. A 95% confidence interval (CI) of 0.80-0.90 suggests that there is a 95% probability that the actual AUC value in the population falls within that specific range. This demonstrates the reliability of the study's findings and their applicability to a broader population.

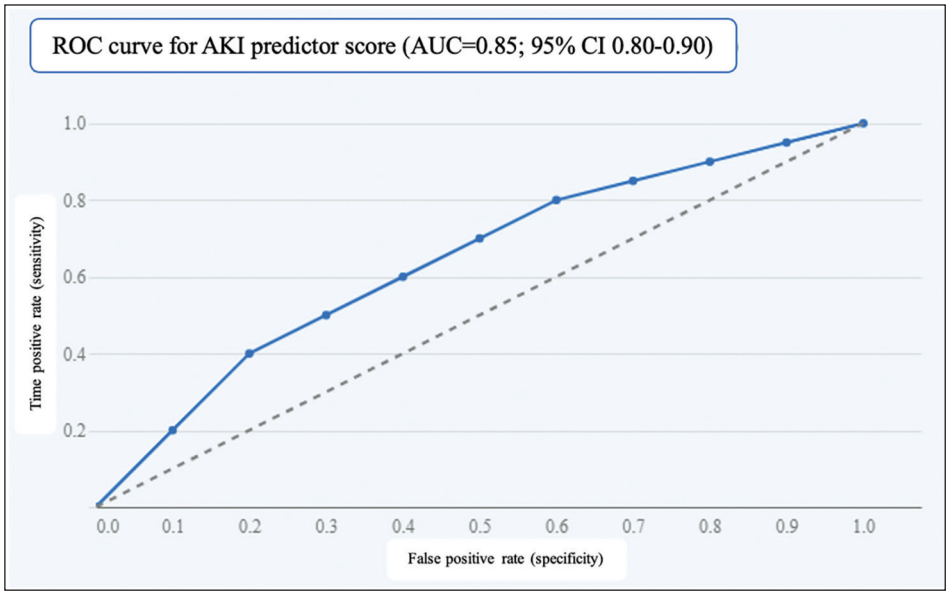

Figure 2. ROC curve for AKI predictor score.

Discussion

This study successfully identified five important independent predictors of acute kidney injury (AKI) development in intensive care unit (ICU) patients in Padang City, Indonesia. Individuals aged over 60 years, those with a history of chronic kidney disease, those with sepsis, those who have used vasopressors, and those with a serum creatinine level exceeding 1.0 mg/dL at admission to the intensive care unit are among the predictors. The findings align with current scientific evidence and enhance our understanding of the etiology of AKI and its therapeutic implications. Aging is an intricate and multifaceted physiological process that impacts different organ systems, including the kidneys. As individuals get older, their kidney function gradually declines (13). This includes a reduction in glomerular filtration rate (GFR), a decrease in kidney mass, and a decrease in the ability to concentrate urine. The decline in kidney function in elderly adults increases their susceptibility to stressors that might induce AKI, such as hypovolemia, infections, and drug-induced nephrotoxicity (14, 15). Furthermore, older adults frequently have comorbidities such as hypertension, diabetes mellitus, and cardiovascular disease, which heighten the risk of AKI. These coexisting medical conditions can hasten the deterioration of kidney function and heighten the vulnerability to sudden kidney damage. People with chronic kidney disease (CKD) have limited renal function, which makes them more likely to have their kidney function decline due to things like low blood volume, infections, and medications that damage the kidneys. Even a slight reduction in GFR can result in medically significant acute kidney injury in individuals with chronic kidney disease (14). Also, people with chronic kidney disease often have problems with their electrolytes, metabolic acidosis, and anemia. These can make the patient's condition worse and increase the risk of complications related to AKI.

Sepsis is a primary contributor to the development of AKI in the ICU (9, 16). Sepsis induces a multifaceted systemic inflammatory reaction that involves the liberation of several inflammatory agents, including cytokines, chemokines, and free radicals (17). The presence of these inflammatory mediators can lead to impaired endothelial cell function, reduced kidney blood flow, and tubule damage, all of which contribute to the development of AKI (18, 19). Also, people with sepsis often have low blood pressure, less blood volume, and are given vasopressor drugs, all of which can make it harder for the kidneys to work and increase the risk of AKI. Vasopressors are administered to elevate blood pressure in individuals experiencing hypotension or shock (20, 21). Nevertheless, the administration of vasopressors can induce renal vasoconstriction, diminish renal blood flow, and initiate renal ischemia. Renal ischemia can cause tubular cell damage and initiate AKI. Furthermore, vasopressors can affect renal endothelial cell function and increase glomerular capillary permeability. This might result in the presence of protein in the urine and worsen damage to the kidneys.

The body eliminates serum creatinine, a byproduct of the breakdown of creatine phosphate in muscle, through the kidneys. Rising levels of serum creatinine are indicative of a decline in the GFR and serve as an early indication of compromised kidney function (22). Patients who have serum creatinine levels greater than 1.0 mg/dL upon admission to the ICU are at a heightened risk of developing AKI due to pre-existing impairment of their kidney function. Numerous causes during care in the ICU, such as hypovolemia, sepsis, and the administration of nephrotoxic medications, can exacerbate the compromised kidney function (22, 23). Compelling evidence of biological plausibility supports the conclusions of this investigation.

Aging leads to a decline in the kidneys' normal functioning, while a previous diagnosis of chronic kidney disease implies past kidney damage (14). Sepsis initiates a widespread inflammatory reaction that can harm the kidneys. The use of vasopressors can cause the kidneys' blood vessels to narrow and blood flow to be reduced, resulting in tissue damage (20). Elevated levels of creatinine in the blood suggest impaired kidney function. The

results of this investigation are consistent with prior studies conducted in various regions around the world. Multiple studies have identified age, previous chronic kidney disease, sepsis, and the use of vasopressors as separate risk factors for AKI among patients in the ICU (24-26). Additional research has also demonstrated that the levels of serum creatinine upon admission to the ICU are a significant indicator of AKI (27).

This work has significant therapeutic implications for the management of patients in the intensive care unit. This study establishes a solid foundation for the creation of prevention and early intervention programs by identifying separate risk factors for AKI. Once generated, the AKI predictor score serves as a valuable tool for identifying patients who are at a higher risk of developing AKI and assessing their individual risk levels. AKI prediction scores can serve as a helpful tool in guiding clinical decision-making in the ICU. We might subject patients with elevated scores to enhanced monitoring, enabling prompt intervention to avert or mitigate the severity of AKI. Possible therapies may involve adjusting fluid volume, refraining from using nephrotoxic medications, and constantly monitoring renal function.

However, our study has limitations. We conducted the study in Padang city, West Sumatra, where the population primarily consists of Malay and Minang ethnicities. While this research makes a significant contribution to the understanding of AKI in Indonesian ICU patients, additional research is still required. The AKI predictor score that has been created must undergo external validation in a larger and more diversified group of patients in the intensive care unit in Indonesia. There is a need for future studies that have specific plans to assess the effects of using AKI predictor scores on patient clinical outcomes, including death, length of hospital stay, and the requirement for renal replacement medication. Novel biomarkers like NGAL, KIM-1, and IL-18 could be used to help predict AKI, but more research needs to be done on this topic. Utilizing both biomarkers and clinical predictor scores can improve AKI prediction precision and provide more targeted therapies (12, 24). Additional investigation could reveal additional risk factors that may lead to AKI in ICU patients, including genetics, environmental variables, and pharmacological interactions.

Conclusion

This study clearly shows that people over 60 who have had chronic kidney disease, sepsis, or used vasopressors in the past, or who had serum creatinine levels above 1.0 mg/dL when they were admitted to the ICU in Padang City, Indonesia, are at a higher risk for acute kidney injury. The newly created AKI predictor score has the potential to enhance ICU patients' treatment by facilitating prompt identification, risk assessment, and early intervention in patients with a high likelihood of developing AKI. Additional research is required to confirm and improve the use of these prediction scores in clinical practice.

What Is Already Known on This Topic:

Several AKI diagnostic tools to assist doctors in identifying individuals at heightened risk for AKI has been established. The scores utilize a range of clinical and laboratory factors, including age, comorbidities, administration of nephrotoxic medications, and fluctuations in blood creatinine levels, to determine the likelihood of AKI for individual patients. These ratings are limited in their ability to accurately predict AKI in patients with specific circumstances, including chronic kidney disease, multiple organ failure, and those receiving renal replacement therapy.

What This Study Adds:

This study establishes a solid foundation for the creation of prevention and early intervention programs by identifying separate risk factors for AKI. Once generated, the AKI predictor score serves as a valuable tool for identifying patients who are at a higher risk of developing AKI and assessing their individual risk levels. AKI prediction scores can serve as a helpful tool in guiding clinical decision-making in the ICU. The newly created AKI predictor score has the potential to enhance ICU patients' treatment by facilitating prompt identification, risk assessment, and early intervention in patients with a high likelihood of developing AKI.

Conflict of Interest: The author declares that she has no conflict of interest.

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Original Article
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Online Workshops Versus Live Medical Education on Self-Medication Literacy for Middle School Students. What Is the Best Pedagogic Method?

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Abstract

Objective. This study aimed to determine which pedagogic method, online workshops or live medical education, was the better way to teach about self-medication for middle school students. **Methods.** The following groups were formed: group O (students receiving online education), group L (students participating in live medical education animated by a medical practitioner and a science teacher) and group C (students without any medical learning). To compare them, the students answered three multiple choice questions before and after the educational intervention. The students in group L were evaluated immediately after the live medical training and group O immediately after the online workshops (t1). Group C was only evaluated at t0. **Results.** Group C N=195), group L (N=219) and group O (N=200, but 101 students who participated in the online workshops students dropped out before the end) were equivalent in terms of gender, but their ages and school grades were statistically different (P<0.001). A post-hoc test revealed that students in group O were older and in a higher grade than those in the other two groups (P<0.001) but the mean ages and school grades were equivalent in group L and group C. At t0, the results obtained were equivalent in the 3 groups. At t1, school students obtained better results in both groups (P<0.001) but these same results were significantly better in group L than those obtained in group O (P<0.001). Age, gender, school grade and school level had no effect on the students' results. Conclusion: The study's findings suggest that live medical education is a superior approach for imparting self-medication knowledge to middle school students.

Key Words: Online ▪ Medical Education ▪ Workshop ▪ Self-Medication ▪ Middle School.

Introduction

Abuse of self-medication is a major public health problem, especially in the teenager population (1, 2). Although self-medication and improvement of health care literacy could be useful tools in reducing medical overuse in high income countries, inappropriate self-medication can cause potential adverse events, leading to the need to create boundaries (3). For example, self-medication using antibiotics and psychoactive agents seems to give limited symptom relief, while potentially causing adverse events in cases of inappropriate use, which must be dealt with by a health care professional (4).

Paracetamol is one of the most widespread and accessible over the counter (OTC) drugs not requiring a medical prescription or medical advice. Self-medication with paracetamol should be well understood, especially in the young population, in terms of its posology, its secondary effects and its potential liver toxicity. However, Miao et al. (5) highlighted the fact that teenagers with low medical knowledge and literacy were most likely to use

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self-medication to treat pain and unfortunately to be more likely to misuse medication.

Self-medication misuse seems to focus on younger aged individuals (6). However, medical education regarding self-medication in middle school has been poorly studied (5). Nevertheless, many different pedagogic methods could be used given that the young population should be more receptive and more skillful in relation to multimedia approaches. Recently, online education, especially for health care professionals and schoolteachers, has spread, as a result of the epidemic context of COVID-19 (7). It is therefore consistent to wonder if online web education could not be a more relevant pedagogic strategy than conventional methods, such as live face to face education, for targeting the teenager population.

The present comparative study aimed to determine which pedagogic method, online workshops or live, in-person medical education, is better for teaching middle school students about self-medication. Our hypothesis was that students receiving live medical education would have higher post-intervention knowledge scores than those receiving online workshops.

Methods

The Population

This prospective study employed a pre-test and post-test design to evaluate the effectiveness of different educational methods on self-medication literacy among middle school students. Three different groups were constituted prospectively and randomly. All students were in the 7th to 9th years of the same middle school. The present study was accepted by the middle school management team. The "control group" (group C) corresponded to the students who had not undertaken any medical education. The second group was named the "live group" (group L) and included the students who received in-person, live medical education. The third group was named the "online group" (group O) and included school students receiving online medical education without any interaction with the medical practitioner or the science teacher. All the results were pseudonymized.

Procedure

Group L (Figure 1) and group C

In group L, each school student received medical education organized in small groups (10 to 15 students) for 40 minutes. The medical education was provided by a senior resident in general internal medicine, along with a middle school science teacher. Each class was divided into two different stages. The first stage focused on self-medication with OTC analgesics. Recommendations regarding the duration of paracetamol use and its posology, according to the symptoms, disease and the morphology of the patient, were presented in an interesting manner, in the form of clinical cases using fake patients presenting with different symptoms. Competition between the groups was used to engage the teenagers proactively. The teachers emphasized the temporary aspect of self-medication. In the context of the clinical cases, different OTC drugs, posology and treatment duration were proposed, and the school students had to choose between the suggested possibilities. The second session focused on the challenges related to the healthcare pathways used, with an explanation of the different routes in France. The clinical cases were presented (as role play) and the care pathways were debated by the students. In group C, the school students received no medical education nor online workshops. To ensure that differences in results were not due to handling, we carefully managed each group. Group C students were assessed only once at the beginning of the study to establish baseline knowledge. Group L students received consistent medical education through live sessions conducted by a senior resident and a science teacher in small groups, with assessments before and after the sessions. Group O students completed the online workshops individually, with engagement monitored to ensure completion, and they were also assessed before and after the intervention. This approach allowed us to isolate the effects of the educational interventions, providing a clear comparison between the different methods

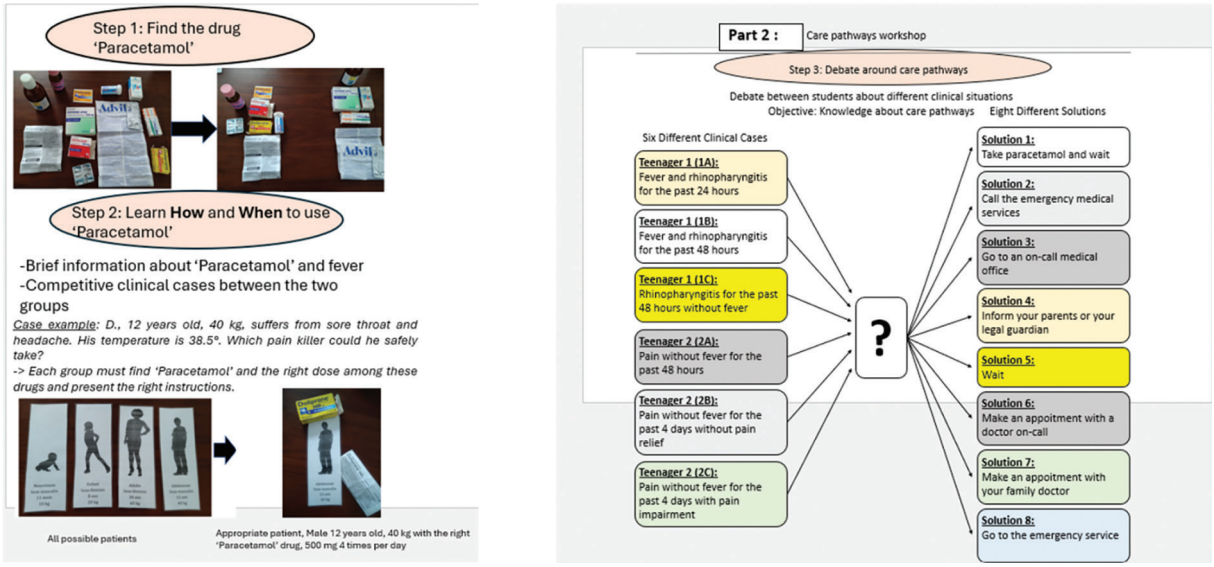

Figure 1. The medical education procedure: First part of the medical education given to group L students: self-medication workshop (Part 1). Second part of medical education given to group L students: self-medication workshop (Part 2).

Group O

In group O, the selected school students connected online to a specially created website. The interactive website was dichotomized into 4 different workshops. The first workshop (Figure 2) consisted of ranking six different medicines as either an OTC drug or other. The students had to decide in six cases involving a different drug either "I can take it without medical advice" or "I cannot take it before seeing a medical doctor". The

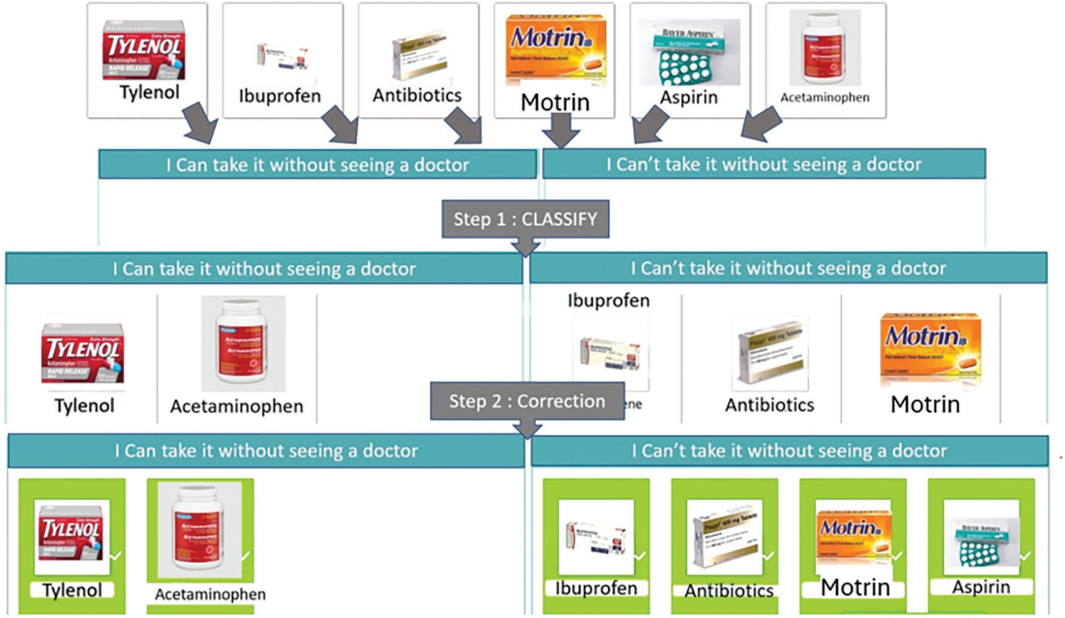

Figure 2. The first workshop for group O consisted of ranking six different medicines as either OTC drug or other. The students had to slide six cases containing a different drug either under "I can take it without seeing a doctor" or "I can't take it without seeing a doctor".

second workshop consisted of identifying the elements in the paracetamol notice (Physicians' Desk Reference) which allowed them to answer three questions regarding the use of paracetamol such as 'What time interval do I need to respect before taking another paracetamol tablet?' (Figure 3). In

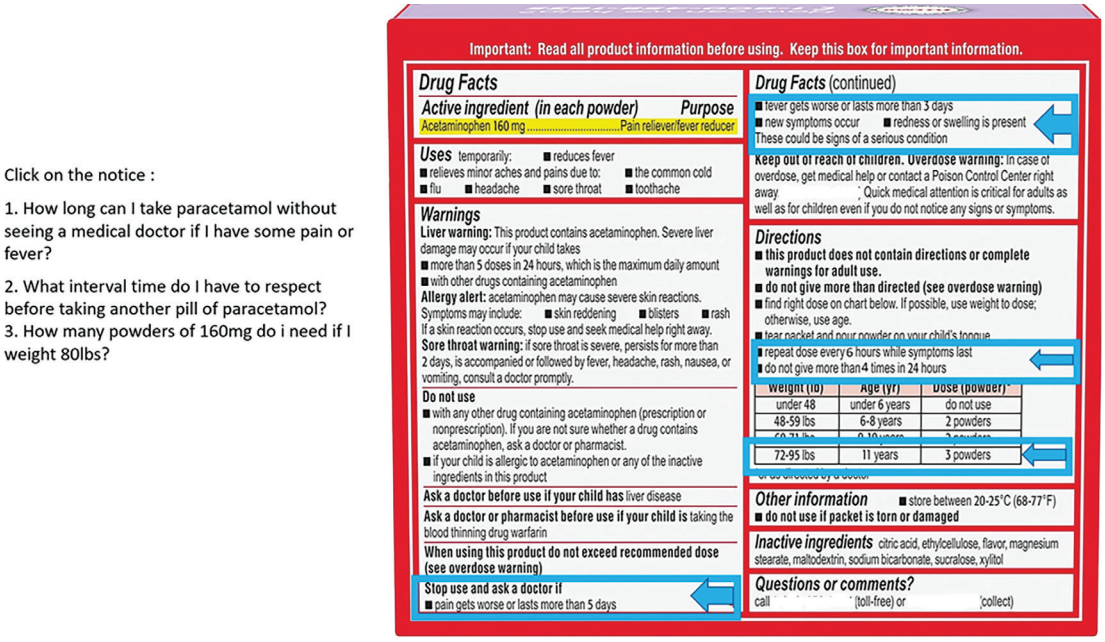

Figure 3. The second workshop consisted of identifying the elements in the paracetamol notice (Physicians' Desk Reference (PDR)) by clicking on the adapted part of the PDR which required answering three questions regarding the use of paracetamol (blue arrows and blue frames):
1. How long can I take paracetamol without seeing a medical doctor if I have some pain or fever?
2. What time interval do I need to respect before taking another paracetamol tablet?
3. How many 160 mg powders do I need to take if I weigh 80 lbs?

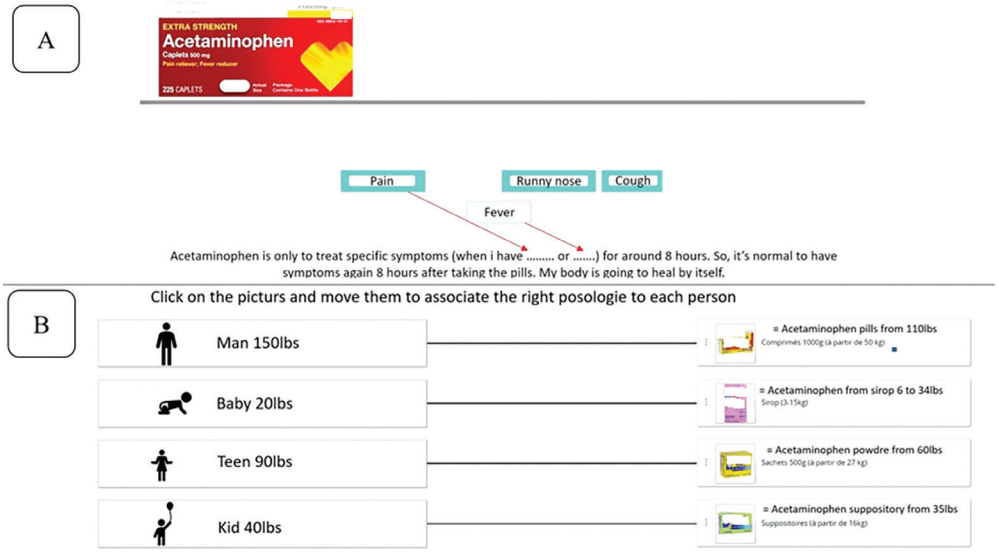

Figure 4. In the third workshop, the school students had to find the symptoms which allow someone to take paracetamol (A). In the fourth workshop, the school students had to find the appropriate posology for paracetamol according to the age and weight of the proposed patient (B).

the third workshop, the school students had to find the right symptoms which authorized the taking of paracetamol. In the fourth workshop (Figure 4), the school students had to find the appropriate posology for paracetamol, according to the age and weight of the patient in question.

School Student Evaluation

Two assessments of the students' knowledge about self-medication were conducted: before the medical education for group L and before the online workshops for group O (t_0). Subsequently, the students in group L were evaluated immediately after the in-person medical education and group O immediately after the online workshops (t_1). Each time, the same assessment was used, containing 3 multiple choice questions (MCQ) based on knowledge of self-medication and corrected. Each MCQ contained between 5 and 7 items. The students included in both groups L and O completed the assessment twice (t_0 and t_1). Regarding group C, the students were only assessed once (t_0). The correction scale was as follows: each MCQ was scored according to the number of items (Question 1: 7 points / Questions 2: 6 points Question 3: 5 points). Each question with a correct answer (can either be true or false) was scored 1. For wrong answers, the score was set at 0. If the student did not give any responses to a question, the assigned score was 0. The students' evaluations were never corrected. In group C and group L, each student had 15 minutes to answer the questions. In group O, no time limit was imposed but the response time was measured for each question. The school standard of each middle school student was given *a priori* by the relevant teacher of each class. The school standards were graded as follows: level A indicated a very good student, level B a good student, level C an average student and level M students with a low level of school success.

Statistical Analysis

Categorical data were expressed as percentages and were compared using Pearson's Chi-square test; continuous data were expressed as the mean and 95% confidence interval (CI) and were compared using one way ANOVA. To compare the observed results from the three groups constituted (group C, group O and group L), four-way (age, sex, school level and school grade) repeated measure ANOVA was performed. A post hoc Bonferroni test was conducted to find differences between the three groups. The statistical significance of all variables was set at a P-value of < 0.05. Data were anonymously recorded in Excel 2008 (Microsoft, Richmond, WA, USA). Statistical analysis was performed with SPSS Advanced Statistics 20.0 software (IBM, Armonk, NY, USA).

Results

The characteristics of each group are presented in Table 1.

One hundred and ninety-five students were enrolled in group C, 219 in group L, and 99 in group O. Initially, 200 students connected through the website for the online workshop in group O, but 101 dropped out, leaving 99 students who completed the final evaluation (t1). The students who dropped out of group O were younger (P<0.001) and in a lower school grade (P<0.001) compared to those who completed the study, but there was no gender difference. The mean ages were 12.1 years (95% CI [12-12.3]) for group C, 11.9 years (95% CI [11.8-12]) for group L, and 12.7 years (95% CI [12.5-12.9]) for group O, with a statistically significant difference in age between the groups (P<0.001). A post-hoc test indicated that students in group O were older than those in the other groups (P<0.001), but the mean ages for groups L and C were equivalent. Gender distribution was similar across the three groups. School grades were significantly different between group O and the other groups (P<0.001), but equivalent between groups L and C. Additionally, the school standard differed between groups O and L (P<0.001).

Comparison of the different pedagogic methods (Table 2).

At T_0, the results obtained before the medical education (group L) and the online workshops

Table 1. Characteristics of Each Student Group

Characteristics	Groups			P-value*
	Control (N=195; %)	Live learning (N=219; %)	Online workshop (N=99; %)	
	$\bar{x}$ [CI]	$\bar{x}$ [CI]	$\bar{x}$ [CI]	
Age	12.1 yr [12-12.3]	11.9 yr [11.8-12]	12.7 yr [12.5-12.9]	P<0.001
Gender (N; %)				
Male	109 (56)	116 (53)	41 (41)	0.06
Female	86 (44)	103 (47)	58 (59)	
Middle scholar grade (N; %)				
6th year	59 (30.3)	81 (37)	16 (16.7)	P<0.001
7th year	71 (36.4)	70 (32)	21 (21.9)	
8th year	65 (33.3)	68 (31)	34 (35.4)	
9th year	0 (0)	0 (0)	25 (26)	
Scholar level (N; %)				
Level A	No data	68 (31.1)	25 (25.3)	P<0.001
Level B	No data	92 (42)	34 (34.3)	
Level C	No data	57 (26)	21 (21.2)	
Level M	No data	2 (0.9)	16 (16.2)	

*Four-way ANOVA.

Table 2. Results of the Multiple Response Questions Evaluations in Each Group. T_0: before the Medical Education for Group L and before the Online Workshop for Group O. T_1: Immediately after the Medical Education for Group L and Immediately after the Online Workshop for Group O

MRQ* evaluation		T_0† Mean Points [CI 95%]	T_1† Mean Points [CI 95%]
Live learning group (N=219)	Question 1	5.7 [5.6-5.8]	6.7 [6.6-6.8]
	Question 2	4.6 [4.6-4.7]	5.2 [5.1-5.4]
	Question 3	4.3 [4.2-4.5]	5 [4.8-5.1]
	Cumulative result (Q‡1 to Q3)	13.7 [13.4-14]	16.9 [16.6-17.1]
Online group (N=99)	Question 1	6 [5.8-6.1]	6.1 [5.9-6.4]
	Question 2	3.5 [3.3-3.7]	3.8 [3.5-4]
	Question 3	4.3 [4.1-4.5]	4.6 [4.3 -4.8]
	Cumulative result (Q‡1 to Q3)	13.7 [13.3-14.1]	14.5 [14-15]
Control group (N=195)	Question 1	6 [5.1-6.2]	-
	Question 2	3.8 [3.6-4]	-
	Question 3	4.2 [4 -4.3]	-
	Cumulative result (Q‡1 to Q3)	[13.6-14.4]	-

*Multiple Response Questions;†Time; ‡Question.

(group O) were equivalent with the control group (group C). At T_1, after participating in the online workshop or the medical education, the school students attained better results in both groups (P<0.001) but these same results were significantly better in group L than in group O (14.5 (95% CI [14-15]) in group O versus 16.9 (95% CI [16.6-17.1]) in group L (P<0.001)). Age, gender, school grade and school standard had no effect on the school students' results. As regards group O, the

cumulative response time of the evaluations was 166 seconds (95% CI [137.5-195.5]) and 84.6 seconds (95% CI [71.2-99.2]) at T0 and T1, respectively. The cumulative response time tended to fall after the online workshops, but this was not statistically significant. Age, gender, school grade and school level had no effect on the response time in the different evaluations.

Discussion

The present study highlights the superiority of live medical education given by a practitioner and a science teacher in comparison with open access online workshops in relation to education regarding self-medication for middle school teenagers. Another advantage of live, in-person medical education is that it avoids any loss to follow-up. In fact, in group O, only one third of the students initially connected finished the entire online workshop, while all the students in group L participated completely in the medical education. Furthermore, the students who dropped out of the workshop in group O were mainly younger than the other students who finished the entire online workshop, suggesting that online pedagogic support does not seem to be relevant for young teenagers. Moreover, despite the fact that the school students in group O were older and hypothetically more experienced than those in group L, they obtained worse results than the students in group L.

Face to face, live medical education represents the gold standard as a pedagogic method to improve teenagers' medical literacy, giving better results than online education (8). Previously, Hudson et al. (9) demonstrated in the same way that a brief educational intervention, similar to our live medical education for middle and high school students statistically improved the teenagers' cancer literacy and knowledge. Miletics et al. (8) in a study comparing online versus live seminars relating to obesity, also noted that the live seminar had a more positive effect than the online seminar, leading to an increase in office visits and bariatric surgeries after participation in live seminars. Live medical education allows a direct link between the teachers and the students, and person-to-person interaction. Live medical education is a more interactive and didactic pedagogic method than online workshops, which are more passive and discouraging, especially for young teenagers. Nevertheless, live medical education has some drawbacks. It is a time-consuming pedagogic method, requiring one or more health care professional. Thus, as a result of the interactive and participative aspect of live medical educations, sessions have to be organized in small groups, which increases their time-consuming character.

To the best of our knowledge, no study has compared conventional live education with online remote education for middle school students. Most of the published studies comparing both pedagogic methods in the literature concern health care professionals, undergraduate or graduate students, or an older targeted population (10). For example, Jain et al. (11) compared tele-education versus classroom training as regards neonatal resuscitation, in relation to nurses. No difference was found. Most published studies on this topic found equivalent results from both educational procedures, and mentioned some of the advantages of online education, such as open access and its apparent flexibility (10). In the literature, it is stated that online education gives equivalent results because, we believe, these studies included more experienced and older populations, whereas in our study we focused on a young population, less accustomed to using online educational support. Online educational support certainly requires a more mature target population, such as adults and younger students after completing their high school education.

In the present study, even if the results obtained after the online workshop were not as good as the results obtained after the live medical education, the students in group O did progress somewhat thanks to the online workshop. Despite the lower efficiency of online workshops in comparison with live medical education given by a health care professional, online pedagogic support has several advantages (12). Online workshops are openly accessible, less time consuming, because they do not

need a teacher, and less expensive than conventional medical education. Online education could be used in conjunction with conventional medical education to create a positive synergetic effect regarding the medical knowledge of middle school teenagers, and online education could also allow teenagers' knowledge to be up-dated and maintained over time.

Limitations of the Study

This study has certain limitations. The populations in the three different groups were heterogenic and these differences can introduce confounding variables that affect the comparability and validity of the results. In fact, the students in group O were older than those in the other two groups. This is explained by the fact that 101 students were lost to follow-up in group O who did not finish the online workshops. The students lost to follow-up were younger than the remaining students who mainly finished the online workshops. This high attrition may indicate that the students who remained in group O were those most enthusiastic about online education. This age difference between the groups underlines the importance of considering variations in school achievement and grades between groups. In addition, including a follow-up test for group C could have provided additional insights into the natural progression of knowledge retention without any intervention. The other limitation concerns the self-medication knowledge of the three groups because only short-term follow-up was possible. Long-term evaluation of their self-medication literacy is needed in order to determine clearly if the medical education and online workshop had any impact on the teenagers' knowledge. Finally, the four way ANOVA used could present some limitations, including the complexity and difficulty of interpreting interactions, the risk of multicollinearity, and the potential issues with the generalizability of the findings.

Conclusion

Live medical education seems to be the better pedagogic method in order to improve self-medication practices. Online workshops certainly improve school students' knowledge, but seem to be less efficient than conventional medical education. The significant problem of students dropping out of the online pedagogic method remains. However, online education is currently taking a growing place and gives access to education to underprivileged and remote populations (13). Even if online pedagogic support does not seem to be able to be a substitute for conventional live medical education, it could have a synergistic effect together with online pedagogic support that is easily reusable, openly accessible and less expensive.

What Is Already Known on This Topic:
In the literature, online medical education, either as e-learning or interactive medical workshops, has not proven its superiority over conventional live medical education.

What This Study Adds*:*
Significantly better results were achieved by conventional live, face to face medical education at school in comparison to online medical education using online workshops.

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Selenium and Triple Negative Breast Cancer

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Abstract

Background. The deadliest, most dangerous subtype of breast cancer is triple-negative, which lacks treatment targets and accounts for 30% of all breast cancer-related deaths worldwide. TNBC is characterized by the expression of no estrogen, progesterone, or human epidermal growth factor 2 receptors. This suggests that new treatment modalities with fewer adverse effects are required. **Objective.** The aim of the present study was to investigate the therapeutic potential of selenium compounds as an adjuvant therapy for Triple Negative Breast Cancer (TNBC), either on their own or in conjunction with nutritional supplements and chemotherapy medications. **Methods.** Using the keywords "selenium" and "triple negative breast cancer", a thorough search was conducted in the PubMed database, yielding 23 articles. The following factors were taken into consideration for inclusion: studies using TNBC cell culture lines or in vivo tumors/specimens; full-text articles from the PubMed database; studies published in the English language; experiments with statistically significant results; and selenium used alone or in combination with other antioxidants or chemotherapy. This led to the evaluation of 13 articles in this review. **Results.** The results show that selenium therapy increased the anti-cancer drug's effects and produced tumor cytotoxicity, while reducing the cellular features of the cancer (hyperproliferation, growth, and metastasis). **Discussion.** This study evaluated the various selenium compounds tested, the cell lines and model organisms used, the assays performed, and the cellular pathways affected. **Conclusion.** Examining the possible benefits of selenium in TNBC treatment highlights the need for more studies to confirm selenium compounds as viable co-therapeutic agents.

Key Words: Selenium ▪ Triple Negative Breast Cancer ▪ Antioxidants ▪ Oncology.

Introduction

Breast cancer ranks among the top causes of cancer-related fatalities worldwide (about 600,000 deaths yearly) (1). Triple negative breast cancer (TNBC) is a highly aggressive subtype, due to the fact that human epidermal growth factor receptor 2 (HER2), progesterone (PR), and estrogen (ER) receptors are not expressed molecularly. TNBC is characterized by significant invasiveness, a high potential for metastasis, and an unfavorable prognosis.

Due to the lack of therapeutic targets (ER, PR, HER2), treatment options for TNBC are limited (2). According to studies, selenium (Se) may be used as a successful treatment for TNBC. Se components, in particular, have the potential to be used in both organic and inorganic forms, as well as conjugated with other antioxidants or conventional chemotherapeutic agents. They have the potential to significantly inhibit tumor growth (2-8), and improve the efficacy of chemotherapeutic agents by influencing multiple molecular pathways involved in cancer cells, while posing minimal toxicity to healthy cells.

This narrative review aims to investigate the potential therapeutic applications of selenium compounds as a customized treatment for TNBC,

either in isolation or in combination with dietary supplements and chemotherapy.

Methods

This review's objective is to evaluate selenium and its constituents' potential as treatments for TNBC. A comprehensive search for the terms "selenium" and "triple negative breast cancer" was done on the PubMed database in August 2023. This search turned up a total of 23 articles that were published between the years 2012 and 2023.

The papers were assessed using the following criteria: (a) use of selenium alone, or in combination with other antioxidants or chemotherapy; (b) research employing in vivo specimens or TNBC cell culture lines; (c) experimental research yielding results that are statistically significant; (d) full-text publications that can be found in the PubMed database; (e) research articles that are available in English. Thirteen articles remained after elimination of others from consideration for this review on the basis of these criteria.

Results

It was found that selenium compounds augment the effects of anticancer medications and induce notable morphological changes in tumor models, which leads to a decrease in tumor development (2-8). The cells displayed apoptotic body formation, along with enlargement, shrinkage, increased granularity, and rupture of the cytoplasmic membrane (1, 8, 9, 10, 11). Increased oxidative stress, superoxide, and ROS production were also noted (1, 3, 8, 10). The tumor models showed reduced growth, viability, and proliferation of cells, which enhanced cytotoxicity against TNBC cells and resulted in cell cycle arrest (2, 5, 7, 12, 13). Numerous investigations, executed at various periods of the experimental process, revealed increased autophagy at first, followed by necrosis, and apoptosis (1, 3-11, 13). Moreover, tumoral metastasis was decreased by treatment with selenium compounds, either by themselves or in combination with anticancer therapy (1-3, 6, 11-13). This is an highly noteworthy result, especially in patients of TNBC with extensive metastases. The results that were reported were dependent on the treatment's dosage and/or duration (1-12). Furthermore, while most research showed little to no negative effects, a few detected cytotoxicity in normal epithelial cells when using selenium compounds alone (7, 8, 11).

Discussion

In the current study, we assessed papers that employed different kinds of selenium compounds. Specifically, some studies used different selenium compounds or derivatives alone, while the remaining studies used selenium compounds in combination with different chemotherapeutic medications and/or supplements. To be more precise, the anticancer medications most often utilized in the second group of research included the chemotherapeutic medications Trastuzumab, Bevacizumab (Avastin), Doxorubicin (Adriamycin), and Paclitaxel (Taxol), as well as the reverse transcriptase inhibitor AZT (azidothymidine). The selenium compounds used in this category of studies were: Se-modified Bolton-Hunter reagent in combination with Trastuzumab and Bevacizumab (1), fish oil with Se (FO/Se) in combination with Doxorubicin (2), Se with AZT resulting in three drugs (S1072, S1073, S1079) (9), Se with EDA/DHA alone or combined with Taxol, Adriamycin and Avastin (3), methylselenic acid (MSA) with Paclitaxel (4), Se-containing polysaccharides from *Pyracantha fortuneana* (Se-PFPs) in combination with Doxorubicin (5), supplementary marine-based FO and Se yeast (FO/SE) in combination with Avastin (6). In the first category of studies, Se was utilized in the form of: selenoesters EDA-71 (Se-(2-oxopropyl)4-chlorobenzoselenate) and E-NS-4 (Se-cyanomethyl 4-chlorobenzoselenate) (7), selenofolate (conjunction of folic acid and redox selenium adduct) (10), seleno-purine SLLN-15 (4-selenomorpholinophenyl and tetrahydroselenophene- substituted diamino-purines) (12), selenomethionine (organic), sodium selenate and sodium selenite (inorganic), ebselen and diphenyl diselenide (synthetic organoselenium

compounds) (11), Se yeast, methylselenic acid (MSA) and methylselenocysteine (MSC) (8), Se-containing polysaccharides from *Pyracantha fortuneana* (13), and Benzimidazole-Containing Selenadiazole Derivatives (BSeDs: 1a, 1b, 1c, 1d) (13). The compounds mentioned above, along with their chemical formulas, dosages, and effects on TNBC viability, proliferation and metastatic potential are summarized in Table 1.

Table 1. Effects of Selenium on TNBC Viability

Authors	Selenium compound used	Chemical formula	Dosage	Effects on TNBC cells
Khandelwal, Soni et al. (1)	Selenotrastuzumab (Se-TZ) and Selenobevacizumab (Se-BV)	Conjuction of Trastuzumab and Bevacizumab with selenium-modified Bolton-Hunter reagent (redox active and toxic form of selenium)	2 µg Se as Se-TZ or Se-BV	1) Inhibition of cell proliferation by generating superoxide and other ROS (resulting from oxidation of glutathione and other thiols); 2) Cell swelling and shrinking; 3) Induced apoptosis by reducing ΔψM and increasing superoxide generation; 4) Reduced chance of metastasis.
Guo, Chih-Hung et al. (2)	Doxorubicin with FO/Se	Combination of Fish oil (FO) containing $C_{22}H_{32}O_2$ (docosahexaenoic acid, DHA, 22:6 n3) and $C_{20}H_{30}O_2$ (eicosapentaenoic acid, EPA, 20:5 n3), and Se with Doxorubocin	5 mg/kg doxorubicin together with 0.4 g of FO/Se in low, medium, and high concentrations (8.8 mg/2.7 µg/g, 16.9 mg/4.0 µg/g, and 19.0 mg/6.7 µg/g, respectively)	1) Reduced tumor growth; 2) Fewer metastases detected; 3) Alteration of tumor cytoplasmatic signaling pathway; 4) Reduction of the expression of tumor oncogenes and proteins; 5) Alteration of immune checkpoints; 6) Decreased cell proliferation, cell cycles and cancer stemness.
Guo, Chih-Hung et al (3)	Nutritional Supplement (NS) containing Se and EPA/DHA used alone or in combination with Taxol (Tax), Adriamycin (Adr), and Avastin (Ava)	Se yeast along with $C_{22}H_{32}O_2$ (docosahexaenoic acid, DHA, 22:6 n3) and $C_{20}H_{30}O_2$ (eicosapentaenoic acid, EPA, 20:5 n3), combined with Tax, Adr, and Ava	TB-Tax-NS group; Tax (5 mg/kg every 4 days) and NS (0.4 mg) by oral gavage twice a day TB-Adr-NS group; Adr (2 mg/kg every 4 days) and NS (0.4 mg) by oral gavage twice a day TB-Ava-NS group; Ava (5 mg/kg every 4 days) and NS (0.4 mg) by oral gavage twice a day for 25 days	1) Reduction of tumor weights and sizes; 2) Reduction of metastatic potential; 3) Alteration of tumor immune micro-environment; 4) Increase of oxidative stress; 5) Suppression of angiogenesis and cancer stem cells; 6) Induction of apoptotic tumor responses.
Qi, Yanfeng et al. (4)	MSA and Paclitaxel	CH_3SeO_2H (Methylseleninic acid, MSA) combined with Paclitaxel	In vitro; MSA (2.5, 3.2, 4 µM) and Paclitaxel (10, 20, 40 nM) in various combinations. In vivo; 3 mg MSA per kg body with 10 mg/kg Paclitaxel.	1) Enhanced inhibition of tumor growth (reduction of tumor weight); 2) Significant decrease in tumor cell proliferation; 3) Induction of apoptosis; 4) Inhibition of tumor re-growth after termination of treatment.
Yuan, Chengfu et al. (5)	1) Se-containing polysaccharides from Pyracantha fortuneana (Se-PFPs); 2) Se-PFPs in combination with doxorubicin	Heteropolysaccharides composed of xylose, arabinose, fucose, mannose, ribose, rhamnose, glucuronic acid, galacturonic acid, glucose, and galactose, along with uronic acid and Se	Escalating concentrations of Se-PFPs; 0, 50, 100, 200 and 400 µg/ml.	1) Inhibition of cell growth in MDA-MB-231 cells, caused by arrest at G2/M phases of cell cycle; 2) Induction of apoptosis; 3) Inhibition of tumor growth in treated mice (decrease in tumor volume and weight); 4) Enhanced effect of Se-PFPs on the sensitivity of MDA-MB-231 cells to doxorubicin.

Continuation of Table 1. Effects of Selenium on TNBC Viability

Authors	Selenium compound used	Chemical formula	Dosage	Effects on TNBC cells
Guo, Chih-Hung et al. (6)	FO/SE with Avastin	Supplemental marine-based fish oil (FO) containing omega-3 fatty acids such as $C_{22}H_{32}O_2$ (docosahexaenoic acid, DHA, 22:6 n3) and $C_{20}H_{30}O_2$ (eicosapentaenoic acid, EPA, 20:5 n3), along with Se yeast (FO/SE) in combination with Avastin (bevacizumab)	Concentrations of EPA, DHA, and elemental Se, respectively in low, medium, and high doses of FO/Se supplements; low; 5.1 mg, 3.7 mg, and 2.7 µg/g medium; 9.1 mg, 6.9 mg, and 4.0 µg high; 10.7 mg/g, 8.3 mg, and 6.7 µg/g 5 mg/kg of the Avastin (once every four days) and 0.4 g of low, medium, and high concentrations of EPA/DHA/Se supplements twice a day for 25 days	Enhanced efficacy of Avastin in 4T1 tumor cells (CRL-2539): 1) Markedly decreased tumor size; 2) Inhibition of EMT (epithelial-to-mesenchymal transition);3) Inhibition of metastasis; 4) Further induction of apoptosis.
Radomska, Dominika et al. (7)	Novel selenoesters EDA-71 and E-NS-4	EDA-71; Se-(2-oxopropyl) 4-chlorobenzoselenoate E-NS-4; Se-cyanomethyl 4-chlorobenzoselenoate	Escalating concentrations of both compounds; 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 µM	1) High cytotoxicity and growth suppression 2) Increased Apoptosis and necrosis 3) Induction of Autophagy 4) Induction of cell cycle arrest (all the above were more significant in MDA-MB-231 cells by using EDA-71)
Guo, Chih-Hung et al. (8)	1) Se yeast 2) Methylseleninic acid (MSA) 3) Methylselenocysteine (MSC)	Se yeast CH_3SeO_2H (methylseleninic acid, MSA) $C_4H_9NO_2Se$ (methylselenocysteine, MSC)	Se yeast (100, 750, and 1500 ng Se/mL) Methylseleninic acid (1500 ng Se/mL) Methylselenocysteine (1500 ng Se/mL)	1) Inhibition of tumor growth; 2) Increased superoxide production and antioxidant enzyme (SOD, GPx) activities; 3) Induction of early and late apoptosis; 4) Loss of mitochondrial membrane potential; 5) Nuclear morphological changes (formation of apoptotic bodies).
Wagner, Mônica Silveira et al. (9)	Seleno-AZT derivatives	Se combined with $C_{10}H_{13}N_5O_4$ (Azido-3´-deoxythymidine, AZT), a nucleoside reverse transcriptase inhibitor resulting in 3 derivatives; S1072, S1073, S1079	Medium containing derivatives S1072, S1073, S1079, and commercial AZT at concentrations 50 and 100 µM	1) Selective decrease in cell proliferation (especially in TNBC cell line); 2) Apoptotic morphology (=loss of attachment to other cells and ECM + having a rounder shape); 3) Cell death rate of a 35% in TNBC line.
Khandelwal, Soni et al.(10)	Selenofolate	2-selenocyan folate (conjuction of folic acid and 2-selenocyanoethanol)	Selenofolate 100 µM (8 µg Se)	1) Morphological changes such as shrinkage, swelling and cell membrane disruption. 2) Loss of mitochondrial membrane potential 3) Apoptosis induction through superoxide generation

Continuation of Table 1. Effects of Selenium on TNBC Viability

Authors	Selenium compound used	Chemical formula	Dosage	Effects on TNBC cells
da Costa, Nayara Souza et al. (11)	Selenomethionine (organic), sodium selenate and sodium selenite (inorganic), ebselen and diphenyl diselenide (synthetic organoselenium compounds)	$C_5H_{11}NO_2Se$ (selenomethionine) Na_2SeO_4 (sodium selenate) Na_2SeO_3 (sodium selenite) $C_{13}H_9NOSe$ (ebselen) $C_{12}H_{10}Se_2$ (diphenyl diselenide)	1, 10, 50, and 100 μM for 48 h	1) Decreased cell viability of MDA-MB-231 by selenate, selenite (100μM), ebselen and diphenyl diselenide (100μM); 2) Decreased cell viability of BT-549 by selenite (100μM), ebselen and diphenyl diselenide (50+100μM); 3) Reduced cell size in BT-549 by diphenyl diselenide at all concentrations; 4) Increased cell granularity by selenate (50+100 μM); 5) Increased number of apoptotic/ necrotic cells in TNBC cell lines by selenite (100μM), ebselen (100 μM); 6) Increased number of apoptotic/necrotic cells only in BT-549 by diphenyl diselenide (50+100μM); 7) Less colonies were formed in all cell lines by diphenyl diselenide; 8) Complete inhibition of colony formation in all cell lines by selenite (10-100μM); 9) Suppression of cell migration in BT-549 by diphenyl diselenide (1 μM).
Chang, Chia-Hao et al. (12)	Seleno-purine SLLN-15	$C_{19}H_{23}N_7Se_2$; (S)-N2-(4-selenomorpholinophenyl)-N6-(tetrahydroselenophen-3-yl)-9H-purine-2,6-diamine	In vivo; 30 mg/kg SLLN-15, 3 times a week for 40 days In vitro; 10 μM SLLN-15 for 24 h	1) Inhibition of cell growth in vitro and in vivo 2) Decreased number of metastases and TNBC cell progression 3) Stimulation of autophagy 4) G2/M cell cycle arrest
Liang, Yuanwei et al. (13)	Benzimidazole-Containing Selenadiazole Derivatives (BSeDs): 1a, 1b, 1c, 1d	1a; 5-(1H-benzo[d] imidazol-2-yl)benzo[c] [1,2,5]selenadiazole 1b; 5-(6-methyl-1H-benzo[d]imidazol-2-yl)benzo[c][1,2,5] selenadiazole 1c; 5-(6-chloro-1H-benzo[d]imidazol-2-yl)benzo[c][1,2,5] selenadiazole 1d; 5-(6-bromo-1H-benzo[d]imidazol-2-yl)benzo[c][1,2,5] selenadiazole	1b (0.5 and 1.0 μM), 1c (0.5, 1.0, and 2.1 μM), 1d (0.5, 1.0, and 1.3 μM)	1) Inhibition of cancer cell growth through cell-cycle arrest (increased sub-G1 and G2/M cell population and enhanced accumulation of cells in G0/G1 phase) and apoptosis (both early- and late-phase); 2) Inhibition of cancer cell migration.

Both in vitro and in vivo experiments were used in the studies evaluated for this review. The in vitro tests employed the following cell lines: HME50-5E (1, 10), MCF-10A (7, 11), CHO (9), HMEC (8), which are normal epithelial cell lines, and MDA-MB-231 (1, 4, 5, 7-9, 11-13), MDA-MB468 (1, 10, 12), BT-549 (4, 11), BT20 (12), which are TNBC cell models. Mice were the model organism used in all of the in vivo investigations. More precisely, MDA-MB-231 triple negative human

tumor cells were implanted into Severe Combined Immunodeficient (SCID) mice (4, 12), nude animals bearing MDA-MB-231 human cells (5), BALB/c mice implanted with 4T1 human TNBC cells, and mice bearing 4T1 human TNBC cells (2, 3, 6).

We also looked at the instruments and assays that each study used. More analytical methods were used to assess cell viability, including the Trypan blue cell exclusion method, the MTT assay (1, 4, 10), the XTT cell viability assay (5, 10, 12, 13), the LIVE/DEAD viability assay with fluorescence microscopy (9), and sulforhodamine B (SRB) (4). Additionally, the MTT assay (1), Brdu-labeling assay (4, 12), and [3H]-Thymidine incorporation assay were used to measure cell proliferation (7). The following techniques were employed to detect apoptosis: annexin V staining (1, 7-11, 13), propidium iodide (PI) staining (7, 8, 13), and other flow cytometry assays (7-9, 13), along with other apoptosis assays (4, 5, 8). In addition, phase contrast microscopy (1), photographic evaluation (10), flow cytometry (11), nuclei staining with DAPI, and fluorescence microscopy were used to evaluate the morphological alterations in the cells (8). Finally, target protein concentrations were assessed via flow cytometry and Western blotting (1, 2, 3-7, 10, 13).

Changes in mitochondrial membrane potential were detected by flow cytometry (1, 7, 8), whereas RNA levels and gene transcription were evaluated by using cDNA (2, 9) and real-time quantitative PCR (2, 6, 9). In regard to cytotoxic activity on cells, MTT assay (7, 9) and LDH assay (5) were most commonly performed. Superoxide generation was measured using lucigenin-amplified chemiluminescence (8, 10), dihydroethidium (10), and oxidation-sensitive fluorescent probe H2DCF (13).

In order to determine the distribution of the cell cycle, cell cycle analysis and flow cytometry were utilized (4, 5, 7, 13). Using the autophagy assay (7) and immunohistochemistry with the autophagy markers MAD1LC3B/LC3B, ATG12, and H+E staining, the quantity of autophagosomes and autolysosomes was estimated. The wound healing migration assay, the cell migration assay, and the colony formation assay were used to assess cell migration (11). Lastly, the colony formation test was used to assess the proliferation of the tumor cells, while flow cytometry was utilized to identify the tumor necrosis using 7-aminoactinomycin D (11) and annexin V-FITC/PI (8).

Cellular Pathways and Structures Affected

The bulk of the research evaluated for this review describes potential signaling pathways, and cellular structures or molecules impacted by using selenium compounds as a therapeutic approach against TNBC cell lines (Table 2). EGFR, FGFR (2, 3, 6), VEGF, phospho-VEGFR2, PDGFR2, TGF-β, and TGF-βR2 (6), as well as variations in VEGFA levels were the first proteins of the growth factor family to show changes in levels. Moreover, elevated p53 (2, 5) and its phosphorylated version (3, 13) levels were observed.

Furthermore, the PI3K-AKT-mTOR pathway was downregulated (2, 3, 6, 13), with higher levels of TSC1/2 (2, 6), whereas the AURKA-AKT-mTOR autophagy pathway was activated (12). The Ras-Raf-MEK-ERK pathway was downregulated (2, 3, 6, 13), the transcription factors c-jun and c-fos were decreased (2, 6), and levels of JNK protein were increased (13). The deactivation of the JAK2/STAT3 signaling (2, 3, 6), as well as the reduction of p-c-Src were detected (2). In addition, the levels of active NF-κB (2, 7), c-Myc (2), HIF-1α (2, 3, 6), and HIF-2α (2, 6) appeared reduced. Regarding the caspase proteins and PARP signaling, the intrinsic apoptosis pathway was induced by the increased levels of caspase 9 (5, 7), and the extrinsic apoptosis pathway appeared activated by the rise of the caspase-3/-8/-10 levels (3, 5-7, 9). The common apoptosis pathway was also activated by the elevated levels of caspase 3/7 (4, 7), along with increased PARP expression (4), and the image of cleaved DARP-1 (6). Additionally, co-signal molecules in T-cell activation were affected as follows: decreased PD-L1 (2, 3, 6), CTLA-4, Foxp3 (2), PD-1 in mammary glands (3), and an augmentation of PD-1 (2, 3) and IL-2 levels (2). The levels of molecules of cell cycle checkpoints (cyclins and CDKs) were also affected: cyclin E (2, 6, 7), cyclin D1 (2, 6), cyclin B1 (5), CDK2 (6),

Table 2. Selenium Compounds and Pathways Affected

Authors	Selenium compound used	Cellular Pathways and structures affected
Khandelwal, Soni et al. (1)	Selenotrastuzumab (Se-TZ) and Selenobevacizumab (Se-BV)	1) Se-TZ and Se-BV: Apoptosis induced by effect on cell membranes and mitochondrial activity (mechanism unknown); 2) Selenite: Cleaved β-actin bands in MDA-MB-468 cells and HME50-5E cells; 3) Se-BV: Cell death in TNBC cells but not HME-50-5E cells when the cell lines exhibited similar levels of VEGFA.
Guo, Chih-Hung et al. (2)	Doxorubicin with FO/Se	1) Lower GPR-40 mRNA levels and higher expression of all selenoproteins,; 2) Decreased expression of membrane EGFR and FGFR; 3) Higher mRNA levels of PTEN and decreased levels of p-PI3K, p-Akt, p-mTOR, p-4EBP1 and p70S6K; 4) Increased expression of TSC1 and TSC2; 5) Decreased levels of Ras, p-Raf1, p-MEK, and p-ERK1/2 proteins; 6) Lower levels of p-c-Src, p-JAK2 and p-STAT3 proteins; 7) Decreased tumor mRNA expression of c-Jun and c-Fos; 8) Lower protein levels of c-Myc and p-NF-κB p65 and lower levels of tumor HIF-1α and HIF-2α protein; 9) Increased tumor levels of p-P53 and lower levels of Ki-67; 10) Decreased mRNA levels of PD-L1, CTLA-4, and Foxp3 and higher mRNA levels of PD-1 and IL-2; 11) Lower expression of tumor PD-L1, CTLA-4, Foxp3, and CD86 and increased expression levels of PD-1, NKp46, and IL-2 proteins; 12) Lower mRNA levels of cyclin E and decreased expression of cyclin D1, CDK4, and CDK6; 13) Lower expression levels of CD24 and CD29.
Guo, Chih-Hung et al. (3)	Nutritional Supplement (NS) containing Se and EPA/DHA used alone or in combination with Taxol (Tax), Adriamycin (Adr), and Avastin (Ava)	1) Increased levels of MDA (oxidative stress biomarker) ; 2) Decreased plasma concentrations of IL-1β, IL-6, IL-10, TNF-α, and VEGF, and increased IFN-γ and IL-2; 3) Decreased expression of tumor HIF1-α; 4) Lower levels of tumor VEGF and decreased CD31, MMP-9, CD24 and CD29 expression; 5) Decreased tumor levels of HSP-70, HSP-90, AXL and p-AXL; 6) Increased levels of phosphorylated p53, cleaved caspase-3 and cytosolic cytochrome c and decreased Bcl-2 and mitochondrial cytochrome c expression; 7) Reduction of PD-L1 (through deactivation of AKT/ERK and JAK2/STAT3 signaling) and augmentation of PD-1 tumor levels; 8) Reduction of PD-L1 and PD-1 levels in mammary glands.
Qi, Yanfeng et al. (4)	MSA and Paclitaxel	1) Further decreased fraction of cells in G0/G1 phase; 2) Further increased G2/M arrest (cause of extensive apoptosis); 3) Activation of the caspase-PARP pathway (enhanced activation of caspase-3, caspase-7 and PARP).
Yuan, Chengfu et al. (5)	1) Se-containing polysaccharides from Pyracantha fortuneana (Se-PFPs); 2) Se-PFPs in combination with doxorubicin	1) Reduction of p-H3 levels in MDA-MB-231 cells; 2) Decreased levels of CDC25C, CDC2 and Cyclin B1; 3) Increase in the activities of caspases 3 and 9; 4) Increases in the protein levels of p53, Bax, Puma and Noxa; 5) Decreased levels of Bcl-2 and, in turn, increased Bax/Bcl-2 ratio; 6) Increased cytochrome C levels (apoptosis via the p53-mediated cytochrome c-caspase pathway).
Guo, Chih-Hung et al. (6)	FO/SE with Avastin	1) Increased SEPW1 in tumor tissues at all FO/Se concentrations and SPEN1 at medium and high doses of FO/se; 2) Lower levels of HSP90, HIF-1α and HIF-2α expression; 3) Lower cyclooxygenase (COX-2), superoxide dismutase (SOD-1) and metalloprotease-9 (MMP-9) levels; 4) Decrease in VEGF+ phospho-VEGFR2, EGFR, FGFR PDGFR2, TGFβ+ TGFβR2 levels; 5) Reduction of growth arest-specific-6 (Gas6) and AXL phosphorylation levels; 6) Lower levels of CXCL12, CXCR4+ CXCR7 (chemokines); 7) Decreased levels of Wnt3α/5α FZD7; 8) Reduction of PI3K, PTEN, AKT, mTOR, phosphor-p70s6K, phospho-4EBP1 and increased in TSC1 and TSC2 expression; 9) Lower levels of Ras, phospho-Raf1, phospho-MEK, phospho-ERK1/2 and higher levels of LKB-1, phospho-AMPK expression; 10) Reduction of phospho-smad2/3, smad 4 and TMEPAI at high concentrations of FO/Se; 11) Lower levels of phospho-c-Src+ phospho-JAK2, STAT3, phospho-GSK-3β, p-S552-β-catenin, p-S33-37-Y41-β-catenin and increase in GSK-3β; 12) Decrease in SNAIL, SLUG (EMT-activated transcription factors), cyclinD1,E, CDK-2/-4/-6 levels; 13) Increased cleaved-caspase-3/-8, phospho-Bcl-2, cleaved-DARP-1 and decreased cofilin-1 (CFL-1);14) Decreased CSC rkermas (CD29/24/44 and CXCR2).
Radomska, Dominika et al. (7)	Novel selenoesters EDA-71 and E-NS-4	1) Increased activation of caspases 8+10 (extrinsic apoptosis pathway); 2) Decreased Mitochondrial Potential (intrinsic apoptosis pathway); 3) Reduction of NF-κB active form (intrinsic apoptosis pathway); 4) Higher levels of caspase 9 (intrinsic apoptosis pathway); 5) Elevation of the caspase 3/7 active form levels (executive common phase of apoptosis); 6) Lower mTOR levels (autophagy pathway); 7) Increased cyclin A2 activity and decreased E1 activity (cell cycle arrest in S phase).

Continuation of Table 2. Selenium Compounds and Pathways Affected

Authors	Selenium compound used	Cellular Pathways and structures affected
Guo, Chih-Hung et al. (8)	1) Se yeast 2) Methylseleninic acid (MSA) 3) Methylselenocysteine (MSC)	-
Wagner, Mônica Silveira et al. (9)	Seleno-AZT derivatives	Mainly affected pathway seems to be the extrinsic apoptosis pathway because of the significant increase in caspases 3 and 8 gene expression (especially by using the S1072 drug)
Khandelwal, Soni et al.(10)	Selenofolate	1) Glutathione triggered superoxide generation; 2) Cellular targets= reduced GSH/other thiols and cysteines of mitochondrial potential; 3) Internalization through FRA (folic acid receptors that are overexpressed in TNBC).
da Costa, Nayara Souza et al. (11)	Selenomethionine (organic), sodium selenate and sodium selenite (inorganic), ebselen and diphenyl diselenide (synthetic organoselenium compounds)	-
Chang, Chia-Hao et al. (12)	Seleno-purine SLLN-15	1) AURKA- AKT- mTOR activation for indication of autophagy; 2) Inhibition of phosphorylation of AURKC; 3) Decreased expression of AURKA+ AURAKB resulting from the proteasome pathway.
Liang, Yuanwei et al. (13)	Benzimidazole-Containing Selenadiazole Derivatives (BSeDs): 1a, 1b, 1c, 1d	1) Induction of intracellular ROS generation; 2) Increased expression levels of P-histone; 3) Increased expression levels of P-ATM, causing the activation of P-BRCA1, which results in the upregulation of P-p53; 4) Downregulation of Bcl-2 and Bcl-xl expression; 5) Upregulation of Puma and Noxa expression; 6) Downregulation of MDM2 expression; 7) Upregulated phosphorylation of pro-apoptotic kinases p38 and JNK (MAPK family members); 8) Suppressed phosphorylation of anti-apoptotic kinase AKT; 9) Suppressed phosphorylation of anti-apoptotic kinase ERK (MAPK family member).

CDK4/6 (2, 6), and CDC25C/2 (5) were reduced, in contrast to the increase in the levels of cyclin A2 (7). This indicates cell cycle arrest in the S phase. Changes in mitochondrial potential and enzymes were also detected: loss of mitochondrial potential (1, 7, 8, 10) and reduction of mitochondrial cytochrome C enzyme (3, 5). Anti-apoptotic and pro-apoptotic protein levels were also impacted: Bcl-2 appeared decreased (3, 5, 13) as well as Bcl-xl (13) in contrast to increased phospho-Bcl-2 (6), Bax (5), Puma, and Noxa (5, 13). Furthermore, IL-2 (2, 3) and IFN-γ (3) were increased, whereas IL-1β, IL-6, IL-10, and TNF-α were deceased (3). Lastly, the heat-shock proteins HSP-90 (3, 6) and HSP-70 (3) appeared to decrease.

In terms of overall TNBC tumoral model findings, all of the trials that were assessed showed that selenium compounds were effective. More precisely, the effects of anticancer medications were increased when selenium compounds were added (1-6, 9). Additionally, it was evident that the use of selenium compounds, either alone or in conjunction with anticancer therapy, resulted in significant morphological alterations in the tumoral models under investigation, by slowing tumor growth (tumor weights and sizes) (2-8).

Regarding the morphology and morphological alterations, the cells showed signs of swelling and shrinkage, along with increased granularity, breakdown of the cytoplasmic membrane, and

apoptotic body formation (1, 8-11). Furthermore, an increase in oxidative stress, as well as the production of superoxide and ROS were noted (1, 3, 8, 10). Regarding the tumor models' cellular activity, it was found that TNBC cells were subjected to increased cytotoxicity and that cell growth (5, 12, 13), viability (7, 11), and proliferation were all markedly suppressed (1, 2, 4, 9).

Additionally, cell cycle arrest was found, usually in the G2/M phase (2, 5, 7, 12, 13). Numerous studies that were reviewed showed a significantly higher induction of necrosis and apoptosis by changes in mitochondrial membrane potential, ROS production, and cell cycle arrest (1, 3-11, 13). The induction of autophagy induced by selenium compounds in several experiments was an intriguing finding (7, 12).

Ultimately, administration of selenium compounds either alone or in conjunction with anticancer therapies, led to a reduction in the potential for tumoral metastasis through the suppression of angiogenesis, EMT (epithelial to mesenchymal transition), colony formation, and cell progression (1, 2, 3, 6, 11-13). TNBC appears to be very metastatic, so this result is of great importance. These outcomes were dependent on either time or dose (1-12).

Most of the investigations also assessed the adverse effects of selenium compound treatment on normal epithelial cells. Some research that examined the utilization of selenium compounds alone revealed cytotoxicity on normal epithelium (7, 8, 11). However, the majority of the other investigations had negligible to no negative effects (1, 4, 5, 8-10, 13).

Conclusion

Since the biomarkers ER, PR, and HER2 are not expressed in TNBC, target therapy is ineffective, and the cancer continues to be one of the most aggressive forms of breast cancer. The aforementioned research shows impressive outcomes when using selenium compounds alone, in combination with nutritional supplements, or as a co-therapeutic agent with common chemotherapy medications such as trastuzumab, bevacizumab, doxorubicin, and paclitaxel. Treatment with selenium compounds inhibited tumor development, caused widespread cell death, and decreased metastatic potential, with minimal damage to normal epithelial cells of in vitro and in vivo TNBC models, according to the publications reviewed in this study. These encouraging findings point to the necessity of additional investigation and assessment, as well as the potential of selenium compounds moving forward in clinical trials as a promising adjunctive treatment for TNBC in the future.

What Is Already Known on This Topic:

Breast cancer is the most frequent type of cancer in women and one of the leading causes of cancer-related deaths globally, accounting for over 600,000 deaths annually. TNBC is a particularly aggressive subtype of the disease due to the absence of molecular expression of human epidermal growth factor receptor 2 (HER2), progesterone (PR), or estrogen (ER) receptors. The characteristics of TNBC include a high risk of metastasis, considerable invasiveness, and a dismal prognosis. Since ER, PR, and HER2 are not therapeutic targets, there are limited treatment options for TNBC. Selenium (Se) has been successfully utilized as a treatment for TNBC, according to studies.

What This Study Adds:

The study presents the remarkable results when selenium compounds were used as a co-therapeutic agent with common chemotherapy drugs such as paclitaxel, bevacizumab, doxorubicin, and trastuzumab, or in isolation with dietary supplements. The papers evaluated for this review indicate that treatment with selenium compounds resulted in a reduction in the potential for metastasis, extensive cell death, and suppression of tumor growth, while causing little harm to the normal epithelial cells of TNBC models, both in vitro and in vivo.

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The Role of FLT3-ITD Mutation, PI3K/AKT Pathway, and Leukemia Stem Cells in D3A7 Induction therapy – the Outcomes of Adult Indonesian Patients with Acute Myeloid Leukemia

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Abstract

Objective. This cohort study aimed to examine the impact of the FLT3-ITD mutation on the downstream signaling pathway of PI3K/AKT pathway, the percentage of leukemia stem cells, and the survival of patients receiving D3A7 induction therapy. **Method.** Bone marrow mononuclear cells were collected from 20 adult AML patients who had completed D3A7 induction therapy at Cipto Mangunkusumo National General Hospital and Dharmais Cancer Hospital. FLT3-ITD gene mutation was examined by the PCR-sequencing method. Expression of phosphorylated PI3K and AKT was detected using the sandwich ELISA method. Flow cytometry was used for detecting the number of apoptosis and proliferation cells, and biomarkers of leukemia stem cells. **Result.** The expression levels of PI3K and AKT proteins were higher in FLT3-ITD, both in the mutant group compared to the non-mutation group, and in the patient group with treatment failure outcomes compared to the patient group with treatment response. The percentage of the leukemia stem cell population did not differ significantly between the FLT3-ITD mutation group and the wild type group, and between the treatment failure outcome group and the response outcome group. **Conclusion.** This study presents the important role of FLT3-ITD mutation via its downstream signaling (PI3K/AKT) in the outcome of D3A7 induction therapy. The FLT3-ITD mutation plays an important role in the 12-month survival of AML patients after D3A7 therapy. However, the outcome of D3A7 therapy and FLT3-ITD mutation were not associated with leukemia stem cells.

Key Words: FMS-like Tyrosine Kinase ▪ Daunorubicin ▪ Cytarabine.

Introduction

The primary treatment for acute myeloid leukemia (AML) has been intensive induction therapy with the purpose of eliminating most leukemic cells to achieve remission. This typically involves a regimen known as D3A7, consisting of three days of an anthracycline (Daunorubicin) followed by seven days of Cytarabine (1-3). In young adults, the complete response (CR) rate for first-line treatment typically ranges from 60% to 80%, while in older adults aged 65 years and above, it ranges from 40% to 60%. However, the majority of patients subsequently experience relapse with poor survival. Recent studies have identified drug resistance as a critical factor leading to treatment failure, ultimately impacting short-term survival outcomes in AML. There are many factors that influence drug resistance, including gene mutations and the presence of leukemia stem cells (4).

The FMS-Like Tyrosine Kinase 3 internal tandem duplication (FLT3-ITD) mutation stands out as one of the prevalent mutations found in AML (5-7). The prevalence of this mutation is

around 20-30% and about 21.5% in Indonesia (8). The FLT3-ITD mutation is typically linked to unfavorable molecular prognostic outcomes in patients and a higher likelihood of relapse in AML. Patients with the FLT3-ITD mutation tend to have a lower one-year survival rate compared to those without the mutation (9, 10). This mutation occurs in the Juxtamebrane Domain, and activates the loop that abolishes the auto-inhibitory function, resulting in persistent activation of FLT3 kinase. Consequently, this activation triggers downstream proliferative signaling pathways, including the PI3K/AKT pathway. This pathway triggers the activation of anti-apoptotic mechanisms, and promotes cell proliferation in leukemia cells, and has become one of many resistance mechanisms in AML therapy (4, 11, 12).

Leukemia stem cells (LCSs), also sometimes referred to as leukemia initiating cells, display specific mutations, epigenetic modifications, and a specific metabolic profile compared to healthy hematopoietic stem cells (HSCs). Leukemia stem cells (LSCs) are typically regarded as resistant to chemotherapy, making them the primary instigators of relapse (13). The intracellular signaling pathways and the niche-driven mechanisms that control quiescence constitute the PI3K/AKT pathway (14). The identification and targeting of LSCs depends on membrane markers, such as CD34+CD38-CD123+, and specific metabolites, such as ALDH1 (13, 15). Both the NCCN (the National Comprehensive Cancer Network) and the ELN (European Leukemia Net) guidelines advocate the incorporation of FLT3 genetic testing into the diagnostic evaluation process. Specifically, the NCCN guidelines propose conducting FLT3 testing alongside cytogenetic testing at the time of AML diagnosis for all patients. This approach aims to identify individuals who could potentially benefit from targeted therapeutic interventions (16).

In Indonesia, testing for FLT3 mutation is not routinely conducted in the diagnostic process of AML patients due to technical limitations. Therefore, the first cohort study in Indonesia was conducted to understand the role of FLT3-ITD mutation and its downstream signaling (PI3K/AKT) in the outcome of D3A7 induction chemotherapy in AML patients, as well as its association with leukemia stem cells.

Methods

Patients

This prospective cohort study was conducted from July 2022 to March 2024 at Cipto Mangunkusumo National General Hospital and Dharmais Cancer Hospital in Jakarta. All patients enrolled in the study received treatment according to the clinical pathway established by the hospital. The inclusion criteria for the study involved de novo AML patients, except those with AML-M3, age above 18 years, and those who had completed induction chemotherapy with D3A7. On the 7th day following chemotherapy, 15 mL of bone marrow was extracted from these patients for laboratory analysis. The chemotherapy outcome criteria used were based on the guidelines of The International Working Group and the European Leukemia Network (ELN). After this procedure, the patients were followed up for one year to evaluate their survival outcomes. The exclusion criteria were: AML patients with a history of transformation from another hematological malignancy, such as myelodysplastic syndrome or chronic myeloid leukemia, and those who were scheduled for bone marrow transplantation. Additionally, individuals with incomplete medical records or who declined to provide informed consent for participation in the study were not included.

Bone marrow specimens obtained from post Induction chemotherapy D3A7 AML patients were analyzed for the presence of FLT3-ITD gene mutation and its downstream pathway (phosphorylated PI3K & AKT protein), the outcome of chemotherapy, early-stage cell apoptosis, late-stage cell apoptosis, proliferation cells, and expression of markers of leukemia stem cells (CD34+CD38-CD123+ & ALDH1).

Isolation of Mononuclear Cells

The isolation of mononuclear cells from the bone marrow blood of patients was performed by means of a gradient centrifugation method, using Ficoll-Paque Plus (Merck). After the mononuclear cells were isolated, the cell count was determined using 90 µL Turks Solution (Merck) in a 10 µL sample in a Neubauer chamber. The cells were then cryopreserved using 10% DMSO in a 1 mL sample, and stored in liquid nitrogen for further analysis.

Detection of FLT3-ITD Mutation

Mutation testing for FLT3-ITD in the mononuclear cells was performed using the PCR-Sequencing method. Genomic DNA was isolated from the mononuclear cells using the spin column method with the Quick-DNA™ Miniprep Kit (ZYMO RESEARCH). Genomic DNA amplification was carried out by PCR using the following primers: FLT3-ITD-F: 5'-GCAATTTAGGTATGAAAGCCAGC-3' FLT3-ITD-R:5'-CTTTCAGCATTTTGACGGCAACC-3' The primers were designed using GeneBank data NG_007066.1. The PCR mix comprised 100 ng/µL genomic DNA, 10 pmol FLT3-F and FLT3-R primers, 12.5 µL MYTAQ HS Ready Mix + Dye, and dH2O to make up the total volume of the mixture of 25 µL. All PCR reactions used the Thermal Cycler (Applied Biosystems 9700) for 35 cycles: pre-denaturation at 95°C for 1 minute, and cycles consisting of a 15-second denaturation step at 95°C, a 15-second annealing step at 56°C, and a 15-second extension step at 72°C, followed by final extension for 10 minutes at 72°C. The PCR products were subjected to electrophoresis on 3% agarose gel. The gel was prepared by dissolving 2% agarose in tris-acetic acid-ethylenediaminetetraacetic acid (EDTA) buffer (TAE) containing 40mM tris, 20mM acetic acid, and 1mM EDTA, supplemented with 0.5µg/mL ethidium bromide. Once the agarose solution was prepared, it was poured into a casting tray and left to solidify. The gels were then electrophoresed for 35 minutes at 100 volts. Following electrophoresis, the gel was visualized using a UV light transilluminator. PCR amplification of genomic DNA for FLT3-ITD yielded a band with a size of 330 base pairs (bp). If a mutation was present, additional bands would appear, indicating products larger than 330 bp. The PCR amplification products targeting the FLT3-ITD mutation were excised from the agarose gel and subsequently subjected to Sanger sequencing to identify and characterize the mutations. Before sequencing, PCR DNA fragments to be sequenced were purified using gel cut extraction. DNA sequencing was performed using the DNA sequencer 3130 × l (Applied Biosystems, USA) on the basis of capillary electrophoresis. The sequencing results were obtained in AB1 and SEQ file formats, which were then analyzed using BioEdit software to identify mutations in the nucleotide sequence of the target DNA.

Detection of Phosphorylated PI3K/AKT Protein

Proteins were isolated from mononuclear cells using the protein isolation procedure with RIPA Buffer (SIGMA-ALDRICH) supplemented with 0.1% protease inhibitor cocktails (SIGMA-ALDRICH) and phosphatase inhibitor cocktails (SIGMA-ALDRICH). To determine the expression of phosphorylated PI3K and Akt proteins, a sandwich ELISA method was performed using the Phospho-PI 3 Kinase p85 + Total In-Cell ELISA Kit (ABCAM-ab207485) and Akt (pS473) + Total Akt ELISA Kit (ABCAM-ab126433) procedure. Finally, the concentrations of phosphorylated PI3K and AKT proteins were then compared to the concentrations of total PI3K and AKT proteins, resulting in the ratio of phosphorylated protein to total protein.

Detection of Proliferation and Apoptotic Cells

The proliferated and apoptotic cells were detected using flow cytometry. A total of 5 x 10^6 mononuclear cells were washed with 2 mL of PBS. After washing, 1 ml of mononuclear cell suspension was transferred and divided into tubes for each test, with 200 µl for the Ki-67 test and 400 µl for

the Annexin V-7AAD test. Each cell suspension for each test was divided into two tubes in equal amounts, one for the test tube and the other for the blank tube. Then, 10 µL (0.5 µg/µL) of Annexin V antibody (Annexin V Apoptosis Kit with 7-AAD, FITC, STEMCELL TECHNOLOGIES) and Ki-67 antibody (20Raj1), APC, eBioscience™ (INVITROGEN) were added to each test tube, followed by incubation for 10 minutes in the dark at 4°C. After the incubation was completed, all the test tubes, as well as the blank controls, were read on the BD FACSAria III flow cytometer machine.

Detection of Leukemia Stem Cells

The percentage calculation of the leukemia stem cell count was calculated using the markers CD34+CD38-CD123+ and ALDH1. A total of 5 x 10^6 cells were washed with 2 mL of PBS. After washing, 1 ml of mononuclear cell suspension was transferred and divided into tubes. Each cell suspension for each test was divided into two tubes in equal amounts, one for the test tube and the other for the blank control tube. Then, 10 µL (0.5 µg/µL) of the antibodies Anti-Human CD34 Antibody, Clone 563, PE, Anti-Human CD38 Antibody, Clone AT-1, FITC; Anti-Human CD123 (IL-3Rα) Antibody, Clone 6H6, APC (STEMCELL TECHNOLOGIES) were added to each test tube, followed by incubation for 10 minutes in the dark at 4°C. After the incubation was complete, all the test tubes, as well as the blank control tubes, were read on the BD FACSAria III flow cytometer machine. To determine the percentage of Leukemia Stem Cells expressing ALDH1, ALDEFLUOR™ assay was used following the ALDEFLUOR™ Kit (STEMCELL TECHNOLOGIES) procedure.

Ethics Statement

This study received ethical approval from the Institutional Review Committees of both Cipto Mangunkusumo National General Hospital and Dharmais Cancer Hospital, with approval number 105/UN2.F1/ETIK/PPM.00.02/2023. Written informed consent was obtained from all patients in compliance with the principles outlined in the Declaration of Helsinki.

Statistical Analysis

Data processing was performed using IBM SPSS Statistics 27 software, with a significance level set at $P<0.05$ (95% CI). Data normality was tested using the Shapiro-Wilk test, while data variance homogeneity was tested using Levene's test. All the obtained data were then subjected to non-parametric statistical testing. For bivariate analysis, unpaired t-tests and Mann-Whitney Tests were used. To examine the relationship between two categorical variable groups, Fisher's exact test was employed. A proportional hazard assumption test was conducted, involving the Kaplan-Meier method, the log-log –In(-ln) survival method test, and Schoenfeld's global test.

Results

Patient Characteristics and FLT3 Gene Mutation Prevalence

The total number of AML patients included 11 males and 9 females. The range of ages was 20-56 years (mean 38.50±SD 11.7). According to FAB classification for AML, two cases were diagnosed as AML-M1 (10%), 10 cases AML-M2 (50%), two cases AML-M4 (10%) and six cases AML-M5 (30%). In addition, 12 patients (60%) had a treatment failure outcome and eight patients (40%) had a response outcome following D3A7 chemotherapy.

Mutations in FLT3-ITD were found in four (20%) AML patients on the basis of the detectable amplicons at 330 bp and larger than 330 bp in 3% agarose gel electrophoresis. All of these patients were classified as FLT3-ITD mutants. The numbers of base pair insertion varied from sample to sample. The lowest insertion was 24 bp and the highest insertion was 84 bp. The results of gel electrophoresis are presented in Figure 1.

The analysis of FLT3 mutations based on the amino acid positions in the four samples showed

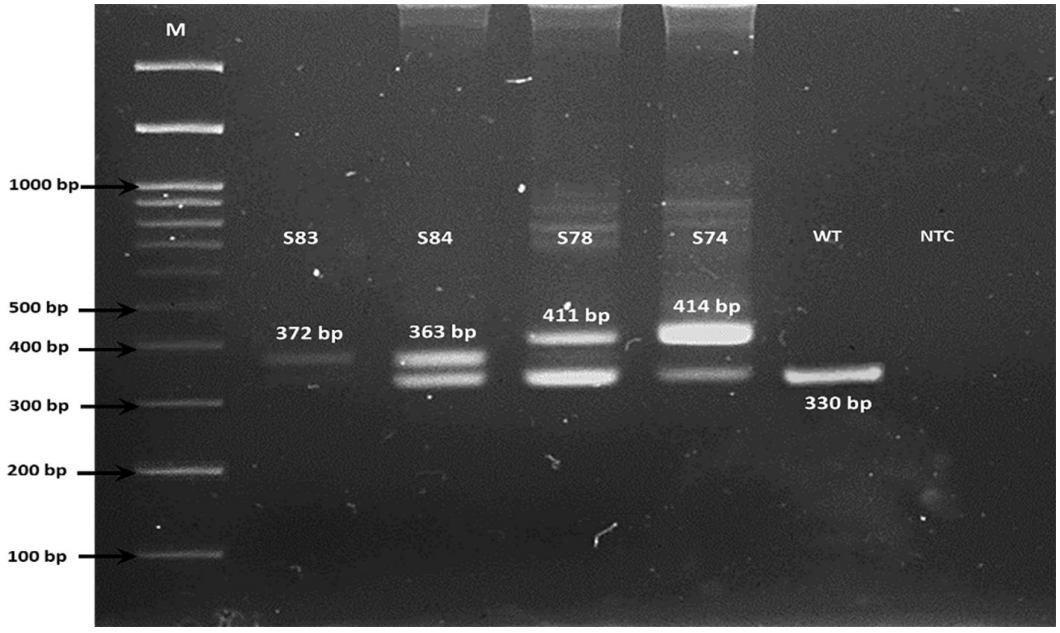

Figure 1. Electrophoresis of PCR products with agarose gel. Sample with FLT3-ITD homozygote allele mutation (S83), Sample with FLT3-ITD heterozygote allele mutation (S84, S78, S74), Wild type (WT) Sample, Non-template Control (NTC), DNA ladder 100bp (M).

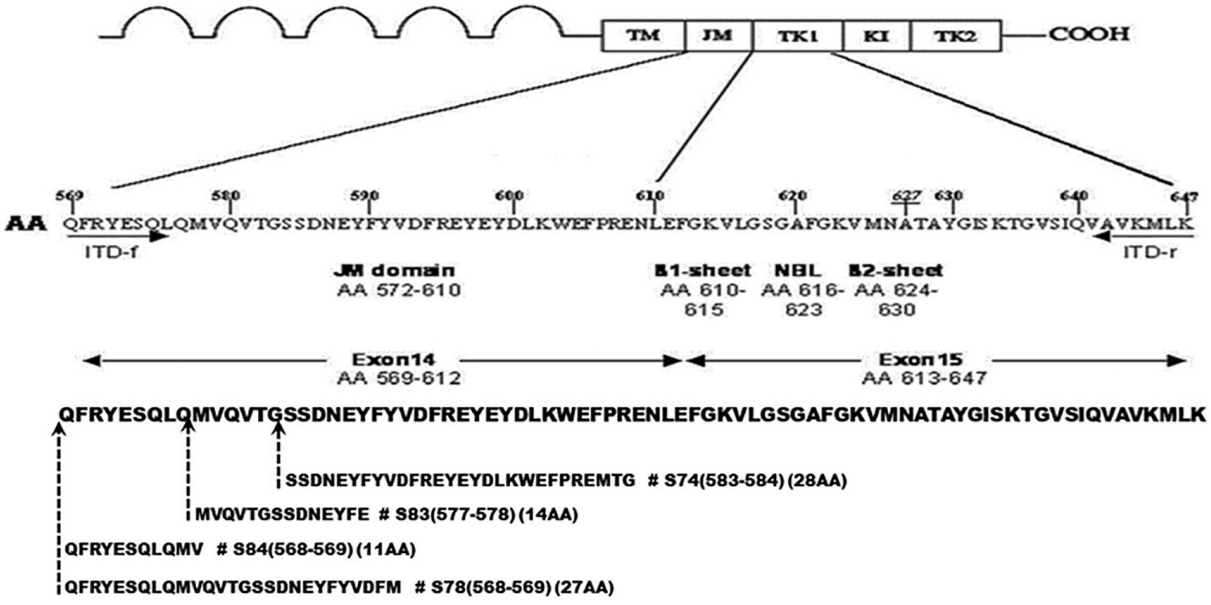

Figure 2. The position of amino acid insertions in samples with FLT3-ITD mutations.

that each mutant sample had mutations at different amino acid positions. In Figure 2, it was observed that samples S84 and S78 had insertions between amino acid positions 568 and 569, where the number of insertions were 11 and 27 amino acids, respectively. Meanwhile, in sample S83, there was an insertion of 14 amino acids between positions 577 and 588. In sample S74, there was an insertion of 28 amino acids between amino acid positions 583 and 584.

Proportional Hazard Assumption test of 12 Month Survival of AML Patients with FLT3-ITD Mutation

The Kaplan-Meier test showed that the survival curves in the Kaplan-Meier plot and the survival lines in the –ln(-ln) survival probability curve did not intersect. The global test (P=0.1) indicated that the assumption of proportional hazards (PH) met the requirements. This meant that the comparison of survival rates between the groups with a FLT3-ITD mutation and the wild type groups was consistent over time. The Cox regression analysis yielded a P-value of 0.03 and an HR: 6.027; 95% CI: 1.61-31.279.

Analysis Comparative of the FLT3-ITD Mutation and the Outcome of D3A7 Chemotherapy

The results of the comparison test between the FLT3-ITD group and the chemotherapy outcome group using Fisher's exact test, with a confidence interval of 95% and a standard deviation of 10%, show that there was no significant difference between the two groups, with P=0.5.

The results analysis in the group of patients with FLT3-ITD mutations and wild type showed that the average values of the variables PI3K, AKT, and late-stage apoptosis were higher in the mutant group compared to the wild type group (P=0.003, 0.009, and 0.023). Meanwhile, the variables early-stage apoptosis, cell proliferation, and leukemia stem cell markers CD34+CD38-CD123+ and ALDH1

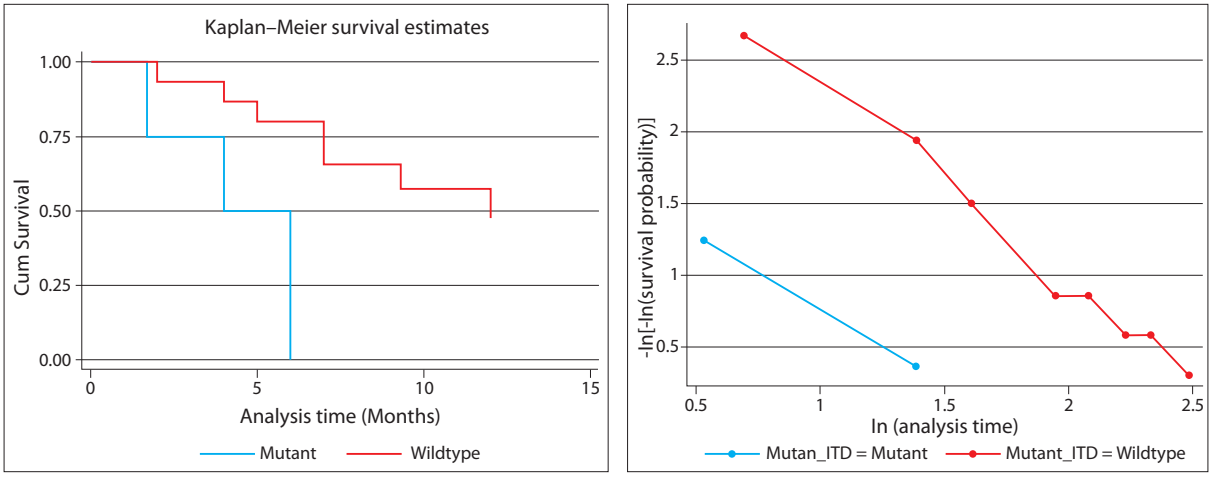

Figure 3. Kaplan-Meier Survival Curve and –ln (-ln) Survival Curve of Patients Based on FLT3-ITD Mutation Status.

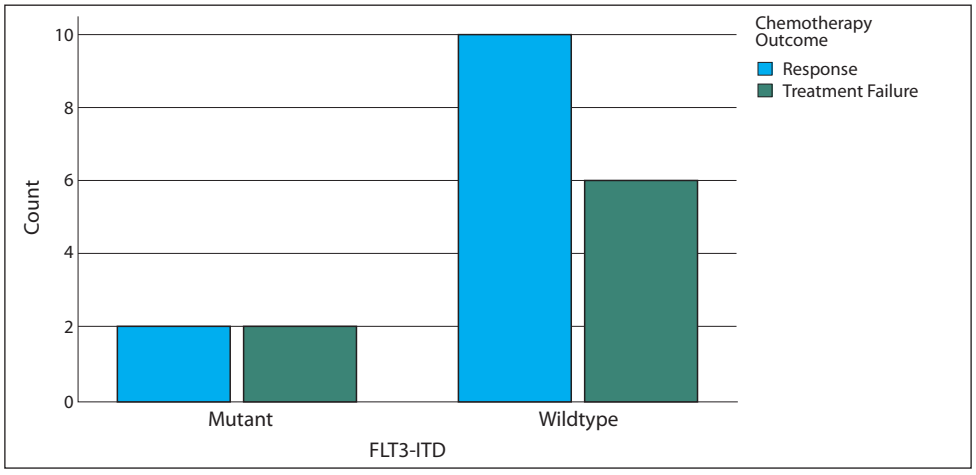

Figure 4. Comparison of the number of mutant and wildtype FLT3-ITD alleles in AML patients with chemotherapy D3A7 outcome using Fisher's exact test.

Table 1. The Results of Mann Whitney Test of the Variables PI3K, AKT, Early-Stage Cell Apoptosis, Late-Stage Cell Apoptosis, Proliferation Cells, CD34+CD38-CD123+, and ALDH1 in AML Patients with and without FLT3-ITD Gene Mutation

Variable	Wild-Type (N=16)	Mutant ITD (N=4)	P-value*
PI3K ratio mean ± SD	0.10±0.09	0.47±0.11	0.003*
AKT ratio mean ± SD	0.12±0.09	0.43±0.19	0.009*
Early-stage cell apoptosis (%) mean ± SD	3.66±5.64	18.35±23.65	0.256
Late-stage cell apoptosis (%) mean ± SD	87.46±20.70	51.62±39.72	0.023*
Proliferation cells (%) mean ± SD	24.56±12.33	32.67±21.80	0.126
CD34+CD38-CD123+ (%) mean ± SD	12.21±9.31	12.62±4.87	0.084
ALDH1 (%) mean ± SD	0.18±0.18	0.43±0.65	0.957

Mann Whitney test, *P-value < 0.05.

Table 2. The Results of Mann Whitney Test of the Variables PI3K, AKT, Early-Stage Cell Apoptosis, Late-Stage Cell Apoptosis, Proliferation Cells, CD34+CD38-CD123+, and ALDH1 in AML Patients with Outcome of D3A7 Chemotherapy

Variable	Response (N=8)	Treatment Failure (N=12)	P-value*
PI3K ratio mean±SD	0.11±0.15	0.28±0.17	0.016*
AKT ratio mean±SD	0.10±0.13	0.29±0.17	0.009*
Early-stage cell apoptosis (%) mean±SD	3.03±5.78	11.97±17.30	0.189
Late-stage cell apoptosis (%) mean±SD	95.63±6.70	57.30±33.23	0.001*
Proliferation cells (%) mean±SD	19.97±14.44	35.52±8.11	0.302
CD34+CD38-CD123+ (%) mean ± SD	9.61±6.96	14.09±9.23	0.352
ALDH1 (%) mean ± SD	0.17±0.09	0.33±0.49	0.571

Mann Whitney test, *P-value < 0.05.

showed no significant differences in average values between the ITD mutant and wild type groups. The analytical data are summarized in Table 1.

In terms of chemotherapy outcomes, the group with treatment failure had higher average values of PI3K, AKT, and late-stage cell apoptosis compared to the response group, with P-values of 0.016, 0.009, and 0.001, respectively.

Other variables in Table 2 do not show any significant differences in average values between the response and treatment failure groups.

Discussion

In this study, the FLT3-ITD gene mutation was most commonly found in patients with the AML-M2 subtype, with a prevalence of 75% (three out of four AML-M2 patients). Additionally, the FLT3-ITD mutation was also present in one patient with the AML-M5 subtype. This result is similar to a study conducted in Japan and Indonesia with AML-M2 as the most frequent subtype in AML patients with FLT3-ITD gene mutation (17, 18). In contrast, in a study in Germany, AML-M5 was the most common FAB subtype in AML with FLT3-ITD gene mutation, and a study on a Thai population showed that AML-M3 was the most frequent subtype (19, 20). Furthermore, we found 60% patients had treatment failure outcome and 40% a response to D3A7 therapy in this cohort. Compared to other studies, about 85% patients had treatment failure with induction therapy (8).

In addition, we found one homozygous allele (S83) of the FLT3-ITD gene mutation and the others

were heterozygous alleles. This homozygous allele is the first reported in the population of AML patients in Indonesia. Previous studies in Indonesia have shown that only heterozygous alleles were present (8, 18, 21). FLT3-ITD is typically found in the heterozygous state, but there is evidence of a partial or complete loss of the wild-type allele in some cases. A hemizygous ITD/- genotype, present in 1% of pediatric patients and 5% of adult patients, is linked to a distinct phenotype that is associated with a significantly worse clinical outcome (22).

In this report, analysis comparative of the FLT3-ITD mutation and the outcome of D3A7 chemotherapy showed no significant difference (P=0.5) between patients with ITD mutation and without a mutation. It may be concluded that statistically, FLT3-ITD mutations have no impact on the outcome of induction chemotherapy, but the graph (Figure 4) shows the trend that patients with an FLT3-ITD mutations had a lower response to D3A7 therapy than patients without an FLT3-ITD mutation. Therefore, we paid more attention to all patients with the FLT3-ITD mutation. In contrast, all the patients with a FLT3-ITD mutation had a poor prognosis and high relapse rate. The sample S83 with 52 bp insertion and S74 with 84 bp insertion had treatment failure as their chemotherapy outcome. In sample S78, with an insertion length of 81 bp, although there was a response to induction therapy, treatment failure occurred one year after induction during consolidation treatment with high-dose cytarabine. Similarly, sample S84, with an FLT3-ITD mutation of 33 bp, showed a good response outcome in induction therapy, but experienced a relapse 12 months after therapy.

Furthermore, the survival analysis in this study showed that the survival rate of patients with the FLT3-ITD mutation 12 months after therapy was lower than patients without the FLT3-ITD mutation, with P=0.03 and HR: 6.027; 95% CI: 1.61-31.279. In other words, each month members of the group were 6.027 times more likely to die compared to the group of patients without the FLT3-ITD mutation. All the results suggest that the presence of FLT3-ITD mutation might affect the outcome of induction therapy, but also have a strong impact on the risk of relapse and survival after therapy. This cohort was similar to the findings of the studies by Grafone et al. and Liu et al. which explained that FLT3-ITD mutations were a significant independent prognostic factor that can influence outcome in terms of survival and duration of complete remission. The reports also stated that the length of base insertions in the FLT3-ITD mutation was associated with high FLT3 kinase activity. Patients with FLT3-ITD insertions >39 bp had worse overall survival and prognosis compared to patients with insertions <39 bp (22, 23). Other studies showed that the presence of an ITD in adults patients had no impact on achieving complete remission (CR) in induction therapy, but it was significantly correlated with an increased risk of relapse (RR), and reduced disease free and overall survival (OS). A systematic review by Rinaldi et.al. also presented the results that an FLT3-ITD mutation was associated with worse prognosis in adult, non-transplant patients with AML, both for overall survival and event-free survival (9).

The research conducted by Griffith et al. described that FLT3-ITD mutations longer than 15 bp in the juxtamembrane domain were known to alter the auto-inhibition conformation of the FLT3 kinase protein, thereby promoting ligand-independent FLT3 receptor dimerization, and resulting in autophosphorylation and activation of downstream signaling associated with cell proliferation and survival (23, 24). The average values of phosphorylated PI3K and AKT in this research were higher in patients with an FLT3-ITD mutation than in patients without the mutation, with a P value of 0.003 for PI3K and 0.009 for AKT. In addition, the average values of phosphorylated PI3K and AKT in the group of patients with treatment failure to D3A7 therapy were greater than in the group of patients with response. Moreover, a significant difference was also found in variable late-stage cell apoptosis, with a P value of 0.023, in patients with FLT3-ITD mutation +/- and a P value of 0.001 in relation to the patients' chemotherapy outcome. The results of this study indicated that the existence of FLT3-ITD gene mutations in AML patients had a significant role in activation

of downstream signaling, which affects the autophosphorylation of protein PI3K and AKT. The increase in phosphorylation of PI3K and AKT resulted in the higher possibility of cell survival and became a mechanism of drug resistance (25).

In the activated PI3K/AKT pathway, PI3K helps convert phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3 recruits AKT to the cell membrane, where AKT is then phosphorylated by phosphoinositide-dependent kinase-1 (PDK1) at residue Thr308 and residue Ser473 by the mTORC2 protein. The phosphorylated AKT is then in an active form and can activate mTORC1 by phosphorylating mTOR at Ser2448, which in turn phosphorylates proteins such as S6K1 (p70S6 Kinase 1) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1). Phosphorylation of 4EBP1 initiates the translation of mRNA coding for proteins such as hypoxia-inducible factor 1α (HIF-1α), Cyclin D1, and c-Myc, which can induce angiogenesis or increase cell cycle activity (12, 26, 27). FLT3-ITD mutations in leukemia cells are known to cause resistance to cytarabine (28). Jin et al. stated that myeloid K562 cells with FLT3-ITD mutations experience a reduction in ENT1 expression through an increase in HIF-1α expression. Equilibrative nucleoside transporter-1 (ENT1) is known to be a transporter protein that plays a role in the uptake of cytarabine into cells, so the decreased uptake of cytarabine due to reduced ENT1 function can lead to cells not responding to cytarabine treatment (29).

In this study, Ki-67 expression as marker of cell proliferation was not statistically significant. However, the average Ki-67 expression values in the FLT3-ITD mutation group tended to be higher than in the wild type group. This is possibly due to the insufficient sample size. Research conducted by Kubota et al. gave the same result, showing that the activity of PI3K had an important role in cell proliferation (30). PI3K/AKT signaling also induces the expression of the BCL2 protein. BCL2 is a mitochondrial membrane protein that can alter membrane permeability, thereby preventing the release of cytochrome c into the cytoplasm. This can prevent apoptosis through the post-mitochondrial caspase cascade. AKT can phosphorylate Ser136 on the BAD protein. AKT can also activate PAK1, which phosphorylates BAD at Ser112, causing BAD to detach from the Bcl-xL complex and inhibit apoptosis. Active AKT can promote cell survival by activating BCL2 and inhibiting Bax (26).

Finally, the leukemia stem cell marker variables from the ITD mutant group had higher average values compared to the wild type group. Similarly, patients with chemotherapy treatment failure outcomes had higher average values of CD34+CD38−CD123+ and ALDH1 compared to the response group, although these differences were not statistically significant. Leukemia stem cells are highly adaptive, aided by a microenvironment that supports the stemness and survival of leukemia stem cells from various chemotherapeutic agents, leading to treatment failure and relapse. The ability of self-renewal is a complex process that involves multiple signal transduction cascades that regulate the balance between self-renewal and differentiation. One important signaling pathway that plays a role in the self-renewal ability in leukemia stem cells is the phosphatidylinositol-3-kinase (PI3K)/AKT pathway, whose activation is induced by ligand-receptor tyrosine kinase (14, 31-33). The expression of ALDH1A1 is known to be influenced by the activity of the transcription factor NF-κB (15). NF-κB activity can induce the expression of miRNA223-3p, which in turn can inhibit the expression of ARID1A. The inhibition of ARID1A expression can initiate histone acetylation at the promoter of the ALDH1A1 gene (11, 34). Meanwhile, in vitro studies have found that the overexpression of FLT3 can increase NF-κB transcriptional activity through the PI3K/AKT/mTOR pathway. FLT3-ITD activation induces NF-κB activity, and FLT3 knockdown or FLT3 inhibition reduces NF-κB activity in patients with MDS and AML (35).

Limitations of the Study

The inclusion of a relatively small sample size due to the strict inclusion and exclusion criteria is one of the limitations of this study. However, this study

provides novel data that shows FLT3-ITD mutation was not associated with leukemia stem cells and new analyses of CD34+CD38−CD123+ and ALDH1.

Conclusion

The presence of an FLT3-ITD mutation might impact the outcome of D3A7 therapy and cause the risk of relapse by the autophosphorylation of the downstream proteins PI3K and AKT. FLT3-ITD mutation also had a strong effect of survival after D3A7 therapy. The FLT3-ITD mutation also affects the downstream signaling associated with survival of the cell, that becomes a resistance mechanism to D3A7 therapy. Due to its prognostic relevance and being a good factor to predict survival in AML patients, we suggest including assessment of FLT3 mutation status for all AML patients before their treatment with D3A7 therapy, as well as the recommendations of the current World Health Organization (WHO) guidelines.

What Is Already Known on This Topic:

The high rates of relapse and refractoriness in relation to D3A7 therapy are classic issues in AML treatment. Several factors play a role in the mechanism of resistance to the D3A7 regimen, including the presence of FLT3-ITD mutations and the existence of leukemia stem cells.

Recent studies have shown FLT3-ITD mutation is associated with poor prognosis and the time of relapse, but the association with the survival of patients is still controversial. It also known that it had no impact on achieving complete remission (CR) with induction therapy but it is significantly correlated with increased risk of relapse (RR), and reduced disease-free time and overall survival (OS). Other studies explained that leukemia stem cells (LSCs) were typically regarded as resistant to chemotherapy, making them the primary instigators of relapse.

What This Study Adds:

This cohort study presents the important role of FLT3-ITD mutation via its downstream signaling (PI3K/AKT) in the outcome of D3A7 induction therapy, demonstrated by the number of cells undergoing apoptosis. The results also give the new insight that the FLT3-ITD mutation plays an important role in 12 month survival of AML patients after D3A7 therapy. However, the outcome of D3A7 therapy and FLT3-ITD mutation are not associated with leukemia stem cells.

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

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Narrative Review
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Paraneoplastic Syndromes of the Nervous System in Patients Suffering from SCLC. A Review of the Recent Literature

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Abstract

Background. Paraneoplastic Neurological Syndromes (PNS) constitute a heterogeneous cluster of disease manifestations related to various cancers. Small Cell Lung Cancer (SCLC) is strongly related to PNS. This narrative review conducted a survey in the available PubMed literature to highlight the appearance of PNSs in SCLC cases and discuss published research highlights on the subject so that general practitioners can be acquainted with the medical phenomenon present in SCLC patients. **Method.** A narrative review of the medical literature was conducted as documentary informative research in the PubMed medical database, combined with a survey of the online e-library Google Books. The key words used were: "Paraneoplastic Neurological Syndromes" and "Small Cell Lung Cancer". **Results.** Paraneoplastic syndromes are related to the presence of a malignancy and are not secondary to treatment. Paradoxally, both a malignancy and its therapeutic approach may cause a series of PNSs. Paraneoplastic cerebellar degeneration, motor neuron disorders, peripheral neuropathies, hyponatremia, and syndromes such as myasthenic Lambert-Eaton, ectopic Cushing's, Stiffman, and Opsoclonus-myoclonus syndrome may also appear in SCLC cases. Diagnosis follows specific criteria, and they are caused by tumor-directed antibodies known as onconeural antibodies. Immunosuppressants, intravenous immunoglobulins, plasma exchange, rituximab, cyclophosphamide, azathioprine, and tocilizumab could be considered as treatment agents. **Conclusions.** Most patients demonstrate poor PNS treatment results with common relapse. The time for beginning treatment of PNS is discussed. A multidisciplinary team is needed for potentially earlier diagnosis and PNS improvement, better prognosis, and increased overall survival and quality of life.

Key Words: Immune Response ▪ Neuro-Oncology ▪ Onconeural Antibodies ▪ Multidisciplinary Team.

Introduction

Paraneoplastic syndromes of the nervous system represent a group of uncommon disorders that develop in some people who have cancer. It is believed that they are a neurological response, which has been demonstrated in studies of lung malignancies in recent years (1). The pathophysiology of paraneoplastic neurological syndromes involves a complex interplay between the immune and nervous systems. While the exact mechanisms vary depending on the specific syndrome, common features have been observed, such as the immune response, which is believed to arise from the antigens expressed by both the tumor and the nervous system. These antigens are often referred to as onconeural antigens. In most cases, the immune system recognizes and eliminates cells expressing these antigens, but the acute and high volume production of tumor-directed antibodies, known as onconeural antibodies, dysregulates homeostasis. This cross-reaction results in the mistaken targeting of normal neural tissues, leading to neuronal dysfunction or damage. The cytotoxicity found in cells such as cytotoxic T cells, can infiltrate neural tissue in response to the presence of onconeural antigens.

The presence of such immune reactions may alter or disrupt normal neuronal function through various mechanisms, including blocking synaptic transmission, receptor function, induced

apoptosis, and inflammation within the nervous system, further contributing to neuronal dysfunction and tissue damage. Certain genetic factors may predispose individuals to developing PNS in response to specific tumors or immune triggers. However, the role of genetics in PNS is still not fully understood. Overall, the pathophysiology of PNS is multifactorial and involves a complex interplay between neuro- and tumor cells. Further research is needed to elucidate fully the underlying mechanisms and develop targeted treatments for these challenging disorders. Although this concept is still under survey, the fact that malignancy may cause the nervous system to produce various disorders such as torpor, stomach hyperactivity, swallowing difficulty, dementia, and motor misbalance has been mentioned in medical literature since the 18th century (2-3).

Small cell lung cancer (SCLC) is a malignant entity among the most aggressive lung tumors, known for its poor survival rates. Initially positive results of treatment are followed by a rapid development of drug resistance and fatal disease progression. Lung cancers remain the most common cause of cancer-related death in the world, while SCLC in the vast majority of cases causes relapse, with a 1-year survival rate of about 40% and a 5-year survival under 5%. SCLC, strongly related to tobacco smoking, accounts for 15-20% of all lung cancers, with an aggressive evolution, poor prognosis and limited treatment options. Paraneoplastic neurological syndromes (PNS) occur in about 0.1% of patients affected by cancer. Meanwhile, approximately 30% of SCLC patients present a serious neurological disorder during the evolution of their disease. Among them, 75% appear due to brain metastases, as these affect 24.8% of all patients with SCLC. However, a total of 25% are related to metastases in other areas than the brain, localized outside the central nervous system (1, 4). This narrative review aims to highlight the emergence of PNS in SCLC cases, and note opinions on the subject by surveying the recent medical literature, through an assessment and analysis of the already published material in a novel way.

Method

Reviews do not present new data, but do provide a summary of knowledge of what has already been published or presented on a subject. Narrative reviews constitute a type of literature synthesis partially framed as systematic, which implies a state-of-the-art, critical, and integrative review, by conducting a subjective examination and critique of the entire body of medical papers inside the database PubMed related to the subject in question (5). The search conducted for the sake of this narrative review included some informative documentary research inside the online library Google Books. The key terms used, were: "Paraneoplastic Neurological Syndromes" and "Small Cell Lung Cancer". Among the 466 articles found in the period from 1997 to 2024, 33 papers in English were included in this review. The title of the paper was examined, as well as full text availability. Furthermore, by reviewing the abstract, papers discussing exclusively SCLC and PNSs were included. We chose to use only the board term Paraneoplastic Nervous Syndromes due to its clinical significance.

Results

Although PNSs appear rarely and are manifested through complex clinical symptoms occurring in association with a tumor, SCLC association with them is more frequent. Some researchers have reported that the use of immunotherapy in SCLC cases is strongly related to a concomitant increase in autoimmune neurological syndromes (1). The main criterion for diagnosis of PNS is the absence of a direct trigger or compression. PNSs arise from tumor secretions of hormones, peptides or cytokines, or from immune cross-reactivity between malignant and healthy tissue (6). Paraneoplastic cerebellar degeneration is one of the most prevalent PNSs associated with SCLC (6, 7). Motor neuron disorders, peripheral neuropathies, hyponatremia and syndromes, such as myasthenic Lambert-Eaton, ectopic Cushing's and Stiffman were among the first to be recognized as PNS (5, 8).

Opsoclonus-myoclonus syndrome (Kinsbourne or Dancing Eyes Syndrome) may also appear in extremely rare cases (9). Opsoclonus-Myoclonus Syndrome, followed by the rapid progression of cerebellar ataxia, is the most common form of PNS in children, and is usually associated with neuroblastoma (10).

SCLC is equally prevalent among males and females, while the percentage of the elderly who suffer from it is increasing. Its main characteristic is its rapid response to chemotherapy and sensitivity to radiotherapy. However, due to its early treatment resistance the 5-year overall survival is <10% (11). Various poor prognostic factors in SCLC include impaired performance status, weight loss, older age, male sex, elevated lactate dehydrogenase, and low sodium. Due to its rapid progression and the early treatment resistance, combined with the long term toxicity of chemotherapeutic and radiotherapy methods, neurocognitive decline and PNS soon emerge to further complicate disease progression (12, 13). PNS have an immune-mediated pathogenesis that is supported by the frequent presence of specific neuronal antibodies. This involvement of the nervous system may provoke reactions which mimic infections, autoimmune non-paraneoplastic diseases, other tumors, neurodegenerative disorders, general toxicity, or metabolic alternations. Specific antibodies may be detected and provide for the possible identification of PNS, but the majority of SCLC PNS cases are difficult to diagnose (14-16). To aid diagnosis of PNS, a cluster of criteria was formed in 2004, and updated with a clinical scoring system in 2021 (Table 1) to increase diagnostic accuracy in complicated clinical cases. In the 2021 update, diagnostic certainty was divided into 3 levels (possible, probable, and definite PNS), taking into consideration the coherence between the clinical phenotype, antibodies, and the cancer (17-18). Even in cases demonstrating detectable onconeural antibodies, the suggestion is that a diagnosis of PNS is definite only after other possible causes of a particular neurological syndrome have been excluded (19). Radiology and nuclear medicine neuroimaging (CT, MRI, 18FDG-PET) and electroencephalography are only used for PNS that affect the central nervous system to differentially diagnose and exclude metastasis or meningeal malignancy, and other diseases, such as encephalitis (14). Meanwhile, the broad differential diagnosis for PNS renders their recognition a challenge at

Table 1. 2004 Criteria for PNS and 2021 Criteria Scoring Update

N/A	2004 Criteria		2021 Scoring Update
	Definite PNS	Possible PNS	Definite, Probable, Possible, Non-PNS
1	A classical post cancer syndrome which develops within five years of the neurological onset diagnosis	A classical syndrome with no onconeural antibodies, no cancer but with a high risk of an underlying malignancy	-
2	A non-classical post cancer treatment syndrome which later resolves or significantly improves, provided that the syndrome is not susceptible to spontaneous remission	A classical or non-classical neurological syndrome with partially characterized onconeural antibodies detected and no cancer manifestation	High-risk antibodies, Intermediate risk antibodies, Lower risk antibody Negative for antibodies
3	A non-classical syndrome with onconeural antibodies (well characterized or not) detected, while cancer develops within a five-year period of the onset and diagnosis of the neurological disorder	A non-classical syndrome with no onconeural antibodies detected and cancer appearance within a two-year period of the onset of diagnosis of the neurological disorder	Cancer presence plus +/- antibodies Non-cancer, a two-year period of follow-up No cancer found within a two-year period of follow-up
4	A classical or non-classical neurological syndrome with well characterized onconeural antibodies detected (anti-Hu, Yo, CV2, Ri, Ma2, amphiphysin) and no cancer manifestation	-	-

N/A=Number of Answer; PNS= Paraneoplastic Neurological Syndromes.

the very least. Infections, toxic and metabolic etiologies, brain metastases, leptomeningeal disease, spinal cord and nerve root compression, and the adverse effects of treatments (radiation therapy, platinums, taxanes, vinca alkaloids) may all mimic PNS (20).

Cancer treatment itself and/or cortico-therapy are used to combat PNS. Most PNS patients with classical onconeural antibodies do not improve, with the exception of some cases which demonstrate some improvement due to the immediate initiation of treatment after the onset of symptoms. Even though in the majority of the PNS cases with onconeural antibodies immunosuppressants, intravenous immunoglobulins and plasma exchange/cyclophosphamide are used, they have showed no statistically significant therapeutic results, while about 30% to 50% remain severely disabled. On the other hand, PNS patients with onconeural surface-binding antibodies have responded to immunosuppressive treatment. If first-line immunosuppressive agents do not show results, then rituximab, cyclophosphamide, azathioprine, tocilizumab (anti-interleukin-6 antibody) should be considered as options for escalation of treatment. Although some responses do actually occur, in 15% to 39% of the cases where a response occurred, there was a subsequent relapse (21-23).

Discussion

An estimated 1% to 7.4% of patients with cancer will develop PNS, while up to 30% of those with SCLC will manifest it (24). Among the cluster of PNS disorders related to SCLC, Lambert-Eaton syndrome may signify this type of cancer (25). Paraneoplastic motor neuron disease may rarely appear in SCLC, but when it does, it is usually related to the SCLC. Sensory neuronopathy, cerebellar ataxia and/or limbic encephalitis, signify SCLC. Among them, encephalitis is the most common manifestation of a PNS (26). Although sex is referred to as a non-indicating factor, various reviews note that male sex is prevalent (18). PNS in patients with lung cancers mainly progress with malfunctions in endocrine, neurological, dermatological and rheumatological regulation. Meanwhile, the less common PNSs manifest in hematological and ophthalmological syndromes. PNSs are detected before a cancer is diagnosed in 80% of cases (24, 27). Rapid PNS diagnosis may indicate an association with a cancer type, resulting in an opportunity for early stage cancer diagnosis and intervention (24).

There is a debate about whether to allow a PNS to progress until the diagnosis of the basic disease is made, or to treat it immediately. Moreover, there is an ongoing discussion as to whether more aggressive early immunosuppression is needed to reduce PNS relapse rates. There are still no definitive study results in the literature (21). There are reports about patients experiencing a worsening of the symptoms after the initiation of PNS immunotherapy (28). Various pathologies may appear as PNS, such as amyotrophic lateral sclerosis, or a PNS may appear soon after the therapeutic intervention (27). Moreover, a report exists of a durvalumab-related PNS, with the development of paraneoplastic myelitis after immune activation by durvalumab (28). As neurology is implicated, pregabalin and antidepressant agents are used to improve the patient's numbness in some cases (29). The heterogeneous group of PNS manifestations signals the need for interdisciplinary/multidisciplinary interaction between oncology, pathology, neurology, radiology, nuclear medicine, surgery, endocrinology, nursery, physiotherapy and palliative care, for prognosis and symptom stabilization. This approach has been highlighted in recent decades (30, 31). A study conducted by Graus et al. in 1997 stated that cases of SCLC presenting with anti-Hu antibodies are more likely to achieve a complete response after treatment than those without. This observation, that anti-Hu antibodies recognize the antigens expressed by neurons and SCLC, raises the possibility that treatment of PNS with immune modulation may surprisingly result in further cancer progression.

This hypothesis, however, has not yet been clinically demonstrated (19, 32). Various PNSs are allied with a series of antibodies against both central nervous (CVS), or peripheral nervous system

(PNS) targets. These sets of antibodies may be broadly divided into those which set their targets intracellularly finding neuronal antigens and those targeting neuroglial cell surface antigens. In the cases when more than one set of CNS autoantibodies are detected, the likelihood of a malignancy is increased. The presence of anti-Hu antibodies increases the possibility of a SCLC up to 83%. Meanwhile, reports noted that when anti-Hu antibodies co-exist with either Collapsin response-mediator protein-5 antibody, or P/Q type voltage gated calcium channel antibodies, the likelihood of SCLC rises up to 100% (33).

In another aspect of PNSs in SCLCs, a PNS may perform as a surrogate marker for clinically significant patient defense against the tumor. This intriguing theory may represent a potential rationale for immunotherapy, while at the same time immunotherapy may also provoke a PNS as a side defect. This dilemma should always be in physicians' minds, even though most studies report no neurological toxicity, recording patients' deaths due to pneumonitis and disease progression. Some anecdotal reports on spontaneous regression of SCLC cases without treatment, in patients with onconeural antibodies, may only signify that an effective host immune response was directed against both the cancer and the nervous system, altering the course of the malignancy, without however saving the patients from a fatal outcome (34).

It is nowadays clear that PNSs occasionally appear with multifocal involvement and plural antineuronal antibodies which can be found in a single patient with SCLC, testifying of a heterogeneous autoimmune mechanism, resulting in strong neurological involvement (35). Still, it seems that PNSs are still a medical mystery among physicians, requiring better knowledge and more research (30, 31).

Strengths and Limitations

Those syndromes may be well known among neurologists and oncologists, however, they constitute a riddle for the general practitioner, who should be aware of PNSs and bear in mind that PNSs and SCLC are closely related in some cases. Although this review presents the not fully understood neurological entities in SCLC, it does not include all the medical literature as in the case of a systematic review, remaining simple in the terms researched and avoiding fully presenting phenotypes and categorization as indicated in various other studies (22).

Conclusions

Advances in recent studies have directed health professionals towards a greater understanding of PNS development, and improved diagnostic tools and therapeutic options. PNS awareness in SCLC may promote earlier diagnosis, to potentially improve patients' overall survival and prognosis. Their heterogeneous pathophysiology, the continuous discovery of antibodies, patients' recovery failure, and the mysterious triggers of the immune system require further PNS-related research.

What Is Already Known on This Topic:
Paraneoplastic syndromes are related to the presence of a malignancy and are not secondary to treatment.

What This Study Adds:
This study gives a review of the recent developments in the study of paraneoplastic syndrome in patients suffering from SCLC.

Authors' Contributions: Concept and design: EG and TZ; Acquisition, analysis and interpretation of data: EG and TZ; Drafting the article: EG, TZ and EM; Revising it critically for important intellectual content: EG, TZ and EM. Approved final version of the manuscript EG, TZ and EM.

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

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Keystone Flap Type IV in Breast Reconstruction: A Case Report

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Abstract

Objective. The objective of this paper is to present and document a specific case of breast reconstruction using an adapted Type IV Keystone Flap technique, with a droplet-shaped design with a reduced flap ratio, and to identify the qualities of this method. **Case Report.** A 41-year-old woman, with a history of myocardial infarction and low ejection fraction, underwent a lumpectomy, resulting in a lower medial quadrant deficit in her left breast. After she developed skin and tissue necrosis and infection, implementing the Type IV Keystone Flap effectively addressed the deficit, ensuring sufficient coverage. The flap extended dropwise beneath the deficit, progressing anteriorly towards the upper rectus abdominis, with a ratio of 2.5:1. The flap's novel droplet shape allowed for the utilization of fewer perforators, while ensuring adequate blood supply and tissue coverage, leading to improved perfusion and aesthetic outcome. **Conclusion.** The application of the adapted Type IV Keystone Flap highlights its capacity as a versatile and effective method for breast reconstruction post-lumpectomy. With the advantages of a short learning curve, easy execution, and acceptable risk profile, it offers a valuable alternative for patients who may not be suitable for more complex surgeries. Further research is recommended to confirm its broader applicability and to conduct a comparative analysis with other techniques.

Key Words: Breast Cancer ▪ Skin Deficit ▪ Perforator Flap ▪ Mastectomy ▪ Lumpectomy.

Introduction

Breast reconstruction poses significant challenges due to the complexity of addressing large defects requiring precise coverage for optimal aesthetic outcomes. Keystone Flaps (KF) have gained popularity, as they are an easy-to-perform and sophisticated technique for reconstructing deficits in various anatomical regions, benefiting from their capacity for similar tissue substitution (1). The term "Keystone Design Perforator Island Flap (KDPIF)" was introduced by Behan in 2003 to describe this curvilinear-shaped trapezoidal design. This method provides a simple and efficient solution for wound closure, presenting a practical alternative to complex flap closures or skin grafting, particularly in cases of melanoma (2). The KDPIF is a multiperforator advancement flap, comprising two conjoined V to Y island flaps. It creates redundancy and effectively releases longitudinal tension, resulting in increased laxity within the flap, which allows for successful advancement toward the specific defect (3). Abraham and Saint-Cyr (2017), in their analysis of perforasome principles, noted that hyperperfusion through a single perforator could capture multiple adjoining perforasomes. On the basis of these principles, they acknowledged the effectiveness of the "Pedicle Perforator Flap (PPF)" and the "Keystone Perforator Island Flap (KPIF)" in facilitating the transfer of considerable volumes of soft tissue for reconstruction purposes. Furthermore, PPF and KPIF methods have decreased donor site morbidities, and have obviated the need for intricate microsurgical free-flap

reconstructions, thereby achieving improved aesthetic results (4). These advantages enable patients with significant comorbidities to undergo crucial, complex surgical reconstructions, and avoid the risks associated with prolonged general anesthesia. The implementation of these methods has also resulted in less postoperative monitoring, diminished patient-reported pain, and shorter periods of hospitalization (5). Advancements in understanding vascular anatomy will improve surgical flexibility for reconstructive flaps, enhancing patient care and outcomes (4).

In the case described below, an adapted KF technique was used for the first time in breast reconstruction.

Case Report

A 41-year-old female patient presented with a deficiency in the lower medial quadrant of her left breast after a lumpectomy for breast cancer. The patient had undergone a cosmetic breast augmentation 15 years previously, and had a medical history of myocardial infarction one year earlier and a low ejection fraction (less than 35%). She had also previously undergone radiotherapy and chemotherapy due to her illness. Following lumpectomy, skin and underlying tissue necrosis developed, and the wound was infected with Gram-positive enterococcus (GPE). The patient was immediately treated with the appropriate antibiotics, and underwent surgical debridement, resulting in a 5 cm × 4 cm skin deficit (Figure 1).

The reconstruction was performed with a local KF, type IV, designed dropwise caudally to the deficit, anteriorly to the upper rectus abdominis, with a ratio of 2.5:1 (Figure 2).

This flap was chosen due to its suitability for providing adequate blood supply and tissue coverage. This specific design utilized fewer perforators than typically employed in such procedures. The flap was precisely dissected up to the superficial fascia of the rectus abdominis muscle, and was then mobilized in a clockwise manner towards the deficit. Part of the flap was then placed carefully into the deficit, avoiding undue tension. The suture techniques chosen were 3-0 Monocryl single sutures for the subdermis, 4-0 Nylon single sutures for the deficit side, 4-0 Nylon running sutures for the rest of the flap (Figure 3).

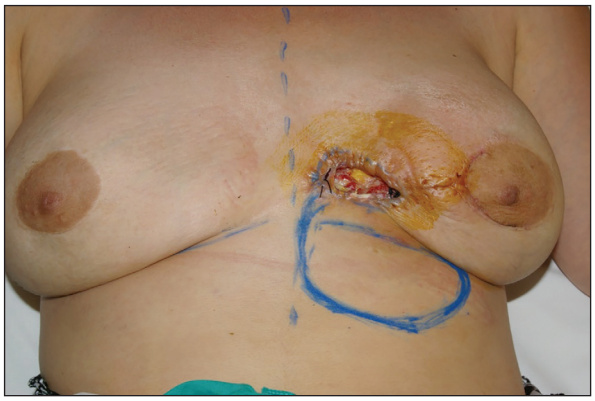

Figure 1. Keystone Flap Type IV, designed dropwise caudally to the deficit, anteriorly to the upper rectus abdominis, with a ratio of 2.5:1.

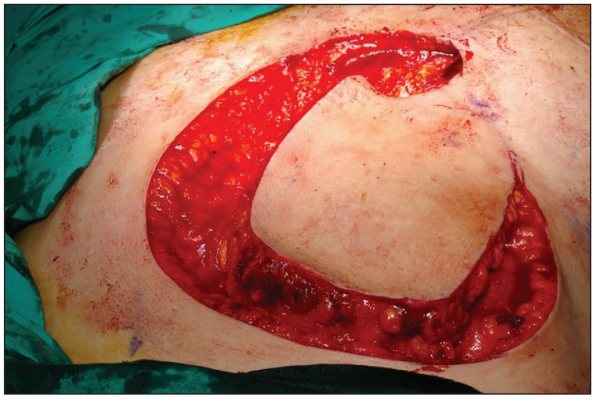

Figure 2. Comprehensive view of the incision surrounding the flap, highlighting the droplet-shaped design with a ratio 2.5:1.

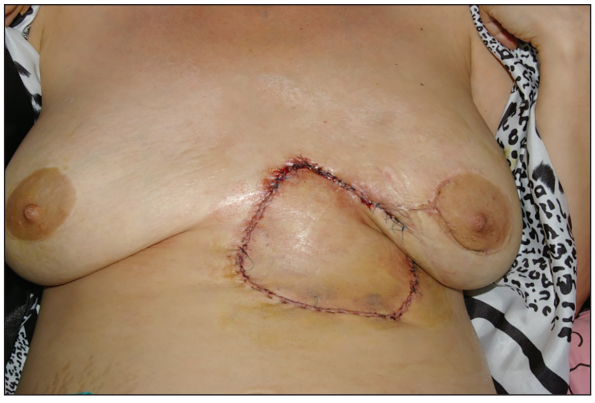

Figure 3. Immediate postoperative result of the adapted Keystone Flap Type IV.

The postoperative course proceeded smoothly, without any complications or signs of congestion to the flap, while wound healing progressed satisfactorily. The patient was able to continue her chemotherapy treatment, which is crucial for managing breast cancer. If not managed properly, surgical resection can lead to a significant defect that can negatively impact the patient's quality of life. However, no widely accepted or ideal approach exists for resurfacing defects in reconstructive surgery (6). The KPIF may be considered a viable method for reconstructive surgery following mastectomy. Nevertheless, more exhaustive research and well-designed prospective cohort studies will provide valuable insights into the outcomes and benefits of using the KPIF for breast reconstruction.

Discussion

Koshima and Soeda (1989) made significant contributions to the field of reconstructive surgery by introducing the concept of perforator flaps, employing a musculocutaneous flap with an inferior epigastric artery–based skin island to restore defects in the floor of the mouth and groin (4). This influential research was pivotal in advancing our understanding of perforator flaps, vascular anatomy, and tissue transfer for reconstructive purposes.

The Keystone Flap, a perforator flap subtype, consists of two V to Y advancement flaps that move in opposite directions. This movement creates additional tissue adjacent to the defect, allowing for primary skin edge approximation (4). Initially proposed for smaller defects, it was later suggested in 2011 that KFs could also address more significant defects in the trunk and limbs (7). Various modifications have been developed to increase their transposition potential for these extensive deficiencies, including double KFs or deep fascia incisions (1, 8). The omega subtype, often overlooked, presents another modification that effectively utilizes excessive laxity in a specific area of the flap during insetting, and capitalizes on the natural laxity of the lateral skin, increasing mobility (6). Surgeons can carefully plan and execute the flap design to advance it into the desired position, ensuring minimal tension or distortion, without compromising flap viability or causing undue complications.

The Type IV Keystone flap is a specific variation of the KF, which includes rotation and advancement of the flap, and is indicated in breast reconstruction and other more complex surgeries (9). The Type IV Keystone Flap has been previously documented for breast reconstruction; however, our case uniquely applies this technique with a specific droplet shape and reduced ratio in the lower medial quadrant. This detail, not previously described, capitalizes on the laxity of the area and minimizes the need for multiple perforators, which is particularly beneficial in patients with limited perfusion capacity.

In addition to the KF, other perforator flap techniques have emerged as valuable alternatives in breast surgery. Hamdi et al. (2006) proposed using Intercostal Artery Perforator (ICAP) flaps as valuable alternatives for breast surgery, which are particularly beneficial when addressing complex defects on the trunk without compromising the underlying muscle (10). Similarly, a study by Orabi et al. (2022) highlighted the reliability of lateral chest wall perforator flaps as a reliable technique for partial breast reconstruction, with satisfactory aesthetic results (11).

These advancements in perforator flap techniques expand the range of reconstructive options available to surgeons, increasing surgical liberty, and allowing for customized solutions in various clinical scenarios. Ongoing research and exploration of these techniques will lead to further improvements in breast reconstruction and other fields of reconstructive surgery.

Conclusion

KPIF is a viable method for resurfacing significant skin deficits and full-thickness cutaneous defects in various anatomical regions, including the breast. Given its relatively short learning curve, acceptable risk factors, decreased operative times, broad applicability, and positive outcomes, the KF

technique should be considered an invaluable approach, suitable for both novices and experienced surgeons (1, 3, 12). Additionally, it is an advantageous option for more complex wounds in patients unsuited for more intricate surgical procedures, such as microsurgery. As we continue to deepen our understanding of vascular anatomy and refine surgical methods, we can expect even more significant progress in reconstructive surgery. Advanced vascular imaging techniques, such as Magnetic Resonance Angiography (MRA), will provide detailed preoperative assessments, aiding in the accurate planning and execution of reconstructive flaps (13). These advancements will enable a personalized approach, minimizing complications and optimizing patient satisfaction.

What Is Already Known on this Topic:

Perforator flap techniques offer advantages such as reduced complications, diminished pain (14), and quick patient recovery. Keystone flaps (KF), including the Type IV variant, became popular due to their simplicity and effectiveness in wound closure, especially in melanoma cases. The KF Type IV represents an innovative and versatile technique for breast reconstruction following lumpectomy. It provides enhanced perfusion and favorable aesthetic results, making it a reliable option for addressing tissue deficits (15).

What This Case Report Adds:

This case report contributes valuable insights to the existing literature, providing further evidence for the Keystone Perforator Island Flap technique's viability in reconstructing significant skin deficits, including breast reconstruction. The findings underscore the positive outcomes and its suitability for complex wounds, particularly in patients who may not be candidates for more intricate surgical procedures. Additionally, the report highlights the need for further research to validate the extensive applicability of this modified technique and compare it with other approaches in breast reconstruction.

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Author's Contributions: Operation and identification of the case: EK and FB; Drafting the article: VK, TT and NP; Revising it critically for important intellectual content: EK and DC; Approved final version of the manuscript: DC and TT.

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

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Case Report
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Entrapment of the Subscapular Artery between the Radial Nerve and the Posterior Wall of the Axilla: An Anatomical Variation with Clinical Significance

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Abstract

Objective. The subscapular artery vascularizes a substantial region of the thoracic wall, and the significance of its distribution is well depicted in the diversity of reconstructive procedures that rely on its blood supply. The aim of this study is to present an uncommon anatomical variation of the artery and discuss the clinical implications of its presence. **Case Report.** This case report depicts a rare variant of compression and the kinking of the subscapular artery by the radial nerve on the posterior wall of the axilla that was encountered during dissection of a male cadaver of Greek origin. **Conclusion.** The use of autologous tissues in the reconstruction of defects and treatment of lymphedema is expanding, so the need to establish safer surgical dissections is also becoming more apparent. The case of entrapment of the subscapular artery by the radial nerve is extremely rare, however, utilizing tissues perfused by this artery for reconstructive purposes could potentially be futile and unsuccessful due to the inadequate blood supply or vessel thrombosis. Hence, the surgeon should adapt the treatment plan according to preoperative findings, as the presence of anatomical variants should always be suspected.

Key Words: Subscapular Artery ▪ Artery Entrapment ▪ Kinking of Artery ▪ Radial Nerve ▪ Reconstructive Surgery.

Introduction

The subscapular artery constitutes the largest branch of the axillary artery and provides the blood supply to the posterior wall of the axilla, as well as the posterior scapular region (1, 2). The significance of the distribution of the subscapular arterial system has been well established, considering the diversity of surgical procedures of a reconstructive nature that rely on its blood supply. The radial nerve originates from the posterior cord of the brachial plexus and passes through the lower triangular interval between the teres major muscle, the long head of the triceps brachii muscle, and the shaft of the humerus, accompanied by the profunda brachii artery. In relation to the subscapular artery, in most cases the radial nerve runs anterior to the artery (3, 4).

Knowledge of topographical anatomy and clinically relevant arterial and neural variations of the axillar region is crucial to improve safety in surgical procedures involving the vascularized region. The increase in the number of reconstructive procedures using autologous tissues reflects the necessity to minimize the risk of inadvertent complications. A prerequisite for successful transplantation of autologous tissues is the sustenance of blood flow through the pedicle of the flap. Hence, disruption of the longitudinal direction of the vessels, such as kinking of the vessels, could have devastating implications as a result of impaired blood supply.

The aim of this study is to present a rare case of entrapment of the subscapular artery by the radial nerve on the posterior wall of the axilla with subsequent kinking of the vessel, and discuss its clinical implications. This uncommon anatomical

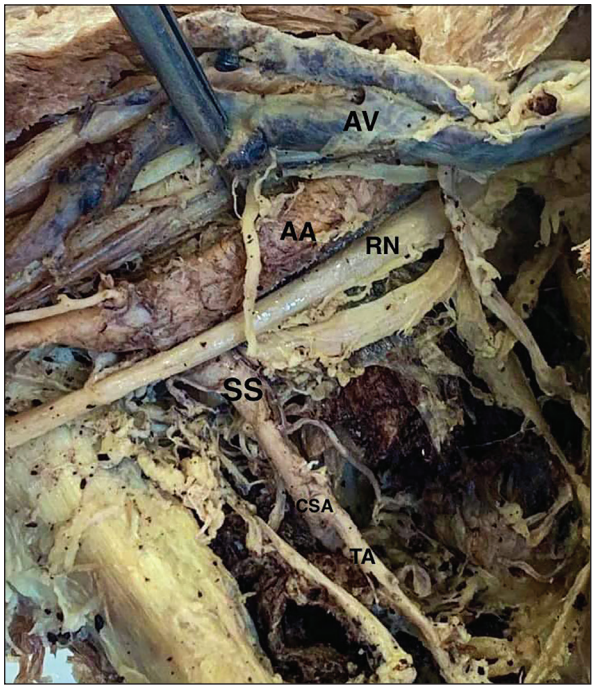
variation was encountered during routine dissection of a male cadaver of Greek origin for educational and research purposes.

Case Report

The anatomic variation in our study was depicted in a formalin-fixed 85 year old male cadaver of Greek origin. The cadaveric dissection took place in the Dissections Hall of the Department of Anatomy, School of Medicine, National and Kapodistrian University of Athens. The axillar region of the cadaver was dissected and cleaned by the contributors, and the anatomical specimen was photographed. While proceeding with the routine dissection of the right axillary region of the Greek male cadaver, we encountered the variation of entrapment of the right subscapular artery by the radial nerve. After preparation and release of the vessel from the nerve, we detected an area of kinking of the subscapular arterial trunk, which corresponded to the point of compression by the radial nerve. The artery presented no further anatomical variations and followed its usual course, dividing into the thoracodorsal and circumflex scapular arteries. The specific distribution and compression of the subscapular artery by the radial nerve were encountered bilaterally. The left subscapular artery presented with an analogous area of kinking as a result of entrapment by the left radial nerve.

Discussion

Traditionally, the subscapular artery originates from the posterior surface of the third part of the axillary artery, then runs along the posterior wall of the axilla, following the inferior margin of the subscapularis muscle for a short distance, and bifurcates into its two terminal branches, the



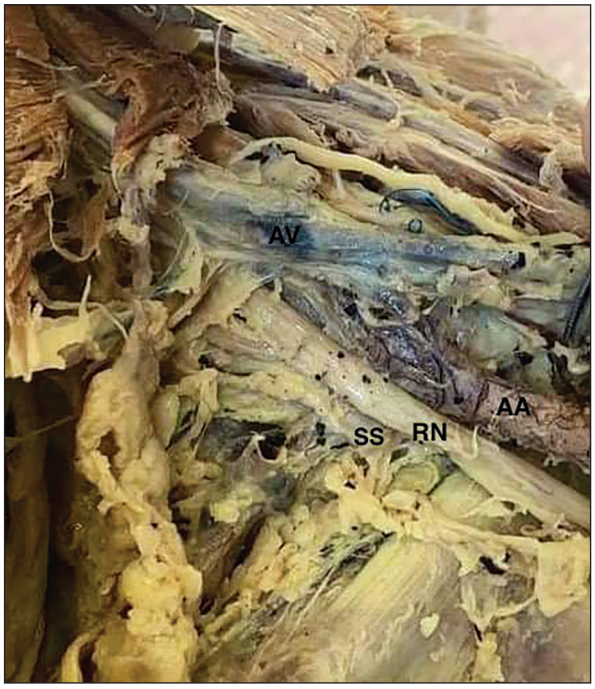


Figure 1. Right axillary region. The right radial nerve compresses the origin of the right subscapular artery from the axillary artery. An area of kinking is detected on the subscapular arterial trunk, corresponding to the point of compression by the radial nerve. AV: Axillary vein, AA: Axillary artery, SS: Subscapular artery, RN: Radial nerve, CSA: circumflex scapular artery, TA: Thoracodorsal artery. Figure 2. Left axillary region. The left radial nerve compresses the origin of the left subscapular artery from the axillary artery. An area of kinking is detected on the subscapular arterial trunk. AV: Axillary vein, AA: Axillary artery, SS: Subscapular artery, RN: Radial nerve.

circumflex scapular artery and the thoracodorsal artery (2).

The branches of the circumflex scapular artery provide the blood supply for regional flaps (scapular and parascapular flaps), which are widely used in the reconstruction of simple and complex defects. Musculocutaneous flaps, as well as perforator flaps based on the thoracodorsal vessels can also be elevated with the purpose of reconstructing defects of areas of the head and neck, thorax and axilla, as well as distant regions, using microsurgical techniques (5). Olinger et al. investigated the branching morphology of the subscapular, lateral thoracic and posterior circumflex humeral arteries, and their relationship to the radial and axillary nerve. According to this study, in 88.5% of cases where the lateral thoracic artery and subscapular artery presented with a classic branching pattern, the two nerves traveled posterior to the lateral thoracic artery, while the radial nerve ran anterior to the subscapular and the axillary nerve posterior to the artery. In 9.2% of cases both nerves were detected posterior to the two arteries, and in 2.3% of the specimens both nerves traveled posterior to the lateral thoracic artery, but anterior to the subscapular artery (4).

Previous studies have reported different variations of the subscapular artery. Lhuaire et al. proposed a classification concerning the subscapular pedicle variations. Three types of arterial variation were presented, where specifically type Ia involved the classic pattern of the artery originating from the inferior border of the axillar artery and bifurcating into two terminal branches. Arteries associated with the type Ib classification presented with a similar pattern, originating, however, from a proximal point of the axillar artery, while type II was associated with the absence of the subscapular artery (6). Cases have also been presented where the subscapular artery terminates into three branches, namely the thoracodorsal, the circumflex scapular, and the posterior circumflex humeral arteries (7, 8). This branching pattern was observed in 12% of cases in a study conducted by Olinger et al.. Dimovelis et al. reported a case of tetrafurcation of the subscapular artery. Variations concerning the origin of the subscapular artery have also been reported. According to Samuel et al. the subscapular artery may also emanate from a common trunk, branching out into the posterior circumflex humeral, radial collateral, middle collateral and superior ulnar collateral arteries (9). A common trunk involving the profunda brachii, subscapular, anterior and posterior circumflex humeral arteries and the superior ulnar collateral artery has also been described (10).

In the context of its relationship with the brachial plexus, entrapment of the subscapular artery between the two roots of the median nerve has also been observed (1, 11). Kuwar et al. described a case where the subscapular artery was encircled by two roots emanating from the posterior cord of the brachial plexus, forming the radial nerve (3). Mistry et al. observed a variant of the radial nerve, which split into two roots following its formation, and encased the subscapular artery (12). To the best of our knowledge no case of compression of the subscapular artery by the radial nerve with subsequent kinking of the artery has been reported previously in the literature.

Successful upper limb revascularization has been documented using parts of the subscapular arterial system as arterial grafts and replacing damaged segments of the arterial systems of the upper extremity (13, 14). According to Malikov et al., patients with critical limb ischemia and large tissue defects may be treated by harvesting a subscapular artery flow-through muscle flap, using the subscapular artery as an arterial graft, and a serratus anterior muscle flap supplied by the distal branch of the thoracodorsal artery to cover the deficit (15). The utility of the subscapular arterial system in the creation of conduits for revascularization has also been established in coronary bypass surgery in cases where the revascularization cannot be achieved using other conventionally used arterial conduits (16-19).

The terminal branches of the subscapular vessels demarcate the posterior limit of axillar lymph node dissection, and they also constitute the pedicles of various regional flaps that can be harvested for reconstructive purposes, such as the latissimus

dorsi and the scapular flaps. The longitudinal direction of the vessels is a prerequisite for successful autologous reconstruction, as twisting and kinking of the pedicle may impair blood flow. According to studies, kinking of the vascular pedicle constitutes the most common cause of vessel occlusion and flap failure (20, 21). An experimental study on rats demonstrated that acute angulation of the vascular pedicles may obstruct blood flow increasing the risk of tissue ischemia and flap failure (22). Vascularized lymph node transfer (VLNT) has, additionally, emerged as a feasible treatment option in cases of lymphedema occurring after lymph node dissection, as the ability of the lymphatics to regenerate after VLNT has been well investigated. Patients suffering from lymphedema previously had to endure conservative decongestive therapies or debulking procedures to relieve their symptoms. Hence, the development of techniques that aim to restore the function of the lymphatic system has revolutionized the treatment of these patients and improved their quality of life. Lymph node flaps supplied by the thoracodorsal artery can be harvested and transplanted in the affected region, while the use of a latissimus dorsi flap and a thoracodorsal artery perforator flap with lymph node transfer has also been introduced as a tool for treating lymphedema (23-26). The thoracodorsal vessels are also used as recipient blood vessels in autologous breast reconstruction, as well as reconstruction of defects of the thoracic wall using microsurgical techniques. Therefore, familiarity with the anatomical variations and branching patterns of the subscapular artery and its spatial relationship to the nerves of the brachial plexus is indispensable for safe surgical dissection and harvesting of flaps.

Conclusion

The widespread use of autologous tissues in the reconstruction of defects renders the optimization of surgical dissections necessary, thus surgeons should be aware of alternate anatomies of the vessels and neural structures of the axillar region before proceeding with any intervention in order to prevent postoperative complications as a result of ischemia of the tissues. This case of entrapment and kinking of the subscapular artery by the radial nerve on the posterior wall of the axilla is extremely rare. However, an attempt to utilize the tissues supplied by this artery for reconstructive purposes could potentially lead to flap loss due to inadequate blood supply or vessel thrombosis, if the surgeon does not suspect the presence of an anatomical variant. Preoperative imaging of the arterial anatomy is suggested to increase the level of safety by revealing unpredictable variations and establishing a more familiar path for the surgeon during dissection.

What Is Already Known on This Topic:

The anatomical variability of the subscapular artery has been elucidated in various case reports. Moreover, the clinical significance of its variants has been depicted in several surgical fields, especially in procedures involving the reconstruction of defects, as well as the treatment of lymphedema.

What This Study Adds:

The case of entrapment of the subscapular artery by the radial nerve with subsequent kinking of the vessel has not been reported in previous anatomical studies. The presence of this particular variation could negatively influence the outcome of reconstructive procedures involving the subscapular artery, thus preoperative imaging of the axillar region could assist in avoiding the use of tissues of this area and turning attention to alternative and more viable reconstructive options.

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

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

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Case Report
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A Rare Case of Retroperitoneal Schwannoma in an Adult Male

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Abstract
Objective. This study aims to illustrate a rare case of retroperitoneal schwannoma by presenting the clinical, imaging, and histological parameters. **Case Report.** A 36-year-old patient visited the outpatient clinic because of back pain experienced over the previous two months. There were no complaints regarding the nervous system or urinary system. Thorough imaging evaluation, including magnetic resonance for the lumbar spine, abdominal computed tomography, and positron emission tomography was conducted. An encapsulated mass was found in the retroperitoneal area, positioned in front of the O4 vertebra and in close proximity to the left psoas muscle, the left common iliac artery, and the left ureter. The lesion exhibited FDG radioisotope uptake, and a CT-guided biopsy confirmed a benign peripheral nerve tumor. The patient underwent laparotomy surgery, where the tumor was removed. The histological investigation, along with immunohistochemistry, confirmed the presence of a retroperitoneal schwannoma. **Conclusion.** Schwannoma is a rare type of retroperitoneal tumor, with nonspecific clinical and radiological characteristics that make diagnosis difficult. Surgical resection is the primary treatment for symptomatic patients, with a favorable prognosis. Long-term follow-up is advised to reduce the chance of late recurrence.

Key Words: Retroperitoneal Schwannoma ▪ Nerve Sheaths ▪ Schwann Cells ▪ Retroperitoneal Tumor ▪ Case Report.

Introduction

Schwannoma, or neurilemmoma, is a rare ectodermal tumor that primarily originates from the sheaths of peripheral or cranial nerves. Retroperitoneal schwannomas are a very uncommon variety, making up about 3% of all schwannomas and 4% of retroperitoneal tumors (1). The sporadic form of retroperitoneal schwannomas is the most common, and predominantly affects females between the second and fifth decades of life (2). The familial form of retroperitoneal schwannomas accounts for 5-18% of all retroperitoneal schwannomas, presents at a younger age, and is often associated with Von Recklinghausen's disease (3). Clinical manifestations are nonspecific, and diagnosis relies mainly on histopathological examination. Prognosis is favorable following surgical resection, however, there is a 5-10% risk of late recurrence in cases of incomplete excision, necessitating long-term follow-up (1).

Here, we present a case report of an uncommon location of schwannoma in a 36-year-old male. The patient was presented to our department due to a two-month history of back pain. The schwannoma was successfully treated using conventional surgery.

Case Presentation

A 36-year-old male was referred to our surgical clinic due to the existence of a retroperitoneal

mass. The pathology department of the hospital discovered this mass during a thorough imaging evaluation in response to a reported two-month history of back pain. It is important to mention that there were no documented issues with urinating or symptoms related to the lower extremities. The patient's medical history, clinical examination and laboratory evaluations were otherwise unremarkable. A lumbar spine Magnetic Resonance Imaging (MRI) scan revealed a well-circumscribed lesion in the left retroperitoneal space, adjacent to the left iliopsoas muscle. The lesion exhibited low signal intensity in T1 weighted images (Figure 1, Panel A) and high signal intensity in T2 weighted images (Figure 1, Panel B).

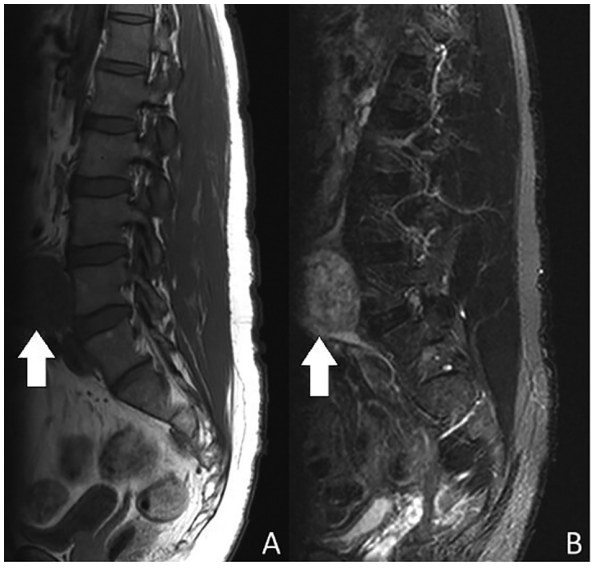

Figure 1. Sagittal lumbar MRI without contrast. A well-circumscribed lesion with signal isointense to the muscle in T1 weighed images (Panel A) and hyperintense signal in T2 weighed images (Panel B).

An abdominal Computed Tomography (CT) scan demonstrated a 52×47 mm mass with heterogeneous contrast enhancement, located anterior to the L4 vertebra, abutting the left ureter and left common iliac artery. Brain and chest CT scans were unremarkable (Figure 2).

The lesion exhibited fluorodeoxyglucose (FDG) avidity (SUVmax 5.2) on Positron Emission Tomography (PET-CT). A CT-guided core needle biopsy was performed, and histopathological analysis of the specimen revealed a nodular mass surrounded by a fibrous capsule, with areas of mixed cellularity and the presence of thrombi in the blood vessels. Immunohistochemistry of the biopsy tissue suggested a benign nerve sheath tumor.

The decision to operate was based on the patient being symptomatic. Beyond the surgical issue, no concurrent diseases were discovered during the routine preoperative examination. During the surgical procedure, the patient had both general and epidural anesthesia. A midline incision was conducted, and a thorough examination of the liver and peritoneal cavity revealed

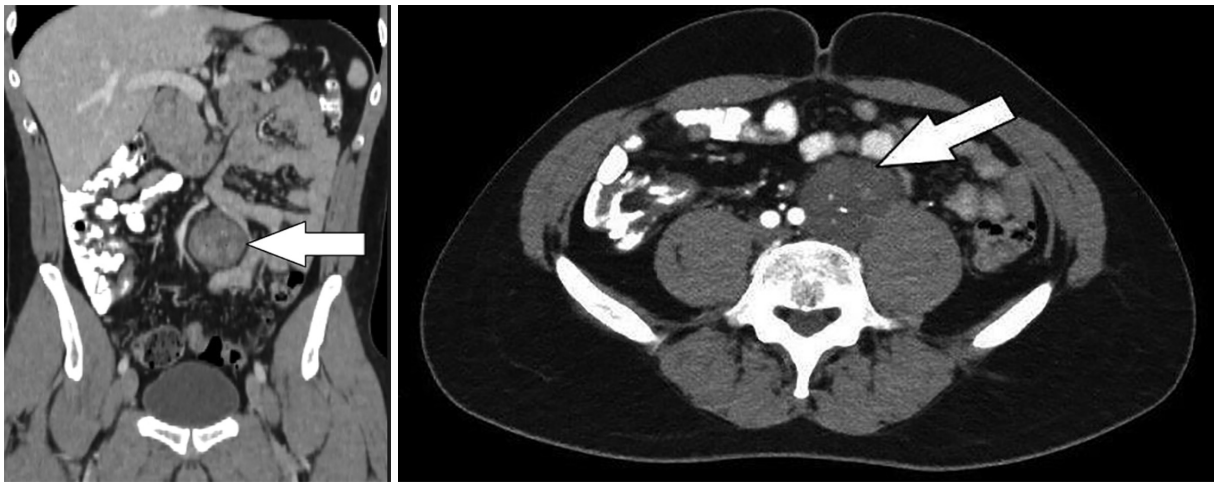

Figure 2. Computed tomography (CT) of the abdomen with intravenous contrast in coronal (A) and axial (B) sections. (A-B) A well-circumscribed mass with heterogeneous contrast enhancement, located anterior to the L4 vertebra, abutting the left ureter and left common iliac artery.

no pathological abnormalities. The posterior peritoneum was incised, we accessed the retroperitoneal area and detected the mass. The left ureter was located superior and lateral to the structure, with outward displacement. The left common iliac artery was situated within it and to its right. The left psoas muscle was positioned posterior to it. Subsequently, we surgically accessed the tumor and securely ligated the lumbar vessels located superior to it. The process entailed surgically removing the tumor and establishing thorough control of bleeding (Figure 3).

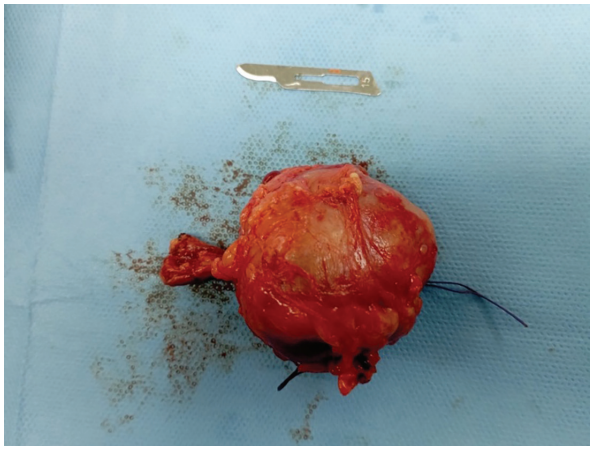

Figure 3. The surgical specimen. Macroscopic examination of the surgical specimen revealed a well circumscribed tumor, encased in a fibrous capsule and surrounded by fatty tissue.

The abdomen was closed in layers. Histopathology of the resected specimen confirmed the diagnosis of a retroperitoneal schwannoma, with negative margins and no evidence of nerve or blood vessel infiltration. Histologically, the lesion consisted of hypercellular (Antoni A areas, Figure 4-Panel A and B) and hypocellular areas (Antoni B areas, Figure 4-Panel A). The immunohistochemistry was suggestive of a benign peripheral nerve sheath tumor. The cells stained diffusely positive for S100 and vimentin. The patient was discharged on postoperative day 8. He did not receive any adjuvant therapy, and was scheduled for follow up with serial MRI scans for a minimum of five years. At the 12-month follow-up, the patient had not developed any recurrence, his condition remained satisfactory, and he reported an excellent quality of life.

Discussion

Peripheral nerve schwannomas are neuroectodermal neoplasms with an annual prevalence of 6 per 1,000,000 people (4). They are the most common peripheral nerve sheath tumor in adults and arise from Schwann cells (1). The retroperitoneal location of a schwannoma is exceedingly rare, accounting for only 3% of all schwannomas (2, 3). The sporadic variant of retroperitoneal

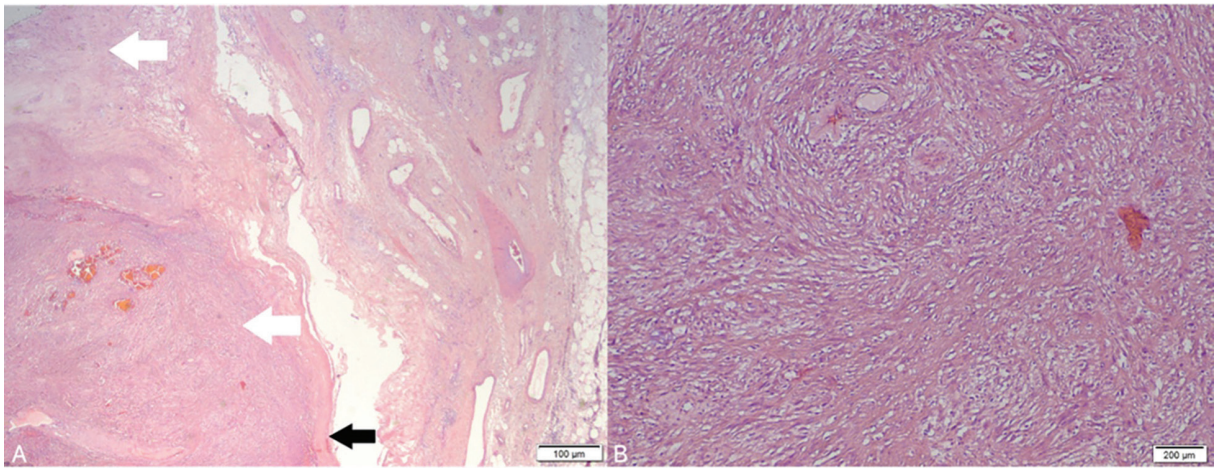

Figure 4. Histopathology of the surgical specimen. A well-circumscribed nodular mass, encased in a fibrous capsule (panel A - thin black arrow). The lesion consists of hypercellular areas (Antoni A areas- bottom white arrow), and hypocellular areas (panel A - Antoni B areas - top white arrow) (magnification ×100). Microscopic image of a hypercellular – Antoni A area (panel B) (magnification × 200).

schwannoma demonstrates the highest occurrence rate during the second to fifth decades of life, displaying a slight predominance in females (4, 5). Conversely, the familial form manifests at an earlier age, exhibiting a higher likelihood of malignancy (with reported rates of up to 60% of cases), and a strong association with von Recklinghausen's disease (3). Schwannomas, which are usually benign growths that develop from Schwann sheath cells, mostly impact nerves in the head and upper limbs. Schwannomas primarily originate from neural crest cells. Except for cranial nerves I and II, which do not have Schwann cells, schwannomas can develop in any other organ or nerve trunk (6). Schwannomas can arise in any anatomical region of the body, with the limbs being the most common site in 53.1% of cases, followed by the trunk in 13% of instances, and the head and neck in 13.9% of cases (7). Additional sites include the posterior mediastinum, retroperitoneum, spinal roots, bone, gastrointestinal tract, pancreas, liver, thyroid, adrenal glands, and lymph nodes (8).

Table 1. The Characteristics of Retroperitoneal Schwannomas in Our Case Report and Cases in Pubmed

Characteristics	Our case report	Cases report in Pubmed (N=28)
		N; (%)
Symptom		
None	-	5 (18)
Abdominal pain	-	16 (57)
Back pain	Yes	12 (42)
Urinary symptoms	-	18 (64)
Neurological symptoms	-	8 (29)
Preoperative imaging		
US*	-	22 (79)
CT†	Yes	15 (54)
MRI‡	Yes	12 (42)
PET /CT§	Yes	4 (14)
Preoperative biopsy	Yes	13 (46)
Surgery method		
Abdominal resection	Yes	13 (46)
Laparoscopy resection	-	15 (54)

* Ultrasound; †Computed Tomography; ‡Magnetic Resonance Imaging; §Positron Emission Tomography/ Computed Tomography.

A total of 28 cases of retroperitoneal schwannoma were retrieved from the PubMed database (1-3, 5-7, 9-16). These items are documented in the supporting information (Table 1).

Out of the total of 28 patients, the larger portion, specifically 18 individuals, did not exhibit any symptoms. Neurological problems were reported by only eight patients. In general, the predominant method of assessment for patients was ultrasound, with 22 out of 28 patients undergoing this procedure. CT was used for 15 patients, while MRI was used for 12 individuals. A PET scan was employed as a preoperative evaluation method for the tumor in just four cases. Fewer than half of the patients (13/28) had preoperative biopsy, as in our case. Out of the total of 28 patients, 13 patients underwent abdominal resection, while the remaining 15 patients had laparoscopic tumor excision.

Retroperitoneal schwannomas are typically benign and slow-growing in nature (5). They are mostly asymptomatic; however, they can present as a palpable mass, or cause abdominal or back pain, urinary dysfunction, or bowel obstruction due to their location (5, 11, 17). On imaging, retroperitoneal schwannomas often appear as encapsulated lesions with low density and heterogeneous contrast enhancement on CT scans (11, 17). On MRI scans they appear isointense to the muscle on T1-weighted images, and exhibit high signal intensity (similar to fat tissue) on T2-weighted images (2, 18).

Differential diagnosis of retroperitoneal schwannoma includes a wide spectrum of retroperitoneal lesions, including: malignant peripheral nerve sheath tumors, sarcoma, lymphoma, neuroendocrine tumors (pheochromocytoma, paraganglioma), vascular tumors (hemangioma, cystic lymphangioma), fatty tissue tumors (angiomyolipoma, myelolipoma, lipoma), rhabdomyoma, or extragonadal tumors (teratoma or seminoma), among others (9). The need for establishing a diagnosis by means of a preoperative biopsy has been a matter of debate (12). The similarity in the clinical and imaging characteristics between schwannomas and other lesions is countered by the risk of tumor seeding, hemorrhage, or a hypertensive

crisis during a biopsy attempt (5). Nevertheless, in the appropriate clinical setting, a preoperative core needle biopsy of a retroperitoneal mass, in order to guide further treatment decisions, is in accordance with the European Society for Medical Oncology (ESMO), the National Cancer Comprehensive Network (NCCN) and the Trans- Atlantic Retro Peritoneal Sarcoma Working Group (TARPSWG) Guidelines (19).

Surgical excision is the mainstay of treatment for all symptomatic patients, and is generally associated with a favorable long- term prognosis. There have been no reports of retroperitoneal schwannoma with distant metastases, although local recurrence rates of 5-10% have been reported, especially in cases of incomplete resection (9, 10). Malignant transformation is exceedingly rare, but it has been reported in cases of von Recklinghausen's disease (5). Long-term follow-up remains necessary to minimize the slight risk of late recurrence and malignant transformation.

At this juncture, we also note that our article serves as another instance illustrating the limited efficacy of Fluorodeoxyglucose Positron Emission Tomography (FDG PET) in differentiating schwannomas from malignant peripheral nerve sheath tumors (20). Due to the FDG uptake observed in schwannomas, it is not feasible to differentiate these tumors from malignant peripheral nerve sheath tumors, which also exhibit high FDG uptake, prior to undergoing a biopsy or surgery (21, 22).

Conclusion

Retroperitoneal schwannomas constitute a small percentage of retroperitoneal tumors and present a diagnostic challenge due to their nonspecific clinical and imaging features. A multidisciplinary approach and early tissue biopsy are required. Surgical resection is the primary treatment modality for symptomatic patients, with an overall favorable prognosis. However, long-term follow-up is recommended to mitigate the risk of late recurrence.

What Is Already Known on This Topic:

Schwannomas are the predominant neoplasms of the peripheral nerve sheath in adults, originating from Schwann cells. Their vague clinical and imaging features make them difficult to diagnose. Schwannomas located in the retroperitoneum are extremely uncommon, accounting for only about 3% of all schwannomas. The necessity of confirming a diagnosis with a biopsy before surgery has been a subject of discussion. The definitive diagnosis relies on the histopathological examination of the operative specimen. Surgical excision is the primary treatment for all individuals experiencing symptoms, and is typically linked to a positive long-term prognosis.

What This Study Adds:

This study offers thorough insights into the imaging and histological diagnosis and management of these uncommon tumors. The preoperative imaging, which encompassed both CT and MRI scans, afforded us a meticulous depiction of the anatomical components encompassing the tumor. In conjunction with the CT-guided core needle biopsy, this played a pivotal role in the favorable outcome of the surgery. The paper showcases the restricted efficacy of Fluorodeoxyglucose Positron Emission Tomography (FDG PET) in differentiating schwannomas and malignant peripheral nerve sheath tumors. Histology and immunohistochemistry markers were used to identify and study this condition, offering insights for future research in this area. Our primary objective is to enhance the limited body of research on retroperitoneal schwannomas by emphasizing the significance of being cognizant and doing comprehensive assessments in similar cases.

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

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Original Scientific Article
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Family Physicians' Awareness of the Burden of Oral Corticosteroids in Asthma Patients

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Abstract

Objective. The objective of this study was to identify when family physicians decide to prescribe oral corticosteroids (OCS) to treat asthma, to establish the factors affecting their decision, and how familiar family physicians are with the side effects of OCS. **Materials and Methods.** A cross-sectional observational study was conducted among physicians that are members of the Slovenian Family Medicine Society. **Results.** The study included 122 family physicians from all 12 Slovenian regions. The great majority (86.9%) reported they had previously prescribed OCS to asthma patients. The largest share of these (45.1%) tended to prescribe a limited number of tablets, although many (42.6%) also prescribed the entire pack. Regarding the adverse effects associated with OCS, the physicians listed a range of potential problems, highlighting hyperglycemia and exacerbated diabetes, the impact on bone density, a suppressed immune system and increased risk of infection as the most common. **Conclusion.** In the future, it is vital to improve family physicians' awareness of when OCS may be prescribed to treat severe asthma, and to define the clinical pathway for severe asthma, which should also involve interdisciplinary collaboration.

Key Words: Family Physician ▪ Oral Corticosteroids ▪ Referrals ▪ Severe Asthma ▪ Treatment.

Introduction

Asthma is a chronic inflammatory disease of the airways and one of the most common chronic lung diseases. Ten percent of the population in the developed world has been diagnosed with asthma, and physicians at all levels of healthcare deal with the disease. In Slovenia, the prevalence of asthma in adults aged 18 to 65 years is quite high, at 16% (1). Asthma patients may not experience any problems; they may only experience individual symptoms, such as shortness of breath, coughing, and wheezing; or they may suffer from severe asthma, which often worsens over time. Most asthma patients are managed by primary care physicians, and 22% are regularly treated by a specialist (2, 3).

Roughly 17% of asthma patients are prescribed a high-dose inhaled therapy, but their asthma is still not under control. This is referred to as difficult-to-control asthma. Its causes may vary from an incorrect inhalation technique and poor adherence, to treatment of comorbidities that can exacerbate asthma (4). Severe asthma is a subtype of difficult-to-control asthma and comprises cases in which the asthma remains uncontrolled even though all the causes of difficult-to-control asthma have been suitably addressed. It is estimated that around 2.4% to 4% of asthma patients have severe asthma. These patients are also eligible for biological therapy (5). Uncontrolled asthma is characterized by poor symptom control (frequent use of a rescue inhaler, activities limited by asthma, or nocturnal awakening caused by asthma) or frequent exacerbations that require two or more courses of oral corticosteroids (OCS) in a 12-month period, or one or more asthma-related hospitalizations in a 12-month period (3, 6).

The goal of asthma management is to control symptoms and reduce the risk of asthma exacerbation, permanently reduced lung function, and severe exacerbations that may also be fatal. Recommendations by the Global Initiative for Asthma (GINA) (3) focus on the latest high-quality evidence available, and scientific consistency. Its reports are updated and posted online annually. They are the most frequently cited evidence-based recommendations for the optimal management of asthma in adults and children. The GINA recommendations also form the basis for the Slovenian guidelines (1).

Inhaled corticosteroids (controllers) are used for the initial anti-inflammatory treatment of asthma, and they are considered the primary treatment. Relievers are used as needed in the event of problems or disease exacerbations. In addition to short-acting beta antagonists, an ICS-formoterol combination inhaler (i.e., a specific long-acting and rapid-onset bronchodilator) may also be used as a reliever.

In the event of acute asthma exacerbation, the physician should prescribe OCS in the recommended dose of 32 mg of methylprednisolone for 3 to 5 days (1). An extensive study conducted among the general population showed that even a single OCS treatment episode was associated with an increased risk of major adverse events. OCS are among the most common causes of adverse drug events (7, 8). Due to various adverse effects, such as hypothalamic–pituitary–adrenal (HPA) axis suppression, osteoporosis, arterial hypertension, diabetes, and increased risk of infection, OCS may only be used for acute asthma exacerbations and by severe asthma patients that do not respond well to other therapies. OCS use is usually short-term, and the goal is to minimize it once stable asthma control is achieved. Sadatsafavi et al. documented the trends in OCS use, establishing that maintenance OCS use declined from 9.12% between 2000 and 2002, to 6.35% between 2011 and 2013. In turn, episodic OCS use increased, averaging from 0.82 episodes per patient-year between 2000 and 2002, to 0.93 episodes per patient-year between 2011 and 2013 (9).

Asthma is one of the most common chronic lung diseases. The heterogeneity of its phenotypes and the varying burden of the disease are the main challenges to achieving optimal asthma control. The goal of asthma management is to achieve good control of symptoms and minimize the risk of exacerbations, persistent airflow limitation, and asthma-related death. The primary medications used to treat asthma are inhaled corticosteroids and bronchodilators. If asthma worsens or the patient suffers from severe asthma, OCS treatment is required. Due to the well-documented side effects of OCS, it is recommended that patients that use OCS frequently be identified and referred to a pulmonologist. To achieve the best possible clinical results in asthma patients, several guidelines provide recommendations for patients that require a specialist referral. The Slovenian recommendations for treating asthma also recommend that the diagnosis of asthma be confirmed or excluded by a pulmonologist. If suitable asthma control cannot be achieved at the primary care level, the patient needs to be referred to a specialist (1). Such referrals can help clear up any uncertainty in the initial diagnosis, offer tailored treatment options to patients with persistent symptoms, and provide patients access to healthcare providers with expertise in asthma management. Hence, specialist referrals have a significant impact on disease prognosis and the patient's health status (10).

The aims of this study were as follows: to identify in which cases family physicians decide to prescribe OCS to treat asthma, to determine the factors affecting their decision, and to study Slovenian family physicians' familiarity with OCS side effects.

Methods

A cross-sectional, non-interventional (observational) study was conducted among members of the Slovenian Family Medicine Society, which is part of the Slovenian Medical Association. The empirical part was based on a quantitative non-experimental explorative method, using a questionnaire. The questionnaire for examining the

awareness of the burden of OCS was developed in collaboration with the International Primary Care Respiratory Group (IPCRG) and Slovenian pulmonologists. A cross-sectional study was conducted among family physicians across Slovenia, who were invited to participate in the study by email. Their email addresses were obtained from the Slovenian Family Medicine Society member database. The members that agreed to participate in the study were sent a link to the online questionnaire (https://www.1ka.si/a/aa266bc7).

Respondents

The study was carried out from October 1st to November 27th, 2023, and it included 122 family physicians (out of 143 invited) from all twelve Slovenian statistical regions, the majority of whom came from the Central Slovenia region (Table 1).

Among the 122 respondents that completed the questionnaire, there were 33 men (27%) and 89 women (73%). The oldest respondent was 72 years old and the youngest was 28. The average respondent age was 45.8 years, and over three-quarters were included in the on-call roster. Most were employed at health centers (94 respondents or 77%), and 28 (23%) were concession holders.

Ethics Statement

The study was approved by the Slovenian Medical Ethics Committee (decision no. 0120-172/2023/4).

Statistical Analyses

We processed the collected data statistically. Descriptive statistics were used, such as proportions and/or absolute numbers. In some cases we also calculated averages or arithmetic means. When correlating the variables, we used cross tabulation as a technique to investigate the relationship between two variables. The Chi-square test

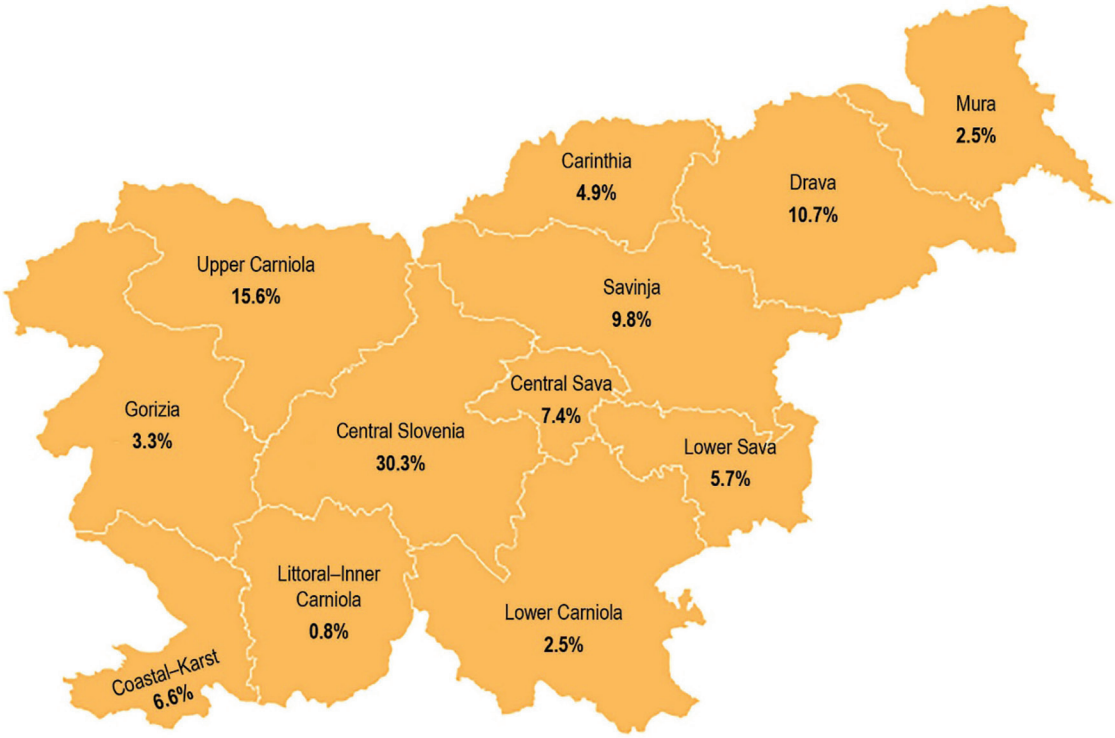


Figure 1. Respondents by statistical region.

was used. All calculations were performed using the SPSS software package, version 12.0. Since the survey used a sample and not all doctors in Slovenia, the results obtained do not automatically lead to population-level conclusions. Therefore, we used a measurement known as the p-value to determine statistical significance. The results were considered statistically significant if there was a confidence level of 95%, which means the P-value is 0.05 or less.

Results

Most respondents (86.9%, or 106 out of 122) reported they had already had experience prescribing OCS to asthma patients. The largest share of these (45.1%) tended to prescribe a limited number of tablets, which means that they dispensed the tablets to patients themselves, but many (42.6%) also prescribed the entire pack.

When asked whether they check how many times a patient had already received OCS (e.g., at the ER or when hospitalized), the respondents provided the following answers: "yes, always" (43.4%), "sometimes" (54.9%), and "no, never" (0.8%). They check this in various ways: they check e-prescriptions (87.0%), they ask the patient (82.1%), or the patients tell them about this themselves or they present their medical results (69.1%). The next question asked the respondents what they felt constituted excessive frequency of OCS use among asthma patients. Their answers were as follows: when they require it once a year (45.1%), two to three times a year (45.9%), or more than four times a year (5.7%). Under "Other," the respondents also provided the following answers: "if they require it twice a day"; "any time they require it"; and "in principle, they should never receive OCS because that is a sign of uncontrolled asthma." There were only statistically significant differences between responding doctors in terms of gender, whereas the differences between the age variables were not significant (Table 1).

Family physicians consider a referral to a pulmonologist a useful mechanism, which they use in various cases, mainly when OCS are prescribed for the first time or if the patient needs OCS to control asthma, especially more than twice a year. The p-value was 0.7, which means we could not draw conclusions about the entire population (Table 2).

In terms of the adverse effects associated with OCS, the respondents listed a range of potential problems. They were asked to what extent they associate certain side effects with taking OCS (1-not at all, 10-significant influence). In their opinion, OCS have the highest influence on diabetes control and osteoporosis. The respondents assigned the lowest average score to adverse effects related to risk of stroke/heart attack (Figure 2).

Table 1. Doctors' Opinion about the Excessive Frequency of Oral Corticosteroid Use in Asthma Patients

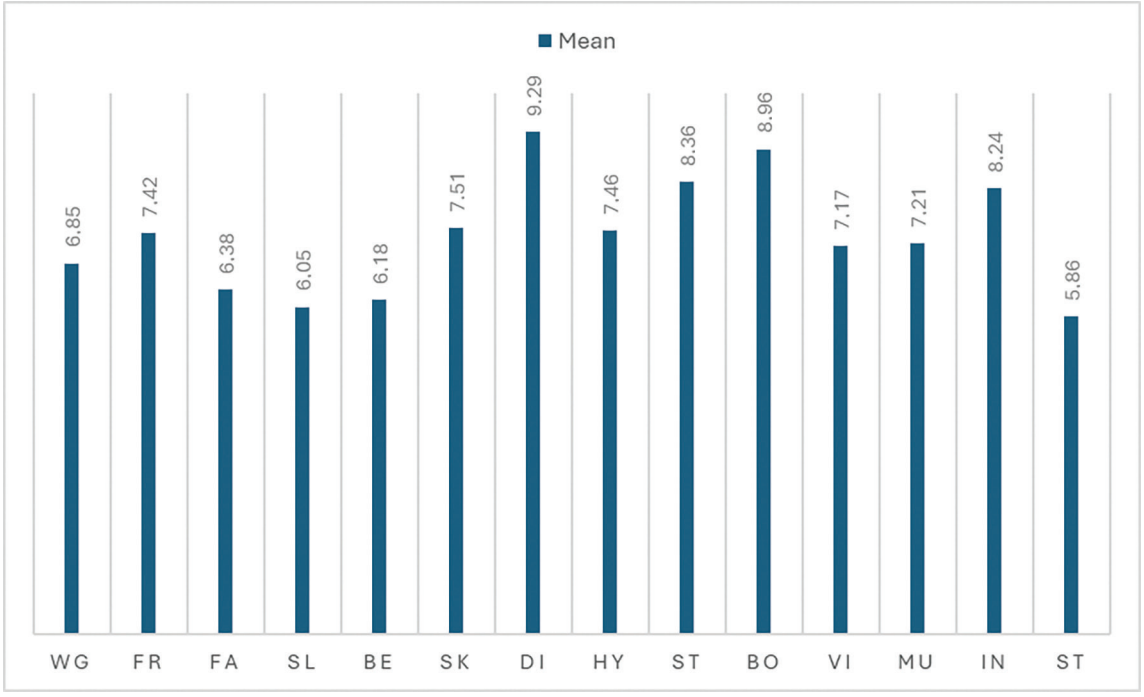
Characteristics of responding doctors	1 a year (N; %)	2 to 3 times a year (N; %)	4 times a year (N; %)	Other (N; %)	Total (N; %)
Gender					
Male	21 (63.6)	10 (30.3)	2 (6.1)	0 (0.0)	33 (100.0)
Female	34 (38.2)*	46 (51.7)	5 (5.6)	4 (4.5)	89 (100.0)
Total	55 (45.1)	56 (45.9)	7 (5.7)	4 (3.3)	122 (100.0)
Age (years)					
18–30	1 (14.3)	6 (85.7)	0 (0.0)	0 (0.0)	7 (100.0)
31–50	41 (54.7)	28 (37.3)	3 (4.0)	3 (4.0)	75 (100.0)
> 51	13 (32.5)	22 (55.0)	4 (10.0)	1 (2.5)	40 (100.0)
Total	55 (45.1)	56 (45.9)	7 (5.7)	4 (3.3)	122 (100.0)

*P=0.039. Chi-square test.

Table 2. When Patients taking OCS* are Referred to a Pulmonologist

Characteristics of responding doctors	When OCS are first prescribed	If patients require OCS twice in previous year	If asthma cannot be controlled without OCS	If patients are already managed by a pulmonologist	Total
Total					
N; (%)	49 (40.2)	36 (29.5)	32 (26.2)	5 (4.1)	122 (100.0)
18–30 yrs					
N; (%)	3 (42.9)	1 (14.3)	3 (42.9)	0 (0.0)	7 (100.0)
31–50 yrs					
N; (%)	32 (42.7	23 (30.7)	18 (24.0)	2 (2.7)	75 (100.0)
> 51 yrs					
N; (%)	14 (35.0)	12 (30.0)	11 (27.5)	3 (7.5)	40 (100.0)

*OCS=Oral glucocorticoid



WG=Weight gain; FR=Fluid retention, swelling, bloating; FA=Fatigue; SL=Sleep disorders; BE=Behavioral changes, excessive irritability, agitation; SK=Thinning skin, skin problems; DI=Impact on diabetes control; HY=Hypertension; ST=Stomach problems, indigestion, pain, ulcers, gastritis; BO=Weak bones, osteoporosis; VI=Visual problems, cataract; MU=Muscle weakness; IM=Suppressed immune system, increased risk of infection; SR=Increased risk of stroke, heart attack.

Figure 2. Family physicians' association of specific adverse effects with Oral Corticosteroids.

Discussion

According to the Medical Chamber of Slovenia, 1,295 family physicians are integrated into the public healthcare system in Slovenia, 983 of whom work at health centers. Our study included 9.4% of all family physicians. The response rate was good: 85%. Only a small share of physicians reported having had no experience prescribing OCS. On the basis of the recommendations, the use of OCS is limited to 3 to 5 days in the event of asthma exacerbation, and the following patients should be referred to a pulmonologist: patients with asthma

that cannot be controlled by inhaled therapy; patients that have visited the ER, been hospitalized, or been prescribed OCS at least once over the previous year due to exacerbations; and patients that have been prescribed more than three packs of a reliever over the previous year (1, 3). In Slovenia, methylprednisolone is only available in packs of 20-50 tablets, and as a result most physicians prescribe a limited number of tablets to avoid uncontrolled use. The study showed a good awareness of the OCS burden in treating asthma. Practically all physicians reported that during appointments they check whether the patient has required OCS in the past, either by reviewing the patient's medical history or checking e-prescriptions. Most physicians can identify when asthma is not well controlled, and they refer the patient to a pulmonologist when OCS therapy is required for the second time; nearly half of these already make a referral when OCS is required for the first time. However, it is alarming that 5.7% of physicians only make the referral after the patient has already required OCS for the fourth time, or if the patient has been taking OCS twice a day. There is still room here to raise physicians' awareness of the OCS burden. Physicians that make a specialist referral if the asthma could not be controlled without OCS were in third place in terms of frequency.

The study did not inquire about comorbidities and potential referrals made because of them. However, it is acknowledged that these may have a significant impact on both patient management and referrals. The presence of comorbidities can complicate the diagnosis of asthma or the assessment of symptoms because they can cause respiratory symptoms. Gastroesophageal reflux disease, sinusitis, allergic rhinitis, and nasal polyposis may exacerbate asthma symptoms and contribute to some diagnostic uncertainty. The NAEPP guidelines recommend that patients with sinusitis, nasal polyps, aspergillosis, severe rhinitis, vocal cord dysfunction, gastroesophageal reflux disease, or chronic obstructive pulmonary disease be referred to a specialist (11-13).

The short-term and long-term risks associated with OCS use include: musculoskeletal, digestive, cardiovascular, endocrine, psychiatric, ocular, dermatological, and immunological side effects, and there is undisputable evidence linking long-term OCS use with infections, osteoporosis and bone fractures, cataracts, adrenal insufficiency, diabetes, and hypertension.

In addition, even episodic use of systemic corticosteroids has been proven to be associated with neurological symptoms, such as insomnia, mania, depression, anxiety, or aggressive behavior. Dyspepsia, hypertension, dyslipidemia, increased risk of infection, muscle atrophy, and increased appetite are also possible. There have also been reports of increased use of healthcare services, which may be associated with both the burden of the primary illness and the side effects of OCS. Steroid-dependent asthma patients have a shorter life expectancy and higher mortality due to cardiovascular and lung complications. In addition, they have been reported to have more ER visits and hospitalizations than non-steroid-dependent patients (14-17).

Among the risks associated with using OCS, family physicians ranked hyperglycemia and exacerbated diabetes, impact on bone density, and a suppressed immune system and increased risk of infection in the top three places. The risk of cardiovascular disease (stroke or ischemic heart disease), sleep disorders, and behavioral changes (excessive irritability or agitation) were identified as lower risks.

Here, too, there is still room for increasing family physicians' awareness of the impact of OCS use, including short-term ones. Sleep disorders and behavioral changes, which can be caused by OCS, should not be overlooked. Problems such as insomnia, mania, depression, anxiety, or aggressive behavior can have a significant impact on patients' quality of life, and so special attention should be paid to these in patient management. Moreover, OCS treatment, including short-term therapies, can cause other adverse effects, such as dyspepsia, hypertension, and increased appetite, which can lead to weight gain and metabolic disorders. Therefore, it is key for physicians and patients to monitor and carefully assess any adverse

effects of OCS together. Regular check-ups, laboratory tests, and appropriate guidance based on the patients' individual needs are vital for reducing the risks and ensuring that these medications are safe and effective.

Conclusion

On the basis of the study conducted, it may be concluded that Slovenian family physicians are well informed about the burden of OCS in treating asthma. Nearly half prescribe a limited number of OCS tablets, preventing patients from making arbitrary decisions about their use. This approach is important because it allows physicians to control the risk of any adverse effects caused by OCS, and to encourage patients to take their medications prudently and responsibly.

Physicians actively seek out patients that require OCS and refer them to a pulmonologist, most often after they require OCS for the second time. This shows that they are aware of the importance of specialist treatment, and that they strive for suitable and holistic management of asthma patients. However, a small share of physicians still believe that patients that use OCS frequently do not require a consultation with a pulmonologist. These viewpoints offer an opportunity for educating and informing physicians further about the necessity of specialist control of asthma patients, especially those with a severe phenotype. The study also showed differences in physicians' familiarity with the side effects of OCS. Most can identify the basic adverse effects, such as osteoporosis, diabetes, and increased risk of infections, but only a small share are also acquainted with more specific side effects that may affect an individual. This shows a need for regular training and updating of physicians' knowledge about safety and risk management in using OCS to treat asthma. This would improve the quality of managing asthma patients and reduce any complications associated with long-term OCS therapy.

What Is Already Known on This Topic:
Oral corticosteroids (OCS) are crucial and effective as a short course treatment for asthma exacerbations, and are sometimes used as maintenance therapy in most severe cases. However, OCS treatment episodes have been associated with an increased risk of serious adverse events. OCS are among the most common causes of adverse drug reactions, such as suppression of the hypothalamic–pituitary–adrenal (HPA) axis, osteoporosis, arterial hypertension, diabetes and increased risk of infection. Therefore, OCS should only be used with caution in acute asthma exacerbations and in patients with severe asthma who do not respond well to other therapies.

What This Study Adds:
This is the first study to investigate the use of OCS for asthma, prescribed by family physicians in Slovenia. Slovenian family physicians are well informed about the risks of OCS. They prescribe a limited number of OCS tablets and prevent patients from making their own decisions about their use. However, there is still room for increasing family physicians' awareness of the effects of OCS use and the need for regular training on safety and risk management when using OCS for asthma treatment.

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

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Appendix 1. Questionaire

Oral Glucocorticoid (OCS) Burden Awareness Questionnaire
Association of Family Medicine Doctors

Gender: M F
Age:

Region of Residence*

Statistical region	
Central Slovenia	Drava
Littoral–Inner Carniola	Mura
Coastal–Karst	Central Sava
Gorizia	Carinthia
Upper Carniola	Lower Sava
Savinja	Lower Carniola

*Mark.

Duration of Employment

1 – 5 years
5 – 10 years
10 – 15 years
> 15 years

Type of Employment Organization

Health Center
Concessionaire

Participation in on-call service: Y N
Participation in emergency medical care: Y N
Teaching at the university: Y N

1. **Have you already prescribed OCS to a patient with asthma or do you have patients with asthma in your practice who need or who ever had to introduce OCS?**
   a. Y
   b. N
2. **If yes, did you prescribe them a whole box or were they only given a limited amount of OCS tablets "to take home"?**
   a. Whole box
   b. Limited amount of tablets
3. **Do you have insight into how many times the patient received OCS (if he received the medicine in the emergency room or during hospitalization)?**
   a. Yes, always
   b. Sometimes, depends on _____
   c. No, never

4. **How do you check this?**
   a. E-prescriptions
   b. Ask patient
   c. The patient tells/brings the report himself
   d. I don't check

5. **When do you think your patient with asthma needs OCS too often?**
   a) If once per year
   b) If 2-3 times per year
   c) If > 3 times per year

6. **When do you refer such a patient to a pulmonologist?**
   a. At the first intervention with OCS
   b. If he needed OCS at least 2 times in the last year
   c. If his asthma remains unregulated without OCS

7. **To what extent do you associate certain side effects with taking OCS**
   1-not at all; 10-significant influence

Possible side effects of OCS	A single epsode of taking OCS	Occasional intake (>90 days difference between OCS interventions)	Frequent intake (<90 days difference btween OCS interventions)
	1-10	1-10	1-10
Weight gain			
Water retention/ swelling/bloating			
Fatigue			
Sleep disorder			
Behaviour change (excessive excitement, restlessness)			
Skin problems (thinning)			
Impact on diabetes control			
Hypertension			
Gastrointestinal problems (Stomach problems (indigestion, pain, ulcers, gastritis...)			
Osteoporosis			
Vision problems (glaucoma, cataract)			
Loss of muscules			
Reduced immune response/risk of infections			
Risk of stroke/heart attack			

8. **What is your level of satisfaction with the use of OCS in patients with asthma?**

Degree of Satisfaction	Don't konw	0	1	2	3	4	5	6	7	8	9	10
Efficiency	○	○	○	○	○	○	○	○	○	○	○	○
Easy to take	○	○	○	○	○	○	○	○	○	○	○	○
Overall satisfaction	○	○	○	○	○	○	○	○	○	○	○	○

0=Unsatisfied; 10=Very satisfied;OCS=Oral glucocorticoid.

Original Research Study
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Academician Milivoje Sarvan: The Founder of Modern Paediatrics in Bosnia and Herzegovina

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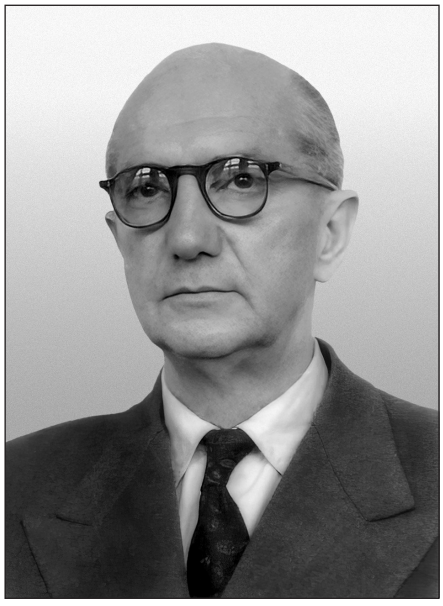
Abstract
The aim of the article is to present to the medical, and then to the general public, the person and work of Milivoje Sarvan (1896–1978)—one of the pioneers of social paediatrics in Serbia and one of the most prominent paediatricians, scientists and organizers of health services in Bosnia and Herzegovina in the second half of the 20th century. Milivoje Sarvan was born in 1896 in Požega, in the Kingdom of Serbia. He completed his medical studies in Lyon (France) in 1921. Upon his return to Serbia, he was a county physician in Aleksinac for three years and, shortly after the establishment of the University Children's Hospital in Belgrade in 1924, he was among the first assistants employed there. Out of the total of 23 years of professional work in Serbia, for 19 years he was an assistant and assistant professor at the Faculty of Medicine in Belgrade. At the end of 1946, at the initiative of the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina, Dr. Milivoje Sarvan was appointed full professor and head of the Department of Paediatrics at the newly established Faculty of Medicine in Sarajevo. At the same time, he was elected head of the Paediatric Clinic in Sarajevo when it was established, and he would later manage it from 1947 until his retirement in 1967. Already at the beginning, Prof. Sarvan developed the activities of the Clinic in several directions: he created the conditions for clinical, teaching and scientific research work. He took care of the education of future paediatricians and child care workers, organized courses in social paediatrics for general practitioners and professional training for paediatricians in the country and abroad. The next period of his activities was marked by the intensive development of the Clinic in all areas of its work. He published more than 120 professional and scientific papers in national and foreign medical journals, and several health education books on mother and child care that have been published in several editions, with large print runs. He was the dean of the Faculty of Medicine and vice-rector of the University of Sarajevo, founder of the Paediatric Section of the Society of Physicians of Bosnia and Herzegovina, lifetime president of the Association of Paediatricians of Yugoslavia, a member of the Scientific Society of Bosnia and Herzegovina from 1955 and the Academy of Sciences and Arts of Bosnia and Herzegovina from its foundation in 1966. He was honoured with high level social awards and recognitions, including the highest state award of the Socialist Federative Republic of Yugoslavia—the AVNOJ award. After his retirement (1967), he lived in Belgrade, where he died in 1978. **Conclusion**. Bearing all of this in mind, there is no doubt that Dr. Milivoje Sarvan is one of the significant figures in the field of professional, scientific and organizational work in the field of children's health care in the former Yugoslavia, leaving a significant and indelible mark in the current states of Serbia and Bosnia and Herzegovina.

Key Words: Milivoje Sarvan ▪ Serbia ▪ Bosnia and Herzegovina ▪ Paediatrics.

Introduction

Milivoje Sarvan was one of the pioneers of social paediatrics in the Kingdom of Yugoslavia (1) and one of the most important paediatricians, scientists and organizers of health services in Bosnia and Herzegovina in the second half of the 20th century (2). Although he worked in Serbia for 23 years and was an assistant and assistant professor at the Faculty of Medicine in Belgrade (3), and a well-known health educator, his name is almost never mentioned in contemporary Serbian medical historiography.

The memory of Sarvan is much better preserved among the Bosnian paediatricians of earlier generations (4, 5). Moreover, in 1979, his heirs



Milivoje Sarvan in the mid-1960. Dossier of Milivoje Sarvan, with permission of the Academy of Sciences and Arts of Bosnia and Herzegovina.

unveiled his bust in the hall of the Paediatric Clinic in Sarajevo, which on that occasion also bore his name, as a sign of permanent remembrance and acknowledgment of his work. Under the name the "Prof. Dr. Milivoje Sarvan Clinic and Polyclinic for Children's Diseases" (6), the Clinic operated until the breakup of Yugoslavia in 1992.

Since the professional and scientific contributions of Milivoje Sarvan have not been fully evaluated, we prepared this article with the aim of presenting his person and contributions, primarily to younger colleagues, but also to a wider public.

Youth and Education

Milivoje Sarvan was born on September 23, 1896 in Požega near Užice, in the Kingdom of Serbia[1] (7). He was the first of the three children born to the couple Teofilo and Jovanka Sarvan born Čkonjović. His father, a merchant by profession, was one of the most respected persons in his area—he was a member of the Board of Directors of the Požega branch of the Serbian Agricultural Society, and he was also involved in politics. The scarce historiographical data available indicate his connections with Nikola Pašić in 1886 (8).[2]

Milivoje probably attended primary school in Požega, and secondary school in Čačak and Valjevo. At that time, the secondary school in Čačak was incomplete, with only six grades, but was considered a very good school. As in other schools, great attention was paid to the upbringing and development of national feelings among students, especially after the Austro-Hungarian annexation of Bosnia and Herzegovina (9).[3] Through the students' literary club "Rajić", which also had its own reading room, the love for literature was nurtured, so the names of the students, including Milivoje's, are on the lists of subscribers to the editions of the Serbian Literary Association (10).[4] After completing the fifth grade in 1912, Milivoje transferred to the secondary school in Valjevo, which became a complete eight-grade high school that year. This meant that students could take the matriculation exam, which was a requirement for admission to university. Although during the 1912/13 school year classes were interrupted for a few months due to the First Balkan War, students were allowed to finish their classes, so Milivoje also finished the sixth, then the seventh grade (1913/14), both with excellent grades (11).[5] He did not have time to enrol in the eighth grade because the First World War broke out in July 1914, which again interrupted the education of Serbian youth. At the beginning of October 1915, on the eve of the invasion of Serbia by the Central Powers, Sarvan and his peers were recruited into the army which began to retreat through Albania, towards Greece. When, in May 1916, the Ministry of Military Affairs made a decision to discharge pupils and students from the army in order to continue their education, he was in a group that set off for France the following month. In Voreppe, a small town near Grenoble, he attended the matriculation course and passed the matriculation exam in November 1916, and then enrolled at the Faculty of Medicine and Pharmacy in Lyon. He completed his studies within the prescribed period and on December 20, 1921, he received his doctorate with the thesis "Les lesions pleuropulmonaires de la maladie de Hodgkin"(12).[6]

The First Years of Medical Practice

In January 1922, after five and a half years spent in France, Sarvan arrived in Belgrade, then already the capital of the new state, the Kingdom of Serbs, Croats and Slovenes (Kingdom of SCS). At first, he served in the Permanent Military Hospital for two months, the time he had left of his regular military service, and then completed the mandatory medical internship at the General State Hospital. At the same time, he also volunteered at the Permanent Bacteriological Station. As he had also completed a six-month course in hygiene at the University of Lyon, it seems that he intended to devote himself to the then most promising branch of medicine—preventive medicine (13).[7] Considerable financial resources were invested in the development of the Preventive Medicine Service, which was managed by Andrija Štampar[8] the head of the Hygiene Department of the Ministry of Public Health and the creator of the health policy of the Kingdom of SCS. The priority given to the development of preventive medical institutions compared to hospital ones led to opposition and division within the medical class, but it did not hinder their progress. However, Sarvan soon decided on a different path: in February 1923, he requested permission from the Ministry of Public Health to start a specialization in internal medicine at the General State Hospital (14).[9] His request was not respected: young doctors were mostly distributed throughout the interior of the country, where they were few in number. Thus, on March 20, Sarvan was appointed county doctor in Aleksinac (15).[10] He spent the next three years in that small town in the southeast of Serbia, which was once an important border town, but it had lost its former importance after the territorial expansion of Serbia (1878). In addition to his regular duties, he performed three other jobs: he was acting head of the county hospital and actually the only hospital doctor (1923), he taught Hygiene in Aleksinac Secondary school and also acted as a school doctor (1923/24 and 1924/25) (16).[11] As can be seen from the elder's report, he performed his duties diligently—it was assessed that he was "professionally well educated, reliable, orderly in his service and of good behaviour" (17),[12] but he himself was not satisfied with his position and life in Aleksinac. He aspired to move to Belgrade, where his family lived at the time, and already in November 1923 he tried again to attain the position of a resident at the General State Hospital, this time in the Children's Department (18).[13] This request was not accepted either, but two years later, when he applied for the position of assistant at the University Children's Hospital of the Faculty of Medicine, he was more fortunate: he was appointed assistant on August 24, 1925 (19).[14]

Assistant and Assistant Professor at the Faculty of Medicine in Belgrade

The University Children's Hospital in Belgrade was founded on September 29, 1924, with the appointment of Franciszek Groër (1887–1965) as a contractual full professor of paediatrics at the Faculty of Medicine (20).[15] Groër, who from 1918 was a full professor of paediatrics in Lviv (then in Poland, now in Ukraine), accepted the invitation of the Faculty of Medicine in Belgrade under one condition: that the construction of the building for the Children's Hospital should begin in the spring of 1925. In addition to the lectures he gave to the first generation of students at the Faculty, immediately after his arrival he devoted himself to organizing and equipping the Hospital, which began operating on January 23, 1925, in temporary accommodation, in a rented apartment, as a Polyclinic (without inpatients). With the admission of young doctors and auxiliary staff, Groër formed the first working team. At the same time, in cooperation with the architect Svetozar Jovanović, he prepared the project of the future hospital building. However, since money and land for the building were not secured in the spring of 1925, he resigned and returned to Lviv in October of the same year (21). Although they only worked together for a month, Sarvan remembered Groër vividly even after 50 years. In the chapter he wrote about Groër's work in Belgrade in the memorial book entitled *Franciszek Groër: life and work*, published in 1973 by the Association of Paediatricians in Poland, he marked him as his first teacher (22).[16]

After the departure of Professor Groër, there were four assistants employed at the Children's Hospital: Smilja Kostić-Joksić,[17] Marija Gajić-Vajs,[18] Ljubomir Vulović[19] and Milivoje Sarvan. Although he was the last among them to be hired, Sarvan was entrusted with the management of the Children's Hospital until the appointment of a new professor. Only a year later, at the end of 1926, with the appointment of Dr. Matija Ambrožić[20] as associate professor of paediatrics, the Children's Hospital had a new head. Ambrožić came from Slovenia, where in previous years he had distinguished himself with extraordinary entrepreneurship and organizational skills: in Ljubljana he founded the Institute for Social and Hygienic Protection of Children with the Queen Marija Children's and Maternity Home, and the first School for Child Caregivers in the Kingdom. Like Groër, he was a student of the famous Viennese paediatrician Clemens von Pirquet (1874–1929) and a follower of the modern paediatric school that combined social protection of children, preventive and curative paediatrics. On this basis, he and his assistants organized work at the Children's Hospital.

In the legacy of Matija Ambrožić kept in the Museum of Science and Technology - Belgrade, there are ten letters from Milivoje Sarvan—seven from 1926 and three from 1930. From them it can be seen that they established contact while Ambrožić was in Ljubljana and was waiting for the completion of his procedure appointments. Sarvan informed him about the situation at the Children's Hospital, about the talks he had with the dean of the Faculty about the Children's Hospital budget, and about the course of negotiations with the management of the "Serbian Mother" Society about the establishment of a kindergarten, in which Štampar also participated, etc. In the summer of 1926, Sarvan travelled to Ljubljana to learn about the organization and work of the Ambrožić Institute, which, as he wrote, *fascinated him* (23).[21] Not only a good professional relationship, but also a lasting friendship was established between them. In the years that followed, Sarvan was Ambrožić's closest collaborator, and after his departure to Sarajevo in 1946, their close cooperation continued.

Two years after his arrival in Belgrade, Ambrožić managed to secure new accommodation for the Children's Hospital, in which a department for bed-ridden patients was also organized. The Institute for the Health Protection of Mothers and Children worked under the same roof, also under his management. Ambrožić formed the Working Community of the Children's Clinic, which included clinical departments and the Institute (with departments: the children's dispensary with a Counselling Centre for infants and toddlers; colonies for infants and young children without parental care, the Department for the Popularization of Children's Hygiene, and the Department of Records of Work in the field of child protection), the Centre for Infants of the Mothers' Association, the Children's Dispensary of the "Serbian Mother" humanitarian society, the municipal

Prof. Dr. Matija Ambrožić (in the middle) with the doctors of the University Children's Hospital, in Belgrade in the early 1930s. Dr. Milivoje Sarvan is marked with an arrow. From the Archives of the University Children's Hospital in Belgrade. Courtesy of Prof. Dr. Mirjana Kostić.

counselling centre for infants and small children and the Neonatal Department of the Gynaecology and Obstetric Clinic. In addition to participating in teaching, the assistants of the Children's Hospital were also assigned to various duties in the institutions of the Working Community. So Milivoje Sarvan also worked in the Kindergarten of the "Serbian Mother" Society (1916–1928), and in the Colony for infants and young children (1931–1933); he collaborated with the organization of the Museum of Hygiene of Infants and Young Children at the Institute and in the Seminar for trainee doctors, and in 1933 he was appointed head of the Municipal Counselling Centre for Infants and Young Children (24).[22] In the meantime, he obtained the title of specialist in children's diseases (1927) and for a little longer than a year, between September 1929 and December 1930, he trained in Germany as a scholarship holder of the Rockefeller Foundation (25).[23]

Being a French student, Sarvan was particularly satisfied that the scholarship allowed him to get to know the German paediatric school and to learn from the most important German paediatricians of the time. First, he spent two months in paediatric institutions in Berlin: at the University Children's Hospital of Professor Czerny (Adalbert Marianus Czerny, 1863–1941), whose school primarily dealt with the physiology of nutrition and metabolic disorders; then in the the Kaiserin Auguste Victoria Haus children's hospital, where under the direction of Fritz Rott (1878–1959), professor of social medicine, and Doctor Leopold Langstein (1876–1933), he studied issues of social hygiene organization and health care for mothers and children; and also in the Kaiser-und-Kaiserin-Friedrich-Kinderkrankenhaus hospital under the direction of Heinrich Finkelstein (1865–1942), who especially dealt with the problems of infant care and nutrition (26).[24] He spent the next year in Düsseldorf, at the Children's Clinic of the Medical Academy, with Professor Arthur Schlossmann (1867–1932), a pioneer in the field of social welfare for children (27).[25] In addition to daily work in the hospital departments, the practical part of the training also included laboratory work, study of autopsy material and work on the organization and implementation of the health care system for children and youth in the Düsseldorf Health Administration. The theoretical part consisted of lectures on paediatrics by Professors Schlossmann, Albert Eckstein (1891–1950) and Adolf Hottinger (1897–1975), and lectures on social medicine by Professors Ludwig Teleky (1872–1957) and Ernst Graf (1869–1938) (28).[26] The issues that Sarvan particularly studied during that time were early mortality, prematurity, tuberculosis therapy, post-vaccination encephalitis, organization of social protection for children, and health education for parents (29).[27] He also engaged in experimental work in the field of tuberculosis and BCG vaccination. Between two semesters, during the summer vacation, he visited child protection institutions in Belgium, the Netherlands and France. In a letter to Ambrožić he wrote some interesting observations: "In terms of work in the purely social and hygienic field [the French are], in some matters, always one quarter of century behind the Germans; in some cases there is something interesting (*Placement familial de tout petits*), which cannot be seen with the Germans".

After returning from Germany, in addition to his regular duties at the Hospital and in social-medical institutions, Sarvan dedicated himself to scientific and health education work. However, as new teaching positions at the University were opened in limited numbers due to the economic crisis in the country, he waited for a promotion to an academic title for 14 years. From 1937, the Department for Children's Diseases had two courses: Pathology and Therapy of Children's Diseases and Hygiene in Childhood, but new teachers were not appointed until December 1939, both to the position of assistant professor—Sarvan for the subject of Hygiene in Childhood[28] and Smilja Kostić-Joksić for the subject Pathology and Therapy of Children's Diseases (30). A few months earlier, assistant professor Uroš Ružičić[29] was appointed associate professor. As the teacher of the newly introduced subject, Milivoje Sarvan had the opportunity to teach students in the areas he was dedicated to during his professional career. However, that did not last long. In April 1941, the

Doctors of the Children's Hospital with members of the administration of the Mothers' Association, 1930s. Dr. Milivoje Sarvan is marked with an arrow, next to him stands Prof. Dr. Matija Ambrožić, and the second on the right is Prof. Dr. Uroš Ružičić. From the Archives of the University Children's Hospital in Belgrade. Courtesy of Prof. Dr. Mirjana Kostić.

Second World War engulfed and dismembered Yugoslavia.

From the beginning of the occupation of Serbia, the Germans, in cooperation with the local collaborationist administration, persecuted communists, freemasons, Jews and opponents of the new regime. The repressive measures were especially intensified after the Uprising of July 7, 1941. In order to intimidate the population, and also due to the suspicion of the existence of connections between members of the Belgrade intelligentsia and the insurgents, in November 1941, the Germans arrested about 200 public and cultural workers, university professors, doctors, lawyers, industrialists and other prominent figures. As "hostages who will guarantee security on Serbian territory with their lives" (31),[30] the arrested were imprisoned in the Banjica concentration camp until the Uprising was suppressed. Among them were professors of paediatrics—Matija Ambrožić, Uroš Ružičić and Milivoje Sarvan. Ambrožić was imprisoned for the longest time, a full two months; Sarvan was first in prison, and then for a week in the concentration camp. Both of them were released on January 5, 1942 (32).[31] Professor Ružičić was imprisoned in the Banjica concentration camp for ten days during November 1941. After the liberation, they continued working at the Clinic, but after one year, their retirements followed. Sarvan retired in December 1942, Ambrožić in January 1943, and Ružićić in June 1943. Until the end of the war, Sarvan worked as a private physician in Belgrade. After the liberation of Belgrade, Ambrožić, Sarvan and Ružičić were reactivated and returned to their positions and duties already in November 1944. Until his appointment to a new position in 1946, Sarvan held the position of head of the Polyclinic Hospital.

Professor of Paediatrics and Head of Department of Paediatrics and the Paediatric Clinic in Sarajevo

On the initiative of the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina (PRB&H), assistant professor Milivoje Sarvan (30) was appointed full professor of paediatrics and head of the Department of Paediatrics at the newly established Faculty of Medicine in Sarajevo at the end of 1946 (33). At the same time, he was appointed as the head of the Paediatric Clinic in Sarajevo, which was being founded at that time (6). He took office after June 17, 1947. He was expected to participate in the broadest sense in the development of the newly opened Faculty of Medicine, especially in the establishment of the Department of Paediatrics and the Paediatrics Clinic in Sarajevo. At that time, there were no children's hospitals in Bosnia and Herzegovina (B&H). In Sarajevo, as part of the State Hospital in Koševo, there was a the Unit for Children's Diseases (VI Department)[32] (34, 35), which was formed after the First World War in a pavilion previously intended for patients suffering from mental illnesses. The head of the department was Prim. Dr. Konstantin Delijanis,[33]

who initially worked alone, with occasional help of medical doctors doing internships. Later, Dr. Jelena Bulić-Adamović worked at the Department,[34] as a specialist in children's diseases. The Second World War founds both these doctors doing the same work (35).

Unlike hospital health care, primary health care for children in B&H was somewhat organized. Immediately after the end of the Second World War, the work of the Institute for Maternal and Child Health Care was activated in Sarajevo, where the paediatrician Dr. Maša Živanović,[35] and Dr. Mara Kurtović,[36] a specialist in school medicine (36) worked. Dr. Kornelija Rakić worked in Mostar, in the Children's dispensary[37] (37), while in other parts of B&H, the primary health care of children was at a very low level in the Public health centres, in which mostly general practitioners worked.

Establishment of the Paediatric Clinic in Sarajevo

After the Second World War, Unit for Children's Diseases of the State Hospital in Sarajevo became the "core" of the future Paediatric Clinic of the Faculty of Medicine in Sarajevo (34). However, it was not possible in terms of space and personnel to meet the professional, teaching and scientific needs of the clinic (6). On the other hand, the severe economic crisis did not allow the construction of a dedicated building, but the decision of the competent authorities was accepted that the existing general hospital of the Railroad Sickness Foundation, newly built before the Second World War on "Jezero", and originally intended for the treatment of adults, be used for the work of the Paediatric Clinic in Sarajevo (38).

Adaptation work[38] started at the end of 1946, and already in the middle of 1947, the Children's Department of the State Hospital was moved to the building of the new clinic, with a capacity of 60 beds, with patients and two doctors: one paediatrician[39] and one doctor on specialization, and several nurses, one trained laboratory assistant, scant equipment and a modest laboratory (34, 38). During the following year, 1948, the young doctors: Dr. Mimi Sejdi, Dr. Katarina Carić[40], Dr. Nevena Stojkov[41], Dr. Suzana Čanji[42], Dr. Žarko Mićić[43] and Dr. Nina Hadžiselimović[44] as residents employed at the Paediatric Clinic in Sarajevo (34).

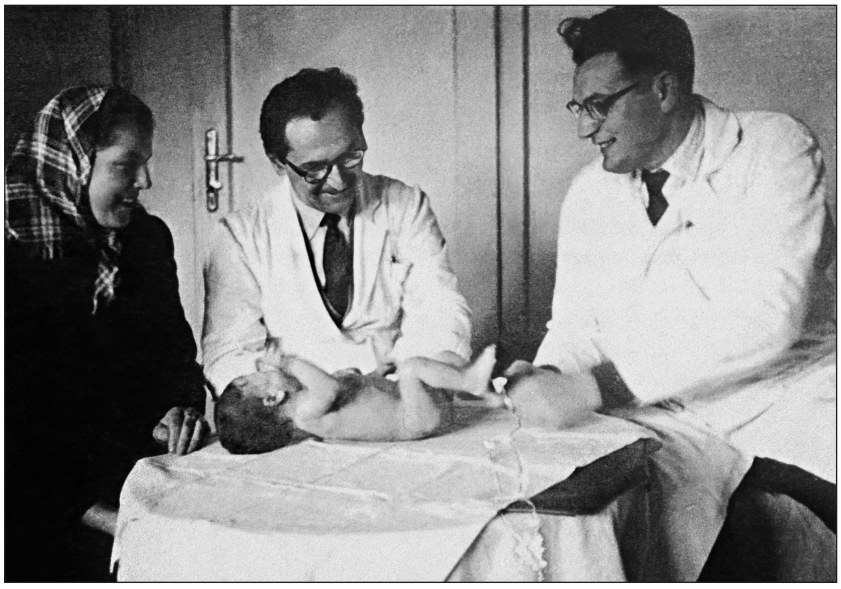
Later Dr. Borivoj Ćurčić[45] in 1951, Dr. Vera Golubović Ćurčić[46] and Dr. Džemal Haverić[47] in 1952, and other doctors were employed at the Clinic for children's diseases.

The first doctors of the Paediatric Clinic in Sarajevo: Prof. Dr. Milivoje Sarvan (in the middle), first from the left, Dr. Nevena Stojkov, and the first right, Dr. Žarko Mićić. From the Archives of the Family of Nevena Stojkov. Courtesy of Prof. Dr. Borislav Stojkov.

Along with the initial solution of pressing spatial problems, Prof. Sarvan gradually solved personnel problems, reflected not only in the lack of doctors, but also of auxiliary medical staff. He saw a long-term solution to the lack of doctors in young doctors graduating from the Faculties of Medicine in Sarajevo, Belgrade and Zagreb who showed an interest in working at the Paediatric Clinic in Sarajevo. In the first five years of its existence, the Clinic had nine doctors, some of whom were paediatricians or doctors

on specialization. Two thirds of them completed their medical studies in Belgrade (39).

The auxiliary medical staff initially consisted of nuns and women who had taken only short courses, which did not meet the needs of the institution (39). Knowing the importance of their expertise and work in paediatric institutions, Prof. Sarvan, after convincing the authorities about the solution to this burning problem, founded the School for Child Caregivers at the Clinic. The first generations of trained caregivers played a significant role in the care of sick children not only at the Clinic, but also in other paediatric institutions in Bosnia and Herzegovina (39, 2).



At the beginning of 1953, assistant professor Dr. Dimitrije Miletić, paediatrician from Paediatric Clinic in Sarajevo at the Dispensary for Children's Diseases in Kasapovići (Novi Travnik B&H).

A relatively favourable solution to the Clinic's accommodation and staffing issues enabled reliable professional and scientific treatment of the pathological conditions of hospitalized patients (39). With this in mind, the management of the Clinic, together with the employees, worked hard to improve the work space, hiring a larger number of educated specialist laboratory staff, acquiring modern equipment, and modernizing existing and introducing new diagnostic procedures. These changes made it possible to begin scientific research into children's pathological issues in Bosnia and Herzegovina (39) in addition to routine examinations and treatment of patients at the Clinic (39). It is interesting to note that as early as 1950, the Federation of Medical Societies of Yugoslavia recognized the successful development of the Clinic in all its aspects and as a result decided that the Paediatric Clinic in Sarajevo would be the organizer of the First Congress of Paediatricians of Yugoslavia.[48]

The concepts applied in the work of the Clinic very quickly proved to be successful in completing the tasks set. However, this was only a partial solution to the pressing problems in children's health care in B&H (39). Namely, Prof. Sarvan very quickly saw that it was necessary for the professional staff of the Clinic, with him at the head, to be involved in solving children's health problems throughout the whole of B&H. He initially tried to solve the problems he perceived with advice, by sending requests to the competent authorities of the Republic to which he insisted that health institutions from the interior of B&H should refer their doctors, as scholarship holders, to courses for specialization in paediatrics.

At the same time, the Clinic's doctors were occasionally sent to the surrounding municipalities—Kalinovnik, Kiseljak, Kakanj, Sokolac, and Novi Travnik—to work in paediatric clinics, counselling centres, and dispensaries. In addition, the doctors from the Clinic, headed by their director, took on some of the responsibilities that arose after the Centre for Health Care of Mothers and Children was unjustifiably abolished: providing advisory, professional, and methodological assistance to regional health care centres in Banja Luka, Brčko, Tuzla, Livno, Mostar, Foča, Zenica, and Bihać (39). This type of activity was especially significant when children's hospital wards or children's hospitals were being opened in these places.

Organization of Postgraduate Paediatrics Courses

One of the most significant visions of Prof. Sarvan in solving the problems of child health care overall in Bosnia and Herzegovina was the strengthening of paediatric dispensares and hospital work in Bosnia and Herzegovina through the organization of various types of postgraduate paediatrics training. Amongst these activities, a significant place is occupied by the organization of Paediatric Days of B&H (PDB&H) in cooperation with the Paediatric Section of the Society of Physicians of B&H.

The idea of Prof. Sarvan was that the PDB&H should be held every year, except for the year in which the congresses of the paediatricians of Yugoslavia were held, and that at those gatherings achievements in the development of health care for children in B&H Yugoslavia should be presented (34). The PDB&H were not intended only for paediatricians but also for other doctors who dealt with the treatment of children within their specialties. The gatherings were held in the cities of B&H, which were seen as the centres of children's health care of a certain region, with the aim that paediatricians and other doctors who dealt with the treatment of children in that area would present current paediatric issues and so that the participants would get to know each other, exchange experiences, and gain new knowledge. So, if these gatherings were started to educate doctors dealing with the treatment of children in Bosnia and Herzegovina, they quickly became interesting to paediatricians throughout Yugoslavia, of which B&H was a part at that time. They were not only members of the audience at PDB&H, but active participants, and they often presented the results of their own research. Prof. Sarvan was the organizer of and an active participant in PDB&H until his retirement in 1967.

According to Prof. Sarajlić, the first PDB&H was held in Sarajevo in 1959. However, when reading today the contents of the lectures that were published in the Almanac marking 25 years of the Paediatric Clinic in Sarajevo (34), it is easy to see that the lecturers were from the former Yugoslav Republics (Serbia, Croatia, Slovenia), but also from European countries (England, Switzerland, and Poland); their titles, functions, and lecture titles are listed, but the location and date of the meetings are not mentioned in detail.

Twenty years later, Professor Rikica Najdanović, one of the closest and longest-term co-workers of Prof. Sarvan, wrote about that gathering: "In 1959, Prof. Sarvan organized the first paediatric days, which he left his stamp on from the beginning by choosing the topics of the most current problems and gathering eminent paediatricians from the country and abroad as guest lecturers. As a result, the Paediatric Days immediately gained a high reputation because they represented a great contribution to the development of paediatric thought in our country, to the modernization of paediatric knowledge, and also to the mutual acquaintance of doctors who work with children in our Republic and with doctors from abroad" (4).

Prof. Sarvan saw the solution to the problem of the lack of primary health care paediatricians in B&H in the organization of postgraduate classes in paediatrics for general practice doctors from the interior of B&H, that is, the training of doctors from smaller towns to work in children's counselling centres and dispensaries. This education started in 1964/65 in the form of four-month courses in Social Paediatrics, which were realized through theoretical and practical classes. Courses were held once a year in December, January, February, and March.[49] (34).

The theoretical classes were held by the teachers of the Clinic, and for the part of the programme related to related paediatric disciplines, teachers from other departments of the Faculty of Medicine and the University of Sarajevo were engaged, as well as prominent experts in the field of health and social legislation in B&H (6, 34). The practical teaching of the course participants was realized through daily work in the clinic's departments and afternoon duty with the clinic's senior doctors.

After the completion of the course, study trips were organized in the country and abroad with the aim of making the participants familiar with the

work of reputable institutions for the protection of mothers and children (40). In the 1970/71 academic year this type of education was abolished because there was no longer a need for this type of professional staff. A total of seven courses were held. Eighty-seven general practitioners completed this type of education. Most of them worked as doctors qualified to work in children's dispensaries in municipal towns in B&H, and some continued their professional activity in other medical disciplines (34).

An interesting observation about this type of education was written by the primarius Dr. Esad Zukić[50], a paediatrician from Gradačac, Bosnia and Herzegovina, who attended the course in the 1965/66 academic year: "The doctors learned so much on the course that they were able to do a great deal in the children's dispensaries. They learned to spot current paediatric problems in a timely manner and to solve them at the level of primary health care. In addition, they were aware of the necessity of continuous professional development because they knew that the time spent on the course and the subsequent six-month work in a children's dispensary would be recognized in a future paediatric residency" (Written announcement. August 10, 2024).

Professor of the Faculty of Medicine, University of Sarajevo (1946 – 1967)

By being appointed head of the Department of Paediatrics immediately after the founding of the Faculty of Medicine in Sarajevo (1946), Milivoje Sarvan was given enough time to make preparations for classes in the subject of Paediatrics, which, for the first generation of students, were not supposed to start until the autumn of 1949. This concept was also applied at other clinics and institutes in order to create at least the basic conditions for the start of classes in terms of personnel, space, and equipment provided for in the study curriculum (6).

In the autumn of 1949, the Paediatric Clinic was ready to start working with students. Prof. Sarvan held an introductory lecture for fourth-year students in an improvised lecture hall on the ground floor of the Clinic's main building (6). At that time, Dr. Jordan Tomić was working at the Department of Paediatrics as an assistant professor.[51]

Practical work with students was initially the task of all Clinic doctors, until the first assistants were chosen: Dr. Nevena Stojkov[52], Dr. Suzana Čanji[53] and Dr. Irina Tomić[54] (34). Student exercises were organized in the afternoons in a very pleasant working atmosphere in which students helped in the care of patients and the application of therapy (34). The admission of the first generation of students to the Paediatric Clinic did not mean that the issue of the formation of the Department of Paediatrics was resolved. On the contrary, training the medical staff to work with students continued with the same intensity, because of the expected increase in the number of students in the coming years, which inevitably implied an increase in auxiliary teaching staff. In the selection of assistants, preference was given to younger staff, including those who are still specializing in paediatrics, in order to provide staff for conducting practical exercises (34).

Training assistant teaching staff in the conditions in which the Paediatric Clinic operated at the time was not an easy task. In addition to the complex and extensive professional work performed by the Clinic's doctors, which by its nature always had to have priority, they were expected to continuously improve their professional skills, to educate intermediate medical staff and doctors on specialization courses, to engage in scientific research work, and to present their results at professional conferences and publish them in journals. After the first few publications in the first years of the Clinic's existence, the number of professional and scientific works in the following period grew year by year, and its associates presented the results of their own research and observations at congresses and symposiums in the country and abroad (6). In the first ten years (1947–1956) of the Clinic's existence, according to the "Bibliography of the Works of Associates of the Children's Clinic Sarajevo" (40), 32 articles were published, mostly in national

Prof. Milivoje Sarvan, the dean of the Faculty of Medicine in Sarajevo, with his colleagues. (From the Archives of the Family of Mirjana Džumhur. Courtesy of Dr. Sead Džumhur).

periodical medical publications. Of that number, Prof. Sarvan published 19, or 60% of the articles, while all his collaborators together published a total of 13 or 40% of the articles.

Although the selection of assistants began as early as the arrival of the first students at the Paediatric Clinic, the selection for teaching positions did not begin until 1958 (34). This is understandable, given that the criteria for these titles were significantly different from today's and, moreover, it was a period when spatial and personnel issues were being resolved simultaneously. Only in the following ten years of work at the Clinic, which was still managed by Prof. Sarvan, was there an obvious improvement in its professional and scientific work, which , among other things, resulted in the selection of doctors for teaching positions. Dr. Dimitrije Miletić[55] was the first to be promoted to the position of assistant professor, then Dr. Izet Hadžić[56], Dr. Ešref Sarajlić[57], Dr. Borivoj Ćurčić[58], and Dr. Izet Čustović[59] Prof. Sarvan produced teaching positions. And not only that, after retiring, as emeritus professor, he proposed the promotion of the most capable doctors and the promotion of three assistant professors to the title of associate professor, which was quickly implemented.

Work at the Medical Faculty and University in Sarajevo

In addition to being the head of the Department of Paediatrics, Prof. Sarvan had other responsibilities at the Faculty of Medicine and the University of Sarajevo. In the academic years 1949/50 and 1955/56, he was vice dean, in 1951/52, and 1954/55, dean, (41), and in 1950/51, vice chancellor of the University of Sarajevo (42). In those periods, these institutions were still at the beginning of their work, which was a good opportunity for the extraordinary organizational and pedagogical abilities of Prof. Sarvan to come to full expression. His work enabled their further successful development.

Academician of the Academy of Sciences and Arts of Bosnia and Herzegovina

At the recommendation of Prof. Dr. Nedo Zec[60] and Prof. Dr. Pavao Štern,[61] on November 12, 1955, Professor Sarvan was appointed a full member of the Scientific Society of Bosnia and Herzegovina[62] (SSB&H) in the Department of Medical Sciences (DMS) (43). He gave the introductory lecture "Evolution of the concept of health and social care for children" on October 31, 1958 (44). At the end of 1959, he was appointed secretary of the DMS, replacing the deceased Blagoje Kovačević (45). In the period from 1959 to 1963, he was also a member of the Presidency and the Supervisory Board of the SSB&H (45). After he moved to Belgrade in 1969, he was no longer a member of the staff of Academy of Sciences and Arts of Bosnia and Herzegovina (ASAB&H) (46).

Admission to the SSB&H was a recognition for his overall professional and scientific

contribution to the development of contemporary paediatric thought in the former Yugoslavia (47). Membership in the SSB&H provided him with wider opportunities for scientific activity and thus also for his co-workers. Very quickly, his scientific, organizational, and management skills were noticed in this institution as well. His scientific and research work was always based on projects previously completed in cooperation with junior doctors of the Clinic for Children's Diseases and the Institute of the Faculty of Medicine, and sometimes with doctors of the Institute of Hygiene in Sarajevo (48). He mainly presented the results obtained and new knowledge acquired resulting from his research, at medical meetings in the country and abroad, and published them together with other project research participants in national and foreign medical publications.

For young doctors, his professional co-workers at the Paediatric Clinic in Sarajevo, this method of research work had multiple significance. In addition to gaining new knowledge, they had the opportunity to participate in the scientific research process and to practically master its basic principles. Moreover, they also participated in collecting and processing the results obtained and writing the press release, which was a good opportunity for them, together with Prof. Sarvan, to master these skills, and the co-authorships they acquired on the basis of this allowed them to advance to higher scientific and teaching positions.

One year after admission to SSB&H, Prof. Sarvan began to publish articles in the journal "Work"[63] with younger colleagues from the Clinic for Children's Diseases, and the results of experimental research done with academician Štern. He published eight articles in the journal Work. The first article, "The influence of general extratherapeutic factors for the prognosis of tuberculous meningitis treated with tuberculostatic agents", was published in 1956, followed by the article "Social and paediatric problems posed by rheumatic disease in childhood" in 1958. Until his retirement in 1967, he published the following articles in the journal Work: "Our experiences in the therapy of tuberculous meningitis", 1961; "Values of biological tests for evaluating the evolution and effect of hormonal treatment of rheumatic disease in children", 1963; "Experimental analysis of convulsions during hyperpyrexia", 1960; "Allergological examinations of children in two schools in Zenica", 1964; "Study of factors that influence the occurrence of poor health status of children in the mining area of the municipality of Kakanj", 1965; "Disturbance of acid-base balance and ion status in acute bronchopathies in the first two years of life", 1966.

At the invitation of Prof. Žarković, he participated in the Symposium on scientific research work in the field of nutrition of the population with the report "Current problems of children's nutrition in the first three years of life: the significance of the problem". The same paper was published in the publication Special Issues 16. Department of Medical Sciences 3. Sarajevo: Academy of Sciences and Art of Bosnia and Herzegovina, 1972.

Medical Literature

Prof. Milivoje Sarvan wrote numerous medical texts of varying content with different goals in different publications. The focus of these texts were always topics relating to healthy or sick children, or research aimed at improving the diagnosis or therapy of sick children closely related to the time and place in which he worked. When he worked as a young doctor in small towns, he was aware that for successful work, the education of his first associates—parents and auxiliary medical staff—was of great importance. That is why he prioritized writing health education texts about current paediatric problems at the beginning of his medical career, which would later remain his permanent preoccupation for the rest of his life.

Health Education Literature

As a socially oriented paediatrician, Sarvan was very dedicated to health education even as a young doctor. In collaboration with Dr. Žika Marković[64] in Serbia, he started and edited the monthly journal "Children and Parents: Care-feeding-raising

children-health of parents" (Deca i roditelji: nega, ishrana, vaspitanje dece – zdravlje roditelja, 1934–1937). It is interesting to point out that in the editorial of the first issue of that journal, perhaps for the first time, the public's attention was drawn to the role of the father in raising a child: "Life's struggle from ancient times, folk customs, and even nature itself placed the father, in terms of raising a child, in second place. Sometimes it goes so far that the father does not have any feel for his duties. Often he considers that he has fulfilled all his duties towards the child if he provides for him economically. This understanding should be considered wrong. A child can only develop completely harmoniously if the male and female sides cooperate in raising him".[65]

In the preface to the first edition of the book "Infant and Mother" (Dojenče i majka, 1960), Sarvan wrote: "The task of a modern paediatrician is not only to treat sick children, but rather that with his work on health education he/she instructs parents on the means by which children's diseases can be most successfully combated. Having understood this task in such a way I started to realize it at the beginning of my medical practice".

It was, in a sense, his conceptual guide, which he would follow for the rest of his life. In order to get as close as possible to the people, from the beginning of his medical work, he consistently wrote health-education articles with an emphasis on disease prevention and published them in the daily press[66] and health-education medical journals in Serbia.[67] Later, in B&H, he continued to do so by publishing popular articles about current pathology in the daily newspaper "Liberation" (Oslobođenje)[68] and journal "Life and health" (Život i zdravlje).[69]

Following this "ideal path", Prof. Sarvan, in simple, understandable and vernacular language, wrote health education books and brochures that were published in the period from 1928 to 1979 in large editions as editions of health institutions in Serbia, Bosnia and Herzegovina, and reputable publishing houses in the former Yugoslavia. Today, it is not easy to determine the exact number of health education books that Prof. Sarvan wrote.

The cover of the first issue of the journal Children and Parents, 1934.

In the list of "popular medical books", which is in his file at ASAB&H, ten references (49) are listed, which are incomplete and incorrectly written.

However, according to our still unfinished research, Professor Sarvan wrote 12 health education books. When compiling this bibliography, we had at our disposal all 12 books that were collected through interlibrary loans from B&H and Serbia. Seven books were published in Sarajevo and five books in Belgrade. We received the books from the libraries of larger and smaller towns in B&H and Serbia, which indicates that they were once widely distributed.

Today, after more than 45 years since the last edition in 1979, his books are preserved as rarities in libraries throughout the former state. Moreover, some of them are also found in home libraries and still serve new generations of parents to nurture their children the way their grandmothers

and mothers did. Overall, his health education books and brochures have been present amongst the wider public for a long time; they have educated generations and generations of young parents, helping them master knowledge for the prevention of children's diseases, but also the timely recognition of child health disorders and undertaking certain health procedures to reduce the duration of the disease and resolve it without consequences. Today, when we read between the lines, we see advice and procedures that can be interpreted as primary, secondary, and tertiary prevention.

Professional and Scientific Literature

Dr. Sarvan decided on an academic career on the day in 1925 when he applied for the position of assistant at the Children's Hospital of the Faculty of Medicine in Belgrade, i.e. when he was appointed honorary assistant (30).[70] As a clinical physician and also an assistant at the Faculty of Medicine, he was presented with a great opportunity to treat the pathological conditions of hospitalized patients professionally and scientifically, which, together with his clinical observations, was later the source from which he drew material for writing professional clinical and scientific papers, which were presented at professional and scientific meetings and published in medical journals in Yugoslavia and abroad. In this way, he successfully transferred his professional and scientific knowledge to younger colleagues and to the general public.

The exact number of his professional and scientific papers published in journals and proceedings of medical meetings has not been determined so far. In the list "Scientific and professional papers of academician Prof. Dr. Milivoje Sarvan", which is in his file at the ASAB&H (49), 116 bibliographic items are listed, according to professor Hadžić (2) 118, and according to the "Bibliography of works of associates of the Children's Clinic Sarajevo (1947–1972)", 82 papers, noting that during his time at the University Children's Hospital in Belgrade he published 34 scientific and professional papers (50), while Professor Kosorić states in the published obituary (5) that the professional and scientific contribution of Professor Sarvan contains 125 bibliographic units published in national and reputable foreign journals. A review of these sources reveals many shortcomings in citing and writing references, which casts doubt on the accuracy of these data and the need for the references to be written again, separately by hand.[71]

Experimental Research Literature

Prof. Sarvan started experimental research while working as a young specialist and assistant at the Faculty of Medicine in Belgrade. According to the commissions that wrote the report for his appointment to the SSB&H (47), his article "On the issue of protective grafting against varicella", which was published in "Medical Journal" (Liječnički vjesnik) in 1928, was evaluated as an experimental article because it had an experimental basis. Even though it was written in an unusual way compared

(Aus der Kinderklinik der Medizinischen Akademie zu Düsseldorf. — Direktor: Geheimrat Prof. Dr. *A. Schlossmann.*)

Über die Rolle der Milz bei der experimentellen Tuberkulose*.

Von

Dr. med. **Milivoj Sarvan,**

Assistent der Universitäts-Kinderklinik Belgrad, z. Zt. in Düsseldorf.

(*Eingegangen am 1. Dezember 1930.*)

Vom klinischen Standpunkte aus wird im allgemeinen die Milz als ein Organ betrachtet, welches in der Abwehr der Infektionen eine große Rolle spielt. Als Beweis dafür wird die bei fast allen Infektionskrankheiten vorhandene Volumenzunahme und Blutfülle der Milz als Ausdruck einer Hyperfunktion im Sinne einer intensiven Antikörperbildung betrachtet. Die experimentellen Untersuchungen liefern allerdings in dieser Hinsicht kein eindeutiges Bild, da den Experimenten, welche die immunkörperbildende Rolle der Milz einwandfrei beweisen, solche gegenüberstehen, bei denen die Abwehrkraft der Milz ganz...

Sarvan M. The role of the spleen in experimental tuberculosis [in German]. Beiträge zur Klinik der Tuberkulose und spezifischen Tuberkulose-Forschung. 1931;77(2): 182-185.

to his later experimental papers, it was significantly methodologically different. A year later, as a Rockefeller Foundation scholarship holder, Dr. Sarvan attended training at the Children's Clinic in Düsseldorf (Germany) from September 1929 to December 1930, where he and his colleagues had the opportunity to engage in experimental research (25). Later, after being admitted to the SSB&H with academician Pavao Štern, he again engaged in experimental research. According to our research, he published a total of six experimental articles. After the first one in "Medical Journal", he published three articles in German magazines: one with Prof. Albert Eckstein in the magazine "Journal of Hygiene and Infectious Diseases" (Zeitschrift für Hygiene und Infektionskrankheiten), one authored and one with his assistant Arno Nohlen in the magazine "Contributions to the Clinic of Tuberculosis and Specific Tuberculosis Research" (Beiträge zur Klinik der Tuberkulose und spezifischen Tuberculose-Forschung), and two experimental papers published in the journal "Work" (Radovi) with Prof. Pavao Štern.

Memberships in Medical Associations

Prof. Milivoje Sarvan was a member of the Section for Paediatric Medicine of the Serbian Medical Association and the founder of the Paediatric Section of the Association of B&H Physicians, founded in August 1945 and its first president until his retirement in 1967 (5). He was the president and lifelong honorary member of the Association of Paediatricians of Yugoslavia (5), a corresponding member of the Association of Paediatricians of France, Switzerland, West Germany, Italy, an honorary member of the Association of Paediatricians of Czechoslovakia and Poland (2), and an active member of the council of the International Children's Centre in Paris (5). As a member of the editorial board, he worked for many years for the newly founded journals "Medical Archives" (Medicinski arhiv)[72], and Work and as editor of the journal "Yugoslavian Paediatrics" (Paediatria Iugoslavica). He was also a member of the editorial board of the journal "Archives for the Protection of Mothers and Children" (Arhiv za zaštitu majke i djeteta). At the National Health Council of the PRB&H, he was the president of the Commission for the Protection of Mothers and Children.

Recognitions and Awards

For his life's work in the Socialist Republic of Bosnia and Herzegovina (SRB&H), Professor Milivoje Sarvan received the most significant federal and republican awards in the former Yugoslavia. He was the recipient of the AVNOJ award, Orders II and I, the Order of the Republic with a silver wreath, the July 27 award of SRB&H. In addition, he received the Memorial Plaque of the Faculty of Medicine in Sarajevo and the Golden Plaque of the Clinical Hospital in Sarajevo. Among foreign decorations, he was a recipient of the high French decoration of the Order of the Legion of Honour.

Concluding Remarks

Academician Milivoje Sarvan was one of the pioneers of social paediatrics in the former Yugoslavia. From his appointment as assistant at the University Children's Hospital in Belgrade (1925), he worked as a paediatrician alongside the first directors of the Clinic—Franciszek Groër, and Matija Ambrožić. Thanks to a scholarship from the Rockefeller Foundation (1929/30), he trained with the best socially oriented German paediatricians of the time. For decades, he remained dedicated to topics that he specifically studied at the time, such as early infant mortality, prematurity, tuberculosis therapy, post-vaccination encephalitis, the organization of social welfare for children, and health education of parents. Although during the interwar period he was engaged in scientific work and was the closest associate of Professor Ambrožić, due to the economic crisis in Yugoslavia, and perhaps for some other reasons, Sarvan held the position of assistant for the entire 14 years. He was appointed assistant professor of Hygiene in Childhood at the end of 1939. During the Second World War and the occupation of Yugoslavia, he was, like many university professors in Belgrade, imprisoned in the Banjica

concentration camp (1941/1942), and then retired (1942). After the liberation of Belgrade, he returned to duty (1944), to the position of assistant professor and head of the Polyclinic.

In the autumn of 1946, Sarvan had the opportunity for academic advancement and full professional affirmation by being appointed full professor and head of the Department of Paediatrics at the newly founded Faculty of Medicine in Sarajevo, in the then PRB&H.

The organization and equipping of the Clinic, the formation of a team of experts and preparations for the start of classes in Paediatrics were his first tasks, but it soon became clear that he needed to engage in a wider field of work. B&H, devastated by the war, but also one of the most underdeveloped parts of the country even before the war, had a shortage of doctors; primary health care was at a low level, and there were almost no in-patient institutions for the treatment of children. The people were largely poor and uneducated about health. Under Sarvan's leadership, the Children's Clinic became a school for professional staff (paediatricians and childcare workers), a centre for the education of general practitioners for the treatment of children and for health education, and also provided advisory, professional and methodological assistance to regional centres in the organization of children's health care at primary and secondary level. In addition to numerous obligations, Sarvan devoted great attention to scientific work and professional development of the Clinic's doctors. Within the Association of Physicians of Bosnia and Herzegovina, he founded the Paediatric Section and established annual professional meetings known as the Paediatric Days of Bosnia and Herzegovina. He also managed to secure scholarships for his young colleagues to train abroad.

He contributed to the development of the Faculty of Medicine by performing the duties of vice dean (1949/50, 1955/56) and dean (1951/52, 1954/55). In 1950/51 he was the vice-rector of the University of Sarajevo. He published more than 120 professional and scientific papers, 12 health education books and a large number of health education articles. As a distinguished scientific worker, in 1955 he was appointed a regular member of the Scientific Society of Bosnia and Herzegovina (since 1966, the Academy of Sciences and Arts of Bosnia and Herzegovina). Among other rewards, he received the highest recognition of the SFRY—the AVNOJ Award (1971) and the French Legion of Honour (1954). The Clinic he founded bore his name from 1979 until the breakup of Yugoslavia in 1992.

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

Notes

[1] State Archives of Serbia, collection of the Ministry of National Health of the Republic of Serbia, Personal file of Dr. Milivoje Sarvan, F-149, No. 50 (hereinafter: SAS, G-216, F-149, No. 50). Although all of Sarvan's documents mention Požega as his place of birth, from the Extract from the Register of Births of the Church of Požega, the Church of St. Konstantin and Jelena in Ravni, issued on August 8, 1907, it could be concluded that he was actually born in the village of Ravni, in Požega county. The surname of the parents is given in the Certificate as Sarvanović, and the same surname is mentioned in the death announcement of Milivoje's younger brother Ignjat (1902–1936), a postal clerk and reserve engineer lieutenant (Source: *Službeni vojni list* 1936, June 23; 55(23):1363-64). In addition to their sons Milivoje and Ignjat, the Sarvans also had a daughter named Slavka.

[2] In Nedeljko V. Radosavljević's article "Simo Sokolov and Serbian emigration in Bulgaria 1883 – 1885 (documents)". Miscellanea 2013, vol. XXXIV:253-4, the text of the receipt was published by which Teofilo Sarvan confirms that he received two packages of documents from Simo Sokolov on the order of Nikola Pašić; Sofia, October 11/23, 1886. "Political friends" of Teofilo Sarvan are also mentioned in a letter sent by Milivoje Sarvan to Matija Ambrožić in 1926. Source: Museum of Science and Technology - Belgrade, Collection of archival materials – Medicine; Legacy of Matija Ambrožić (2014/1033). Letter from M. Sarvan to M. Ambrožić, Belgrade, April 8, 1926. [in Serbian].

[3] Popović D. S. Čačak Secondary school: 1837 – 1937. Čačak: [Community of the home and school of the Čačak Secondary School]; 1939, pp. 189-190.

[4] Archives of the Čačak Secondary school, Reports on work and student progress in the school years 1907/08, 1908/09,

1909/10, 1910/11 and 1911/12, printed in the form of brochures in the printing house of Stevan Matić in Čačak in 1908, 1909, 1910, 1911 and 1912. The authors sincerely thank Mrs. Jadranka Vitas, secretary of the Secondary school, for the copies of the brochures.

[5] Archives of Valjevska Secondary school, Register of students of Valjevska Secondary school for school year 1912–1913. (I – VII grade), No. 21 – Milivoje Sarvan; *Report on work and success in the school year 1913–1914*. Valjevo: Valjevska Secondary school, 1914 [in Serbian]. The authors sincerely thank Prof. Dr. Vladimir Krivošejev on the submitted copies of the documents.

[6] SAS, G-216, F-149, No. 50 – Certificate of the University of Lyon on obtaining the title of Doctor of Medicine; Lyon, December 22, 1921.

[7] SAS, G-216, F-149, No. 50 – Certificate of the Permanent Bacteriological Station in Belgrade on the voluntary work of M. Sarvan between February 1 and April 31, 1922; Belgrade, December 12, 1922 and M. Sarvan's application to the manager of the General State Hospital regarding the acquisition of the general right to practice medicine; Belgrade, December 15, 1922.

[8] Andrija Štampar (1888–1958), completed his medical studies in Vienna in 1910. From 1919 to 1930, as head of the Hygiene Department in the Ministry of Public Health of the Kingdom of SCS, he created a network of over 600 preventive medical institutions. After premature retirement in 1931, he worked as an expert of the League of Nations and he was also a professor of social medicine, dean of the Faculty of Medicine in Zagreb and president of the Yugoslav Academy of Sciences and Arts. Between 1946 and 1948, he was president of the Interim Commission of the World Health Organization. [Štampar, Andrija. Hrvatska enciklopedija, mrežno izdanje. Leksikografski zavod Miroslav Krleža, 2013. – 2024. Accessed on October 8, 2024. <https://www.enciklopedija.hr/clanak/stampar-andrija>]

[9] SAS, G-216, F-149, No. 50 – Request of M. Sarvan to the Minister of Public Health, Belgrade, February 6, 1923.

[10] SAS, G-216, F-149, No. 50 – Decision of the Minister of Public Health dated February 19, 1923.

[11] Stevanović Z. [et al.] Healthcare in Aleksinac and surroundings: 1836-2018. Aleksinac: "Vuk Karadžić" Library; 2018. p. 77. [in Serbian]

[12] SAS, G-216, F-149, No. 50 – Elder's report of the Regional Health Administration of the Niš County Region on the work of M. Sarvan, Niš, March 14, 1925.

[13] SAS, G-216, F-149, No. 50 – Request of M. Sarvan to the Minister of Public Health; Aleksinac, November 22, 1923.

[14] SAS, Faculty of Medicine collection (hereinafter: G-203) – Register for 1925, No. 895, February 24 , 1925 – Request of Dr. Milivoje Sarvan for the position of assistant at the Children's Hospital; SAS, G-216, F-149, No. 50 – Ministry of Education to Ministry of Public Health, August 24, 1925 – Notification that by the decree of the Minister of Education dated August 24, 1925, M. Sarvan was appointed as an assistant at the Faculty of Medicine and that it is therefore necessary to relieve him of his duties in Aleksinac.

[15] SAS, G-203 – Register for 1924, No. 3851 – On October 8, 1924, the Rector of the University reported to the Dean of the Faculty of Medicine about the decision on Groër's appointment, which was made by the Ministerial Council at the session on September 29, 1924.

[16] Sarvan M. Działalność profesora Franciszka Groera w Belgradzie [in Polish]. In: Helena Krukowska, editor. Franciszek Groer: życie i działalność. Warszawa: Państwowy Zakład Wydawnictw Lekarskich; 1973. p. 53.

[17] Smilja Kostić-Joksić (Belgrade, 1895 – Belgrade, 1981) completed her medical studies in Montpellier in 1919. She worked at the University Children's Hospital as an assistant (from 1924), assistant professor (1939–1948) and associate professor (1948–1954). In 1952, she was awarded the French Legion of Honour for her scientific contributions to the study of tuberculosis and the protective value of immunization with the BCG vaccine. (Bondžić D. Kostić Joksić, Smilja. Serbian biographical dictionary, Vol. 5, ed. Čedomir Popov, 299-300. Novi Sad: Matica srpska, 2011. [in Serbian]).

[18] Marija Gajić-Vajs (Sarajevo, Austro-Hungarian Monarchy, 1899 – Belgrade, 1964) completed her medical studies in Vienna in 1924; from 1925 to 1931 she was an assistant at the University Children's Hospital in Belgrade. She earned the title of specialist paediatrician in 1927. Until the occupation of Serbia in World War II, she worked in the health service of the Belgrade municipality, where she advanced to the position of head of the Department for Social Protection of Children and the head of the Children's Home. In the spring of 1941, she joined the National Liberation Movement and during the war she worked as a doctor in partisan units. After liberation, she was an advisor to the Council for Public Health and Social Policy of the Government of the Federal People's Republic of Yugoslavia. (Archives of Yugoslavia, collection of the Ministry of Social Policy and Public Health of the Kingdom of Yugoslavia (F-39), Personnel files (hereinafter: AY, F-39, Personnel files), Folder 41 [in Serbo-Croatian]).

[19] Ljubomir Vulović (Belgrade, 1896 – Belgrade, 1969) completed his studies in Geneva in 1921, and specialized in paediatrics in Vienna in 1923. He was an assistant at the University Children's Hospital in 1925–1926, and then until mid-1932 he was the head of the Central Dispensary and Counselling Centre for infants and mothers of the Belgrade municipality. At the same time, from 1928, he worked at the Children's Department of the General State Hospital, where he founded the Department for Children's Chest Diseases in 1938. He managed that department until 1947, and then until his retirement in the early 1960s, he was the head of the Children's Department of the Railway Hospital in Belgrade. (AY, F-39, Personnel files, Folder 208 [in Serbo-Croatian]).

[20] Matija Ambrožić (Hrastnice, Slovenia, Austro-Hungarian Monarchy, 1889 – Belgrade, 1966) was a pioneer of social paediatrics in the Kingdom of SCS/Yugoslavia. In Vienna, he completed his medical studies (1918) and specialization in paediatrics (1921). Until he was appointed associate professor of paediatrics at the Faculty of Medicine in Belgrade (1926), he worked in Ljubljana, where he founded the Institute for Social and Hygienic Protection of Children (1923) and the first school for paediatric nurses (1924). He was appointed full professor in 1947. He managed the University Children's Hospital in Belgrade and the Department of Children's Diseases of the Faculty of Medicine until his retirement in 1961, with a break during the Second World War (1943–1944). (Tasovac B. In memoriam: Professor Matija Ambrožić (1889–1966). Srp Arh Celok Lek 1967; 95(7-8):755-7. [in Serbo-Croatian]).

[21] Museum of Science and Technology - Belgrade, Collection of archival materials – Medicine; Legacy of Matija Ambrožić (2014/1033) (hereinafter: MST, CAM, MA, 2014/1033) – Letter from M. Sarvan to M. Ambrožić, Belgrade, May 4, 1926.

[22] Archives of Yugoslavia, collection of the Ministry of Social Policy and Public Health of the Kingdom of Yugoslavia (F-39), Personnel files—File of Dr Milivoje Sarvan, f-159, Sheet of personal and official data, Belgrade, November 20, 1946; SAS, collection of the University of Belgrade (hereinafter: G-200), 1939, F IV, r. 27 – Report on the election of Dr. M. Sarvan to the position of assistant professor for the subject of Childhood Hygiene at the Department of Children's Diseases of the Faculty of Medicine in Belgrade, 1939.

[23] Rockefeller Archive Centre, Fellowship Recorder Card for Milivoje Sarvan 1929/1930, RF_FA426_24_710_B19_Sarvan-M_29208. The authors sincerely thank Mrs Bethany J. Antos, Archivist at the Rockefeller Archive Centre, for the copy of the document.

[24] Stürzbecher, Manfred, "Finkelstein, Heinrich" in: Neue Deutsche Biographie 5 (1961), S. 162-163 [Online-Version]; URL: https://www.deutsche-biographie.de/pnd117508152.html#ndbcontent.

[25] Wunderlich, Peter, "Schlossmann, Arthur" in: Neue Deutsche Biographie 23 (2007), S. 108-109 [Online-Version]; URL: https://www.deutsche-biographie.de/pnd117330442.html#ndbcontent.

[26] MST, CAM, MA, 2014/1033 – M. Sarvan – Arbeitsplan [in German]; SAS, fund University of Belgrade (G-200), 1939, F IV, r. 27 – Report on the appointment of Dr. M. Sarvan to the position of associate professor on the subject of Hygiene in Childhood at the Department of Children's Diseases of the Faculty of Medicine in Belgrade, 1939.

[27] MST, CAM, MA, 2014/1033 – Letters from M. Sarvan to M. Ambrožić, Düsseldorf, February 15, 1930 and August 29, 1930.

[28] "Faculty of Medicine – Appointments – Decrees – Milivoje Sarvan". Educational Gazette 1939; LV(12):1447. [in Serbian-Croatian].

[29] Uroš Ružičić (Pljevlja, Kingdom of Montenegro, 1891 – Belgrade, 1966) completed his medical studies in Bordeaux in 1919. As a scholarship holder of the Rockefeller Foundation, he trained in the USA and France (1924–1926). In 1927 he was appointed an assistant at the University Children's Hospital in Belgrade, where he was successively appointed to all the academic positions until his retirement in 1961. From 1958 he was a correspondent member, and from 1963 a regular member of the Serbian Academy of Sciences and Arts. (Jovanović Simić J. Uroš Ružičić. In Giants of Serbian Medicine: 19th century and first half of the 20th century, 93. Belgrade: SASA : Museum of Science and Technology : Serbian Medical Society; 2016 [in Serbian]).

[30] Jovanović N. The attitude of occupiers and quislings towards Freemasonry in Serbia 1941 – 1942 [in Serbian-Croatian]. Godišnjak grada Beograda, knj. XVIII – 1971, p. 88.

[31] Historical Archives of Belgrade, Administration of the city of Belgrade, record books of the Banjica concentration camp, Book 2, 1319 – Matija Ambrožić; Book 3, 3390a – Milivoje Sarvan.

[32] The Department for Children's Diseases was previously part of the Obstetrics and Gynaecology Department of the National Hospital in Sarajevo, located in one room with eight beds, where sick children were admitted, except those suffering from infectious and skin diseases.

[33] Konstantin Delijanis (Sarajevo, Jun. 6, 1882–Sarajevo, Dec. 31, 1961) completed his secondary education in 1901 in Sarajevo and Faculty of Medicine in 1906 in Vienna. His specialization in paediatrics was recognized by the Royal Administration of the Drina Banovina. Until April 6, 1941, he was the head of the Children's Department of the State Hospital in Sarajevo, when he was dismissed from service without the right to a pension. After the liberation in 1945, he worked for a short time in the Ministry of Social Policy, and from February 15, 1946, he was again the head of the Children's Department of the State Hospital in Sarajevo. (AY, F-39, Personnel files, File of Dr. Konstantin (Josifov) Delijanis, f. 28 [in Serbo-Croatian]).

[34] Jelena Bulić-Adamović (Kostroma, Russian Empire, Oct. 29, 1886 – Sarajevo, Aug. 20, 1967) graduated from the Faculty of Medicine in 1917 in Moscow, Russia, where she completed her medical internship in the period from 1917 to 1919. She received the title of Specialist in Children's Diseases from the Royal Administration of the Drina Banovina in Sarajevo in 1933. From that time, she worked as a assistant physician at the General State Hospital in Sarajevo. For her zealous work in the service in 1939, she was awarded the Order of St. Sava from Drina Banovina. (AY, F-39, Personnel files, File of Dr. Jelena Bulić-Adamović, f. 1 [in Serbo-Croatian).

[35] Maša Živanović (Delnice, Dec. 14, 1898 – Beograd, Aug. 12 1960) was a reputed paediatrician in Sarajevo in the interwar years. She was the head of the Children's Dispensary and the Institute for Health Care for Mothers, Children, and Youth in Skerlić's Street in Sarajevo. After World War II,

she performed the same duty. Živanović was a well-known women's activist and president of the Women's Movement in Sarajevo (1924–1936). (Available from: https://pubmed.ncbi.nlm.nih.gov/36943037/).

[36] Mara Kurtović (Belgrade, Jul. 12, 1892 – Sarajevo, Oct. 17, 1979) was the first expert in school hygiene in Bosnia and Herzegovina and the head of the School Polyclinic at the Institute for Maternal and Child Health Care on Skerlić Street in Sarajevo. (Archives of B&H. Dossier Mara Kurtović).

[37] Kornelija Rakić (Ruma, Aug. 19, 1879 – Mostar, Jul. 1952) was the first Serbian female physician in Novi Sad, Vojvodina, and she was employed as an AH official female physician in Bihać (1908–1912), Banja Luka (1912–1917) and Mostar (1917–1918). After World War I, she participated in the establishment and expansion of public health institutions in Mostar and Herzegovina from 1918–1949 against the backdrop of the devastation of the two World Wars. (Available from: https://pubmed.ncbi.nlm.nih.gov/34075776/).

[38] It is worth noting that a few months after the completion of the adaptation of the hospital, in the September issue of the journal Medical Archives in 1947, Prof. Sarvan published an article entitled "Several organizational problems related to the opening of children's hospital wards in Bosnia and Herzegovina". He knew very well that with the development of the paediatric service in Bosnia and Herzegovina, the inevitable opening of children's hospitals or children's hospital departments would soon occur and that his experience in "construction" would be useful to those who were taking up this work in Bosnia and Herzegovina (Sarvan M. Several organizational problems related to the opening of children's hospital departments in B&H, [in Serbian]. Medical Archive. 1947;2;80-6).

[39] Milena Mitrović (Vienna, Austria, Feb. 20, 1895 – Sarajevo, Jun. 10, 1959) graduated from the Faculty of Medicine in Vienna in 1920. She completed her medical internship at the State Hospital in Belgrade. She then worked as a municipal and secondary doctor at the hospital in Livno and then in Skopje, Ohrid, Novi Sad, and Subotica, in various hygiene institutions. She spent the Second World War in Karagujevac working as a children's doctor at the County Hospital and the National Health Centre. After liberation, she was employed at the Children's Department of the State Hospital in Sarajevo as acting head of the Department and physician assistant. After the opening of the Paediatric Clinic in mid-1947, she was one of the first associates of Prof. Sarvan. She temporarily worked at the Children's Hospital in Tuzla in 1949, and after that returned to Sarajevo to the Paediatric Clinic, where she remained until her death. (Protić M. Dr. Milena Mitrović. Oslobođenje. 1959 Jun 11; Col. 4; Historical Archives of Belgrade, Fund of the Chamber of Physicians, no. 2315—Dossier of Dr. Milena Mitrović-Popović).

[40] Katarina Carić (Sarajevo, Jan. 10, 1920 – Dubrovnik, Oct. 14, 2013).

[41] Nevena Bajić, married Stojkov (Bosanski Novi, B&H, May. 5, 1915 – Belgrade Mar. 2, 2008), graduated from the Faculty of Medicine in Belgrade on November 13, 1940. After completing her internship, she was not employed until January 15, 1945 because she believed that her work would benefit the enemy. After that, already in January 1945, she began her specialization in paediatrics at the University Children's Hospital in Belgrade. In February 1948, she successfully passed the specialist paediatrics exam at the same clinic, leading to her selection as paediatrics assistant at the Faculty of Medicine in Sarajevo. She remained in that position until January 31, 1959. From February 1, 1959, she worked at the Pneumophthisiology Clinic in Sarajevo as the head of the Children's Department. In 1964, on December 15, she was appointed consultant at the Federal Institute of Statistics in Belgrade. (AY, F-39, Personnel files, File of Dr. Nevena Stojkov Bajić, f. 175 [in Serbo-Croatian]; Personnel and Family Archives of Nevena Stojkov).

[42] Suzana Čanji (Bački Petrovac, Austro-Hungarian Empire, Oct. 17, 1913 – Sarajevo, Feb. 5, 1969) graduated from the Faculty of Medicine in Belgrade on December 21, 1940. After completing her medical internship in Banja Luka, she worked at the State Hospital there until August 1942, when she was arrested as a member of the People's Liberation Movement by the Ustaša authorities and taken to the Stara Gradiška concentration camp. She was liberated in February 1943. She worked in the Yugoslav Army from September 19, 1944, to December 6, 1945. From March 1946 she was mobilized as a civilian doctor at the State Hospital in Banja Luka, and from July to October 1946 she was a county doctor in Zavidovići. From 1948, she worked at the Paediatric Clinic. (AY, F-39, Personnel files, File of Dr. Suzana Čanji, f. 23 [in Serbo-Croatian]).

[43] Žarko Mićić (Stupari near Kladanj, B&H, Oct. 30, 1918 – Zrenjanin, Serbia, Jul. 17, 2007) graduated from high school in Tuzla in 1937 and the Faculty of Medicine in Belgrade on December 4, 1946. During 1947, he completed a medical internship at the General State Hospital in Belgrade, and in early 1948, he was employed at the Paediatric Clinic in Sarajevo, where he began his specialization in paediatrics. In May 1952, he returned to Tuzla as a specialist in paediatrics, where he opened a children›s dispensary and the Department for Children's Diseases as part of the General Hospital in Tuzla. (AY, F-39, Personnel files, File of Dr. Žarko Mićić, f. 103 [in Serbo-Croatian]).

[44] Nina Delari married Hadžiselimović (Minusinsk, Siberia, Russian Empire, Mar. 18, 1914 – Basel, Switzerland, Mar. 17, 2007). She graduated from high school in Bela Crkva, Serbia, and from the Faculty of Medicine in Belgrade on July 4, 1939. She completed her medical internship at the General State Hospital and the Central Institute of Hygiene in Belgrade in 1940. From April 1941 to February 1942, she worked in Belgrade and then as a doctor in Kovin (Serbia). She specialized in paediatrics at the Paediatric Clinic in Sarajevo with Prof. Dr. Milivoje Sarvan. As a paediatrician, she worked in primary health care clinics in Sarajevo. She

ended her working life in 1979 as a paediatrician with the title of primarius at the Clinic for Eye Diseases in Sarajevo. (Historical Archives of Belgrade, Fund of the Chamber of Physicians, no. 1577—Dossier of Dr. Nina M. Delari [in Serbo-Croatian]).

[45] Borivoj Ćurčić (Sarajevo, May 4, 1924 – Belgrade, Oct. 15, 2016) graduated from high school in Sarajevo in 1943. He enrolled in the Faculty of Medicine in Belgrade in 1945 and graduated from the same faculty in 1951. Then he was employed at the Paediatric clinic in Sarajevo. He completed his specialist internship from 1953 to 1957 in Sarajevo, where he also passed the specialist exam. He was trained in Lyon, London, and Edinburgh. He defended his doctoral dissertation "The relationship between magnesium and magnesia in various children's nephropathies", at the Faculty of Medicine in Sarajevo in 1965. He was appointed associate professor in 1967. After the departure of Prof. Sarvan 1967, he was appointed head of the Paediatric Clinic in Sarajevo. After being appointed full professor from Sarajevo, he moved to Belgrade in 1973 as the head of the "Olga Popović Dedijer" Institute of Paediatrics in the "Zvezdara" Clinical Hospital Centre. At the same time, he was a regular professor of paediatrics at the Faculty of Dentistry in Belgrade. He retired in 1989. (Marković D, Mimica M, editors. Who is Who in Yugoslavia [in Serbo-Croatian]. Belgrade: Savez lekarskih društava Jugoslavije; 1968. p. 525. Andrejević M. They created the city hospital 1936–2006 – 70 years of work of KBC Zvezdara in Belgrade. Belgrade: MG Marketing; 2007, p. 137–138).

[46] Vera Golubović Ćurčić (Belgrade, Jun. 4, 1925 – Belgrade, Oct. 15, 2016) graduated from the Faculty of Medicine in Belgrade in 1952, after which she was employed at the Paediatric Clinic in Sarajevo, where she completed her medical internship and specialized in paediatrics in 1958. In 1960/61, she trained in Cambridge, where she was educated in experimental medicine. She defended her doctoral dissertation under the title "Effect of a diet with different protein percentages in experimental malnutrition and rehabilitation" in 1963 at the Paediatric Clinic in Sarajevo, after which she was appointed assistant professor. As an associate of Professor Sarvan, she participated in work on projects of the Scientific Society of B&H. She moved to Belgrade in 1973, where she started working at the "Zvezdara" Health Centre in November of the same year. (Marković D, Mimica M, editors. Who is Who in Yugoslavia [in Serbo-Croatian]. Belgrade: Savez lekarskih društava Jugoslavije; 1968. p. 529. and Data obtained from Mrs. Nevena Ćurčić, daughter of Dr. Vera Golubović Ćurčić).

[47] Džemal Haverić (Podgorica, Jun. 4, 1919 – Sarajevo, Oct. 10, 1995). He enrolled in medical studies in 1939 in Belgrade. After the start of World War II, he moved to Zagreb, where he graduated in July 1946. He started his first service on the Šamac–Sarajevo youth railway, then he was a doctor for the youth work brigade for the reconstruction of Warsaw and for the suppression of freckles. From 1947 to 1949, he worked as an assistant at the Institute of Physiology of the Faculty of Medicine in Sarajevo under Prof. Aleksandar Sabovljev. After that, he was an assistant at the Dermatology clinic with Prof. Josip Fleger (1950–1951). From February 1, 1952, to December 23, 1955, he specialized in paediatrics at the Paediatric Clinic in Sarajevo. He took the exam in front of a commission composed of Prof. Dr. Matija Ambrožić, Prof. Dr. Milivoje Sarvan, and Prof. Dr. Blagoje Đorđević. After his specialization, he worked from 1956 to 1959 at the Children›s Dispensary in Sarajevo. Then he returned to the Paediatric clinic, where he remained until the end of his working life. (Marković D, Mimica M, editors. Who is Who in Yugoslavia [in Serbo-Croatian]. Belgrade: Savez lekarskih društava Jugoslavije; 1968. p. 531. and Family archive of Tarik Haverić).

[48] The congress was held from September 24 to September 28, 1952, in Sarajevo (Banja Ilidža). The president of the organizing congress committee was Prof. Dr. Milivoje Sarvan. The main topic was "Nutrition and disorders of digestion and nutrition in early childhood in our country". The main lectures were given by eminent professors of clinics for children's diseases in Yugoslavia at the time, and other lectures were given by distinguished paediatricians. Two years later, in 1952, the topics of the Congress were published in the book Sarvan M, Kostić-Joksić S, Skrivaneli N, Avčin M, ed. Nutrition of our children and its disorders. Belgrade - Zagreb: Medicinska knjiga; 1954.

[49] This period was chosen because it best suited the health institutions in the field.

[50] Esad Zukić (Gradačac, B&H, 26 Feb. 1935) completed primary and secondary education in Gradačac, Bosnia and Herzegovina, and then the Faculty of Medicine in Sarajevo, Bosnia and Herzegovina, in 1962. He passed the specialist exam in paediatrics at the Paediatric Clinic in Sarajevo in 1972. He received the title of primarius in 1982. He spent his entire working life at the Health Centre in Gradačac, where he founded the Children's and School Dispensary. He has been a participant in all Paediatric Days of Bosnia and Herzegovina since 1972. (According to Dr. Zukić's oral statement, August 10, 2024).

[51] Jordan Tomić (Tetovo, Ottoman Empire, 1910 – Belgrade, 1998) graduated from high school in Belgrade in 1928 and the Faculty of Medicine in 1935. Two years later, he began his specialization in paediatrics at the Children's Department of the General State Hospital in Belgrade. After completing his specialization, from 1943 to 1945 he worked as an assistant at the University Children's Hospital, Belgrade, Serbia, then moved to Niš, where he was the head and manager of the Children's Hospital. Then he went to Switzerland for training, where he worked for a year at the Children's Hospital of the University of Zürich with Professor Guido Fanconi. He worked at the Paediatric Clinic in Sarajevo from 1949 to 1952. He was an assistant professor at the Department of Paediatrics and a paediatrician at the Paediatric Clinic at the Faculty of Medicine in Sarajevo. Then he went to Belgrade, where he founded the "Olga Popović

Dedijer" General Hospital for Children's Diseases, which he managed as the primarius until his retirement in 1972 (Andrejević M. They created the City hospital in 1936–2006. 70 years of work of the KBC "Zvezdara" in Belgrade. Belgrade: MG Marketing; 2007, pp. 134–135).

[52] Ibid, p. 227.

[53] Ibid, p. 218.

[54] Irina Tomić (Rostov, Russian Empire, 1915 – Belgrade, ?) finished high school in Valjevo in 1933 and the Faculty of Medicine in Belgrade in 1939. She was recognized as a dentist by the Dental Clinic of the Faculty of Medicine in Belgrade in 1942. From the liberation of Belgrade in October 1944 to August 1945, she had a private practice. Then she went to Niš with her husband, Dr. Jordan Tomić, where she volunteered at the Children's Department of the State Hospital. She worked at the Paediatric Clinic in Sarajevo from 1948, and in 1952 she passed the specialist exam in paediatrics. (AY, F-39, Personnel files, File of Dr. Irina Tomić, f. 190 [in Serbo-Croatian]).

[55] Dimitrije Miletić (Sarajevo, Mar. 7, 1920 – Belgrade, Apr. 22, 1989) graduated from the Faculty of Medicine in Belgrade in 1949. He began his specialization in paediatrics at the University Children's Hospital, in Belgrade in 1949 and continued the same year at the Paediatric Clinic in Sarajevo. He was appointed assistant at the Paediatric Clinic of the Faculty of Medicine in Sarajevo in 1953. He defended his habilitation thesis "Neurotoxic infant syndrome" in 1958 at the Faculty of Medicine in Sarajevo, where the following year, 1959, he received the title of assistant professor. After being promoted to the position of associate professor (1962), he moved to the Faculty of Medicine in Novi Sad, where from 1963 he held the position of head of the Department of Paediatrics and director of the Clinic for Children's Diseases. As a professor and head of the Department of Paediatrics, he worked at Makerere University in Kampala (Uganda) from December 1969 to October 1971. His doctoral dissertation entitled "Clinical protein malnutrition of hematological and serum protein changes" was defended at the Faculty of Medicine in Sarajevo in 1974. He was appointed professor of paediatrics and child care at the Faculty of Medicine in Banja Luka in January 1979. In May of the same year, he was appointed dean of the Faculty of Medicine in Banja Luka. He held that function until his retirement at the end of December 1982. (Predojević Samardžić J. The first dean of the Faculty of Medicine in Banja Luka [in Serbian]. Kod. 2020;19(52):61-62; Šuščević D. Prof. Dr. Dimitrije Miletić - Obituaries [in Serbian]. Scripta Medica. 1955;24(1-4):53).

[56] Izet Hadžić (Bileća, Apr. 20, 1920 – Sarajevo, Aug. 11, 1998). He graduated from the Faculty of Medicine in Zagreb in 1951. He passed the specialist exam in pediatrics at the Paediatric Clinic in Sarajevo in 1956. After defending his habilitation thesis entitled "Diagnostic values of cytochemical analysis of cerebrospinal fluid in children" in 1961, he was elected Assistant Professor, and in 1968 Associate Professor of Paediatrics at the Faculty of Medicine of Sarajevo. He undertook additional postgraduate training in Germany, Czechoslovakia and France. His doctoral dissertation entitled "Clinical and electroencephalographic characteristics of febrile convulsions and their relationship to epilepsy" was defended at the Faculty of Medicine in Sarajevo in 1971, followed by his election to a Professor of Paediatrics at the Faculty of Medicine, University of Sarajevo in 1975. He was University Chair of Paediatric Department and Director of Paediatric Clinic in Sarajevo from 1976-1980. He has initiated regular annual meetings of paediatricians from Bosnia-Herzegovina entitled Pedijatrijski Dani. During his academic career he presented his work at several overseas conferences and published over 170 papers in the national and international journals. He was the president of The Medical Doctors' Association of Bosnia and Herzegovina and Yugoslav Society of Paediatricians over many years. He retired and became Emeritus Professor of Paediatrics at the Faculty of Medicine of Sarajevo in 1985. (Marković D, Mimica M, editors. Who is Who in Yugoslavia (in Serbo-Croatian). Belgrade: Savez lekarskih društava Jugoslavije; 1968. p. 530).

[57] Ešref Sarajlić (Sarajevo, Jun. 6, 1923 – Sarajevo, Jan. 24, 1980) graduated from the Faculty of Medicine in Sarajevo in 1953. As a resident, in 1957 he was chosen to be an assistant at the Department of Paediatrics at the Faculty of Medicine in Sarajevo. He passed the specialist exam in paediatrics at the Paediatric Clinic in Sarajevo in 1958. After defending his habilitation thesis in 1963, under the title "Carditis during the primary attack of rheumatic disease in childhood", he was appointed assistant professor in the same year, and in 1968, an associate professor. He defended his doctoral dissertation entitled "Changes in the electrocardiogram caused by abnormal concentrations of potassium in the isolated heart and their significance" at the Faculty of Medicine in Sarajevo in 1973, after which, in 1975, he was appointed full professor at the Department of Paediatrics of the Faculty of Medicine of the University of Sarajevo. He was appointed a corresponding member of the Academy of Sciences and Arts of Bosnia and Herzegovina in 1978 at the Department of Medical Sciences. In the period from 1974 to 1980, he was the dean of the Faculty of Medicine in Sarajevo. (Ešref Sarajlić [in Bosnian]. U: Lincender-Cvijetić L. Editor. Spomenica 70 godina ANUBiH. Sarajevo: Akademija nauka i umjetnosti BiH; 2021. p. 366-367.)

[58] Ibid, p. 228.

[59] Izet Čustović (Trebinje, Jun. 6, 1923 – Sarajevo, Jan. 24, 1979) graduated from the Faculty of Medicine in Zagreb in 1953. After passing the specialist exam in paediatrics at the Children's Clinic in Sarajevo in 1961, he was selected to be an assistant at the Department of Paediatrics at the Faculty of Medicine in Sarajevo. He defended his habilitation thesis entitled "Electrophoretic and immunoelectrophoretic study of some fractions of serum proteins in eutrophic and dystrophic infants in certain pathological conditions" at the Faculty of Medicine in Sarajevo in 1965. He was appointed

assistant professor in 1967, he defended his doctoral dissertation entitled "Modifications of human colostrum and milk immunoglobulins in the preservation process" at the Faculty of Medicine in Sarajevo in 1970. He was appointed associate professor in 1972, and full professor at the Department of Paediatrics at the Faculty of Medicine of the University of Sarajevo in 1977. (Najdanović R, Kosorić D, editors. 40 years of Children's Clinic and Polyclinic "Prof. Dr. Milivoje Sarvan" in Sarajevo [in Bosnian]. Sarajevo: Pedijatrijska sekcija Društva ljekara B&H i Klinika za dječije bolesti – Sarajevo. Sarajevo; 1987. p. 60-61.

[60] Nedo Zec (Mostar, Jul. 12, 1899 – Mostar, Nov. 18, 1971) graduated from the Faculty of Medicine in Vienna in 1927. He was the first Minister of Public Health of the People's Republic of Bosnia and Herzegovina, the Head of the Department of Neuropsychiatry at the Faculty of Medicine in Sarajevo, and an Academician of the Academy of Sciences and Arts of Bosnia and Herzegovina. (Nedo Zec [in Bosnian]. U: Lincender-Cvijetić L. Editor. Spomenica 70 godina ANUBiH. Sarajevo: Akademija nauka i umjetnosti BiH; 2021. p. 488-489.).

[61] Pavao Stern (Varaždin, Mar.17, 1913 – Zagreb, Mar. 20, 1976) graduated from the Faculty of Medicine in Zagreb in 1936. From 1946 he was an assistant professor at the Department of Pharmacology of the Faculty of Medicine in Zagreb, and from 1947 until the end of his life, he was a full professor at the Faculty of Medicine in Sarajevo, where he founded the Institute of Pharmacology. He was appointed a full member of the Scientific Society of Bosnia and Herzegovina in the Department of Medical Sciences in 1952. From the establishment of the Academy of Sciences and Arts of Bosnia and Herzegovina in 1966, he continued to be a full member. From 1976, he was also a corresponding member of the Yugoslav Academy of Sciences and Arts in Zagreb. (Pavao Štern [in Bosnian]. U: Lincender-Cvijetić L. Editor. Spomenica 70 godina ANUBiH. Sarajevo: Akademija nauka i umjetnosti BiH; 2021. p. 400-401.)

[62] The Scientific Society of B&H operated on July 2, 1951, until it became the Academy of Sciences and Arts of B&H on June 22, 1966.

[63] Journal of the Scientific Society of Bosnia&Herzegovina.

[64] Živko Žika B. Marković (Lazarevac near Belgrade, 1889 – ?, after 1953) obtained the title of medical doctor in Basel in 1919. He was a county doctor in Lazarevac and Ub (1920–1924), and then, until his retirement in 1943, he worked as paediatrician of the Institute for Health Protection of Mothers and Children. After the end of the Second World War, in March 1945 he was reactivated and appointed head of the department at the Bacteriological and Epidemiological Institute in Belgrade. He retired in 1946. (AY, F-39, Personnel files, File of Dr. Živko Marković, f. 100 [in Serbo-Croatian]).

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[67] Deca i Roditelji 1934, editors Milivoje Sarvan and Žika Marković.

[68] Bosnia and Herzegovina daily newspaper.

[69] The first Bosnian-Herzegovinian health and educational Journal.

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[71] The bibliography of medical literature of Prof. Dr. Milivoje Sarvan is part of the overall project "Academic Milivoje Sarvan: Founder of Modern Paediatrics in Bosnia and Herzegovina" and will be published as a separate publication in the next issue of the same journal.

[72] The first medical professional and scientific journal in Bosnia and Herzegovina, founded in 1946.

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Lyssa: Goddess, Drug, Illness and Shield in Hellenic Antiquity

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Abstract
The aim of this historical review is to present the beliefs of the ancient Greeks related to lyssa and how the mythology surrounding this disease was created. In Greek antiquity Lyssa was a secondary goddess, a personification of a zoonotic disease which could be transmitted after an animal bite. Also named hydrophobia, the illness lyssa presented with an acute loss of mental stability, offensive frenzy and madness, and fear of water in the patient, who was seen to be possessed by a daemon as a divine punishment. In the Trojan War, lyssa was seen as a drug to Greek warriors, to demonstrate unreal power during battle. Homer was the first to refer to the hound of Orion, who was the greatest ancient Greek hunter. The hound, named Sirius, as a carrier of lyssa, was used as a bio-weapon to inflict death among the Trojans. Soranus of Ephesus and Galen gave descriptions of the disease, and proposed a sponge soaked with various herbal drugs as a therapeutic measure. The Greco-Roman physician Caelius Aurelianus noted that ancient Greeks knew about lyssa, and was the first to suggest that this was a neuro-disease. Lyssa was a figure in Greek Tragedy, depicted as a young female with a dog-like crown, related to Erinyes and Maniae. **Conclusion.** Lyssa was noted as a disease in Hellenic literature more than 2500 years ago. It was used as a bio-weapon to inflict madness. This vignette reveals Lyssa within a historical framework for the reader to understand the disease's origins.

Key Words: Lytta ▪ Rabies ▪ Zoonotic ▪ Iliad ▪ Ancient Greece.

Introduction

Lyssa, a bullet-shaped neurotropic virus with a strong affinity for nerve tissue, was considered to be a daemon which possesses the brain. It is a virus which causes hydrophobia, aerophobia, malaise, anxiety, paralysis, and focal or generalized seizures, followed eventually by coma and finally death. This "bullet" of nature, known since prehistoric times, became a weapon in the era of the Trojan War, and was seen as a goddess whose fatality should be celebrated, and a disease well described by Greek scholars through the ages (1). Many believe that lyssa was unknown to Homer (2). However, a closer look at the war described in The Iliad demonstrates the opposite (3). In ancient Greece Lyssa (Greek λύσσα), also known as Lytta in Attic Greek, meant fury, rage, furious rush, insanity, madness, and mania (4). The modern term "rabies" derives from the Latin "rabere", meaning infection of madness, a patient talking like a raven (5).

Regarding other possible etymological origins of the word lyssa, it may derive from the ancient Greek verb "λύω" (lyo), which among other things means "to leave", "to unleash", while the noun "λύσις" (lysis) means loss and dissolution, rendering the disease a neuro malady, as it leads to an acute loss of rational abilities, unleashing a mental frenzy (6). Lyssa seems to have been highlighted in many works of ancient Greek literature. Epic poems, tragedies and medical treatises celebrate this mental peculiarity. In relation to this concept, it can be argued that, depending on the era, the social ethics and the authors who dealt with it, lyssa was understood and described in various ways.

This historical review aims to accentuate the climax of the use of the term Lyssa/lyssa, ranging

from a disease to a weapon, and finally to a chthonic goddess in Hellenic antiquity.

The Goddess

In Greek mythology, Lyssa was a goddess, the daughter of Nyx (Greek: νύξ, the night, darkness), born from Uranus' blood, which dripped soon after his castration by Cronus (4, 7-9). Lyssa was a kindred form of the Erinyes and Maniae, causing mad rage in any person or animal her ailing hounds hunted (7, 9). An inhabitant of Erebus, the underground world of chaos, the personified spirit of crazed frenzy in both humans and dogs, a daemon, she was a major figure in Athenian tragedy. Aeschylus presented her as an agent of Dionysus, sent to drive the Minyades mad, while Euripides, in his "Heracles Furens", narrates the incident when the goddess Hera sent her to inflict Heracles with madness (7).

On an Athenian Krater (vase), she is implicated in the myth of Actaeon, depicted standing beside him, as he is torn apart by his maddened hounds. In this scene, she appears as a young female, dressed in a short skirt and crowned with a dog's head, signifying her dominion over the hounds (Figure 1) (10). If someone dared to offend Lyssa, she would possess him, resulting in a loss of mind, logic and behavior control. This concept provides further insights about how such behavior was viewed within the ancient Greek culture - as a disease inflicted by gods who possess you (11). Lyssa acted in order to provoke a crime, whereas the action of the Erinyes followed the crime, and she was able to produce terrible deeds, or make the possessed act in a certain way (12).

Figure 1. Lyssa (Λύσα, second figure from the left) and her hounds, a detail from an Athenian red-figure krater vase c. 5th century BC, Museum of Fine Arts, Boston.

The Disease

In the epic poem "The Iliad", Homer uses the word "lyssa" in a series of fragments, mainly to convey the unrestrained rage of war, the furious drive of the warriors towards killing or possible death. The term used is "μαίνομαι" (mainomai, being possessed by mania), meaning to be driven to frenzy and madness by the goddess Lyssa. Ajax, the great warrior, was possessed in order not to cause greater harm to the Trojans, and was driven to attack sheep. In another part of The Iliad, when Achilles learns that Hector has killed his friend Patroclus, he returns to the battlefield fighting "like a rabid dog" (ο Αχιλλέας με λύσσα εντρόπιαζε τον Έχτορα. Rhapsody Ω, verses 22-24) (3).

Medico-philosophers of the era categorized the disease as a zoonosis found in wolves, bears, leopards, horses, donkeys, bats and dogs. Humans could be infected after being bitten by an infected animal. The first description is found in the works of the Greek physician Rufus of Ephesus (late 1st and early 2nd centuries AD), who emphasized mental disturbance, paranoia and fear of water, proposing a sponge should be placed on the wound as treatment, soaked with "absinthe and aristolochia and wolfberry, and a decoction of river crabs, garlic, parsley, and gentian root" (13). Hydrophobia was used as a term by the Atomic philosopher Democritus (c. 460-370 BC) for a fever causing dehydration. The patient was tormented by an intolerable thirst, which resulted in the most obvious symptom, which was the fear of water, also observed in sick animals. Hippocrates (c. 460-377 BC), who without actually naming the disease, claimed that patients "in a frenzy drink very little, are disturbed and frightened, tremble at the least noise, seized with convulsions" (14). This concept was also suggested by the Greek physician

Galen of Pergamon (129-216 AD), naming the disease hydrophobic (Greek: νόσημα ὑδροφόβον) (15). They tried to throw the possessed into deep water, including children who could not swim, so that they would drown (16). All these named the disease "hydrophobia", as an alternative name to lyssa.

The Hoplon

A Hoplite was a citizen soldier of the ancient Greek states, carrying weaponry to defend his position (4). One of these weapons, a hoplon (ὅπλον), was used in the Trojan War, but it was different in nature. Greek warriors who campaigned against Troy used lyssa as a drug, acting as a shield to enable them to achieve impossible results during battle, while they also used dogs as carriers of the disease to inflict death through their saliva. Homer described Sirius (Greek: Σείριος, meaning flaming), Orion's hound, which was sent to attack the Trojans, possessed by Lyssa, to cause traumatic death (3). This use of a bio-weapon in a battle during the siege of Troy was among the first references to a different kind of warfare.

Discussion

Among all the features of mankind's experience, illness has been one of the most constant. The earliest etiological explanation of diseases in ancient Greece was that they were divine in origin, a symptom of a god's displeasure, a punishment for moral misdeeds. The remedy was sought in prayer, purification, votive offerings and sacrifices, to seek the favor of the gods. This attitude towards disease, referring to the supernatural for relief, reflected the paucity of therapeutic measures available in antiquity against diseases of often epidemic proportions. Thus, the goddess Lyssa was the bringer of a neuro-brain disease with chthonic characteristics, related to mental darkness, and fatality, with inevitable death in agony. Her hounds alleviated the nature of the punishment, whilst revealing some understanding of the disease itself (17). The goddess of hunting, Artemis, was thought to be the healer of rabies (18).

The Greco-Roman physician Caelius Aurelianus (c. mid. 5th century AD) testified that ancient Greek physicians knew about the disease. Meanwhile, in his effort to present the most vivid description, he noted that patients "have difficulty performing usual movements, experience light, turbulent, and intermittent sleep, yawning constantly, which is probably an indication of both physical and mental weakness. They become annoyed by the weather, complaining about a dirty southerly wind, despite clear and calm weather, and they can barely tolerate rain". He was among the first to categorize lyssa clearly as a brain disease, and opposed Eudemus (c. 4th century AD) who compared lyssa to melancholy, due to the similarity in the manifestation of fear, by highlighting the acute nature of lyssa and the chronic nature of melancholy, thereby setting apart these two different forms of mental illness (19).

The earliest reference to death from a dog-bite may be found in the laws of Eshnunna from Mesopotamia, dated c. 2200 BC, while in Old Babylonian texts, fragments exist about a dog disease which could render humans mad, or cause them to become frenzied. Even though rabies cannot justifiably be incriminated as the cause of death, these references give strong evidence that enzootic diseases were known many millennia ago. This could suggest that in Homer's era, lyssa was a notorious malady, a disorder of the structure or function of the brain, in a person who seemed to be possessed by a daemon (20).

Dogs in ancient Greece enjoyed an important place, alongside goddesses such as Hecate, while most famous warriors, such as Alexander the Great, were accompanied by hunting and fighting dogs (Figure 2). Alexander's mother Olympia was credited with introducing Molossians from her native Illyria. Meanwhile, Xenophon wrote a treatise entitled "*Kynegetikos*" (The Hunting Dogs), referring to dogs and their use in hunting and war. Thus, Homer, when mentioning Sirius, was referring to a usual habit in ancient Greek culture (21).

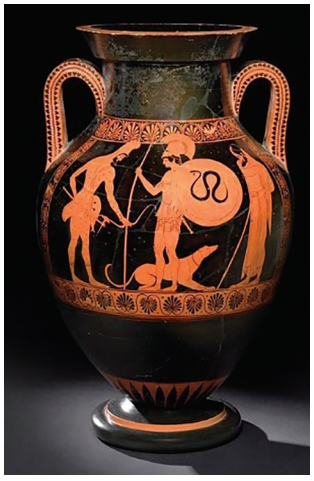

Figure 2. Departure of a Warrior. In the center a young warrior stands, facing to his left a bearded archer in a Phrygian costume, who is looking downward, as if in grief. At his feet lies a large hound of mastiff breed, looking up at him. Attic red figured amphora, c. 510-500 BC, British Museum.

Conclusion

From the archaic period of ancient Greece and throughout the Bronze Age and the Classical Period, lyssa was considered a disease, as well as a secondary goddess, and was used as part of the Greek war arsenal. Homer mentioned the condition known as lyssa (rabies) in hounds, and from that time philosophers and physicians went on to determine its nature. Lyssa epizootics existed in the Greek world, and had direct and indirect consequences for the local human population, also molding ancient Hellenic mythology.

What Is Already Known on This Topic:

Lyssa is a viral disease, transmitted though the saliva of an infected animal soon after a bite. The disease was known in ancient Greece.

What This Study Adds:

This historical review collects references by various ancient Greek philosophers and physicians, in order to present the disease as it was understood in that era. The paper emphasizes a different approach for the reader to comprehend the origins of a disease which is still endemic worldwide.

Conflict of Interest: The author declares that he has no conflict of interest.

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