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Dr. Maša Živanović (1890–1960) in the outpatient clinic of the Dispensary for Mothers and Children on Musala Street in Sarajevo, c. in 1925. Archives of Maša Živanović, with permission of the family.

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The Efficacy and Tolerability of a Fixed Combination of Perindopril and Indapamide in the Treatment of Unregulated Essential Hypertension – a Post-marketing Study

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Abstract

Objective. The objective of this non-interventional post-marketing clinical trial was to analyze the antihypertensive effect and safety of a fixed combination of perindopril and indapamide in the treatment of unregulated essential hypertension. **Patients and Methods.** The prospective clinical trial included patients aged 20 to 75 years with essential hypertension and blood pressure values ≥140/90 mmHg at baseline. On the basis of the investigator's decision, patients received 2 mg perindopril + 0.625 mg indapamide (group 2+0.625) or 4 mg perindopril + 1.25 mg indapamide (group 4+1.25). **Results.** The study included 1173 patients (426 patients in group 2+0.625 and 747 patients in group 4+1.25) at 27 investigational centers in Bosnia and Herzegovina. Mean blood pressure values at baseline and visits after nine months were significantly higher in the 4+1.25 group compared to the 2+0.625 group. There was a significant drop in systolic and diastolic blood pressure in both groups. The target values of systolic and diastolic blood pressure, according to the European Society of Cardiology (2018), were reached after nine months of therapy by more than 80% of patients in the 2+0.625 group, and this number was significantly higher compared to the 4+1.25 group where more than 60% of patients reached target values. Newly diagnosed patients had a better response to therapy. The percentage of patients receiving additional antihypertensive therapy decreased by the end of the study. Age, gender and the existence of diabetes mellitus were identified as negative predictors of target blood pressure achievement. The therapy showed a good safety profile. **Conclusion.** A fixed combination of perindopril and indapamide was effective and safe in the treatment of unregulated essential hypertension.

Key Words: Perindopril and Indapamide ▪ Fixed Combination ▪ Uncontrolled Hypertension.

Introduction

Hypertension affects about 900 million adults worldwide and is the leading global cause of death and disability (1). The Task Force for the Management of Arterial Hypertension of the ESC and the ESH recommend that when blood pressure-lowering drugs are used, the first objective should be to lower blood pressure to <140/90 mmHg in all patients (2). Provided that the treatment is well tolerated, treated blood pressure values should be targeted to 130/80 mmHg or lower in most patients, although in some groups the evidence is less compelling. In older patients (>65 years), systolic blood pressure should be targeted to between 130 and 140 mmHg, and diastolic blood pressure to <80 mmHg. Treated systolic blood pressure should not be targeted to <120 mmHg (3).

The guidelines from the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) from 2018 recommend initiating antihypertensive treatment with a two-drug combination of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARB), plus calcium channel blockers (CCB) or a diuretic, providing more rapid control of blood pressure than monotherapy (2). Use of an ACE inhibitor combined with a diuretic is a well-established antihypertensive combination that is very effective because of their different, yet synergistic, mechanisms of action (3). Contrary to commonly used thiazide diuretics that have negative metabolic effects, in terms of increasing the risk for diabetes and hyperlipidemia, indapamide has neutral metabolic effects (4-6). The antihypertensive effect of indapamide is due to its dual mechanism of action: both natriuretic diuretic and vasodilatory effects. It is highly lipophilic with a tendency to accumulate in the plasmatic membrane of smooth muscle cells, reducing transmembrane calcium flux, with a vasodilatory effect (7).

Although a fixed combination of perindopril and indapamide is standard care (2), there are no studies evaluating the efficacy and safety of this combination in Bosnia and Herzegovina. Also, it is of interest to evaluate independent predictors of target blood pressure achievement, and monitor concomitant medications and comorbidities in patients treated with this combination.

Therefore, the objectives of this study were: (i) to analyze the antihypertensive effect and safety of a fixed combination of perindopril and indapamide in the treatment of unregulated essential hypertension, (ii) to determine independent predictors of target blood pressure achievement, and (iii) to analyze concomitant medications and comorbidities in patients from Bosnia and Herzegovina.

Materials and Methods

Study Design

This prospective, non-interventional, post-marketing clinical trial was conducted in 27 investigational centers in Bosnia and Herzegovina. Patients aged 20 to 75 years with essential hypertension and blood pressure values ≥140/90 mmHg at baseline were included. On the basis of the investigator's decision, patients received either 2 mg perindopril+0.625 mg indapamide (Hypressin Plus® 2 mg/0.625 mg tablets, Bosnalijek d.d., Bosnia and Herzegovina) and were assigned to the 2+0.625 group, or 4 mg perindopril+1.25 mg indapamide (Hypressin Plus® 4 mg/1.25 mg tablets, Bosnalijek d.d., Bosnia and Herzegovina) and were assigned to the 4+1.25 group.

The exclusion criteria were: a positive history of angioneurotic edema, unregulated hypertension after administration of more than three antihypertensives, a mental/emotional disorder, malignant disease, severe liver and kidney damage, dialysis requirements, untreated decompensated heart failure, hypokalemia, pregnancy, breastfeeding, hypersensitivity to drug components, and concomitant use of drugs containing aliskiren, immunosuppressive, allopurinol or procainamide therapy.

The primary objective was defined as a reduction of blood pressure to normal values according to the ESC and the ESH guidelines (8, 9). The secondary objective was evaluation of the safety and tolerability of perindopril + indapamide (Hypressin Plus®) tablets in the treatment of unregulated essential hypertension.

Ethics Statement

The clinical trial was approved by the Agency for Medicinal Products and Medical Devices of Bosnia and Herzegovina. The Helsinki Declaration from 1975 and its amendments from 1983 were followed in all procedures. Before any procedure started, each patient signed an informed consent form.

Evaluation of Efficacy and Tolerability

The efficacy of perindopril + indapamide fixed combination in the treatment of non-regulated essential hypertension was evaluated by measurement of heart rate, and systolic and diastolic blood pressure. Tolerability was evaluated by monitoring the incidence of adverse drug events with an assessment of

the association between the use of the drugs and the occurrence of adverse reactions by the physician. Blood concentrations of potassium, sodium, creatinine, urea, and glucose were also monitored.

Data Collection

Data collection for each patient was performed over a nine month period (baseline, first follow up visit three months after baseline, second follow up visit six months after baseline, and third follow up visit nine months after baseline). At the baseline, demographic data about the patient were collected, together with their heart rate, systolic and diastolic blood pressure, and the results of laboratory tests (blood concentrations of potassium, sodium, creatinine, urea, and glucose). Previous concomitant therapy and newly included therapy were recorded. At the first and second control visits, heart rate, systolic and diastolic blood pressure, together with adverse events and therapy to be used or continued, were recorded. At the third and last follow up visit, the investigator recorded the heart rate, systolic and diastolic blood pressure, and the results of laboratory tests (blood concentrations of potassium, sodium, creatinine, urea, and glucose). Adverse events were monitored at all timepoints.

Statistical Analysis

The Kolmogorov-Smirnov test was used to determine normal distribution of numerical data. The results were presented as mean ($\bar{x}$) and standard deviation (SD) for data that followed normal distribution, or as median and interquartile range (IQR) for data that did not follow normal distribution. The average values of systolic and diastolic blood pressure estimated at different time intervals were shown with a 95% confidence interval. The differences in the mean values of heart rate, blood pressure, and laboratory parameters between the two treatment groups were tested by the Student t-test for independent samples if the variables followed normal distribution, and the Mann–Whitney U test for variables that did not follow normal distribution. To test the differences in blood pressure changes estimated at different time intervals (at baseline, and after 3, 6 and 9 months), the ANOVA (analysis of variance) test was used for repeated measurements, after which an appropriate *post hoc* test was applied. The differences in the proportion of patients who achieved target values of systolic and diastolic blood pressure between the groups were tested by the Chi square test. The logistic regression analysis was used to examine the independent predictors of predefined outcomes. Gender, age, newly discovered/pre-existing hypertension, duration of hypertension, diabetes mellitus, smoking, and concomitant antihypertensive therapy were covariates included in the logistics analysis. Outcome predictors were presented as odds ratio and a 95% confidence interval. Statistical significance was taken to be at the level of $P < 0.05$.

Results

The study was conducted in the period between June 2019 and November 2020. Out of 1373 patients screened, 1173 patients were enrolled (426 patients in the 2+0.625 group and 747 patients in the 4+1.25 group). Patients were monitored for the following nine months at three visits (after three, six and nine months) where blood pressure was assessed at each visit, and some patients changed treatment group, as decided by the investigator. A diagram of the flow of patient distribution into the therapeutic groups is presented in Figure 1.

At the baseline visit, patients in the 4+1.25 group compared to the 2+0.625 group were significantly older, had a higher body mass index (BMI), higher waist circumference, more diabetes mellitus, more pre-existing hypertension that had lasted longer, and higher mean values of systolic and diastolic blood pressure and blood glucose levels. No differences in potassium, sodium, creatinine and urea levels were observed between the two study groups (Table 1).

The antihypertensive concomitant therapy used during the study is shown in Table 2. The most common additional antihypertensive drug was a beta blocker in both treatment groups. The use of additional antihypertensive drugs decreased from baseline to the visit after six months (Table 2).

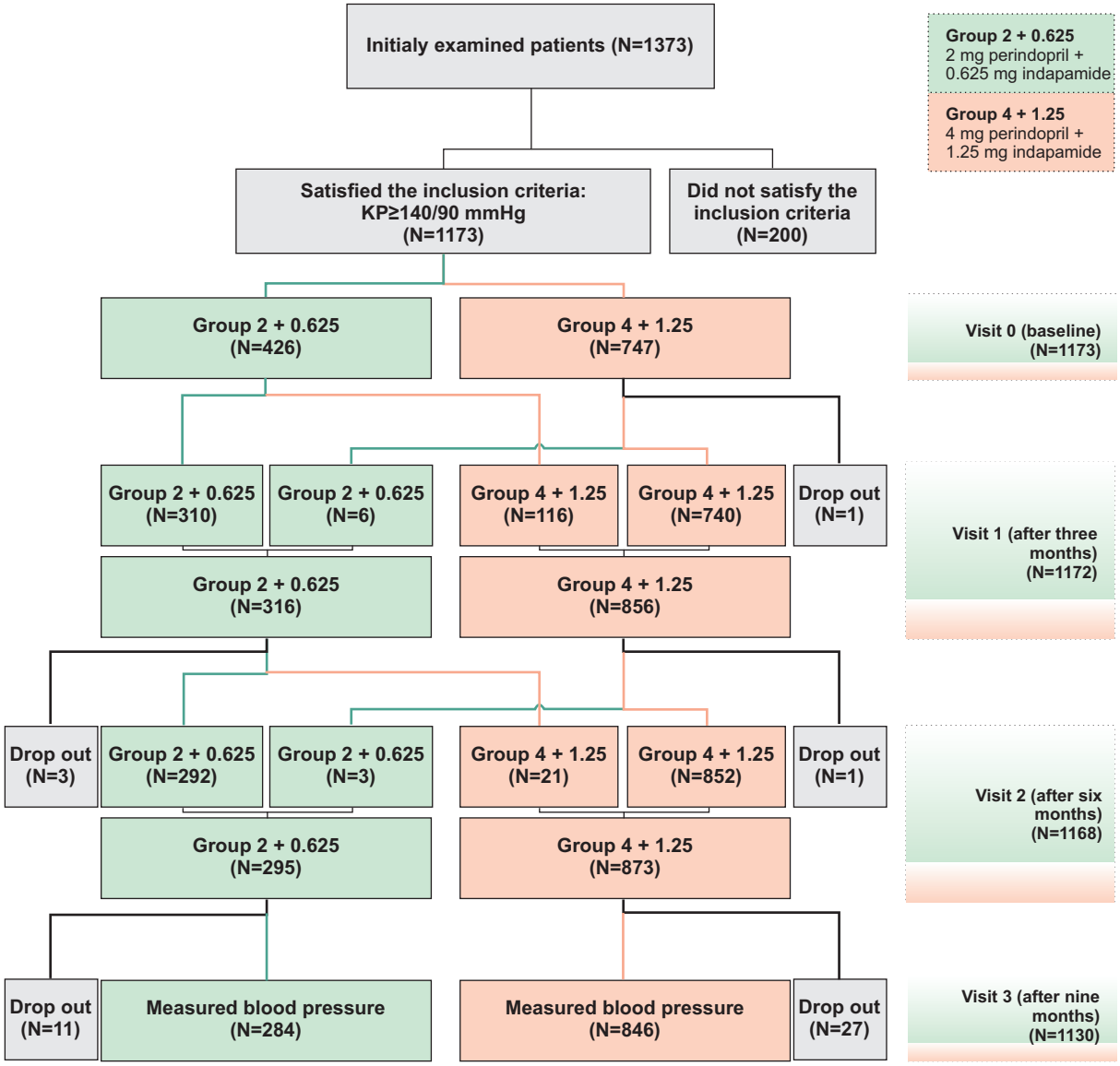

Figure 1. The flow of patient distribution into therapeutic groups

Table 1. Demographic and Clinical Characteristics of Patients with Unregulated Hypertension at the Baseline Visit in Relation to the Prescribed Therapy

Characteristics	Group 2+0.625 (N=426)	Group 4+1.25 (N=747)	P-value
Age (years)	53.6±11.6	59.8± 11.3	<0.001‡
Gender (Male/Female)	198 (46.9%)/224 (53.1%)	386 (51.9%)/358 (48.1%)	0.110‖
Height (cm)	173.2±12.6	174.6± 9.7	0.036‡
Weight (kg)	81.5±11.3	84.6±13.6	<0.001‡
Body mass index	27.1±3.4	27.9±3.8	0.001‡
Waist circumference (cm)	94.1±11.1	99.1±12.5	<0.001‡
Pre-existing HTN*	196 (46.0%)	528 (70.7%)	<0.001‖
Newly diagnosed HTN*	225 (52.8%)	216 (28.9%)	
HTN* duration (years)	5.0 (3.0-10.0)	8.0 (4.0-12.5)	0.001§
Smoker	201 (47.2%)	303 (40.6%)	0.030‖
Former smoker	32 (7.5%)	65 (8.7%)	
Number of cigarettes	21.6±8.8	22.0±8.3	0.650‡
Consumes alcohol	86 (20.2%)	155 (20.7%)	0.820‖
Sedentary lifestyle	186 (43.7%)	370 (49.5%)	0.070‖
DM†	51 (12.0%)	164 (22.0%)	<0.001‖
Type 2	40 (78.4%)	135 (82.3%)	-
Type 1	0	4 (2.4%)	-
Duration of DM† (years)	7.0 (3.0-10.0)	7.0 (5.0-10.0)	0.160§
Systolic blood pressure	156.2±10.6	161.7±14.3	<0.001‡
Diastolic blood pressure	94.7±5.5	97.3±6.9	<0.001‡
Heart rate	82.3±12.8	82.0±12.7	0.700‡
Potassium (mmol/L)	4.4±0.5	4.4±0.5	0.300‡
Sodium (mmol/L)	139.4±8.2	139.5±10.9	0.920‡
Creatinine (mmol/L)	91.6±18.5	93.0±18.4	0.230‡
Urea (mmol/L)	6.8±4.7	6.9±4.0	0.730‡
Glucose (mmol/L)	5.8±2.0	6.3±3.4	0.014‡

*Hypertension; †Diabetes mellitus; ‡Student t-test; §Mann–Whitney U test; ‖Chi-square test.

Table 2. Concomitant Antihypertensive Therapy at the Baseline Visit and after Three and Six Months of Follow-up in Relation to Therapeutic Groups

Visit	Antihypertensive therapy	Group 2+0.625 N (%)	Group 4+1.25 N (%)
Baseline visit	Calcium channel blockers	3 (0.7)	38 (5.1)
	Beta-blockers	23 (5.4)	57 (7.6)
	Diuretic	1 (0.2)	10 (1.3)
	ACE inhibitors	-	12 (1.6)
Visit after three months	Calcium channel blockers	2 (0.6)	52 (6.1)
	Beta-blockers	10 (3.2)	55 (6.4)
	Diuretic	-	9 (1.1)
	ACE inhibitors	-	25 (2.9)
Visit after six months	Calcium channel blockers	1 (0.3)	38 (4.4)
	Beta-blockers	2 (0.7)	34 (3.9)
	Diuretic	-	10 (1.1)
	ACE inhibitors	-	13 (1.5)

Systolic and Diastolic Blood Pressure During Therapy

In the 2+0.625 group, mean systolic blood pressure values dropped significantly from baseline to the visit after nine months, with a mean reduction of -27.9 mmHg; 95% CI (-29.4 to 26.5); P<0.001 (Table 3). Also, mean diastolic blood pressure values decreased significantly between baseline and the visit after nine months, with a mean reduction of -15.3 mmHg; 95% CI (-16.2 to -14.5); P=0.014 (Table 3).

In the 4+1.25 group, mean systolic blood pressure values dropped significantly from baseline to the visit after nine months, with a mean reduction of -29.9 mmHg; 95% CI (-30.8 to -28.9); P<0.001 (Table 3). Also, mean diastolic blood pressure values dropped significantly from baseline to the visit after nine months, with a mean reduction of -16.4 mmHg; 95% CI (-16.9 to -15.9); P<0.001 (Table 3).

Mean systolic and diastolic blood pressure values were significantly higher in the 4+1.25 group versus the 2+0.625 group at baseline and the visit after nine months (Table 3).

The mean reduction in systolic and diastolic blood pressure was significantly higher in the 4+1.25 group compared to the 2+0.625 group at all time points of patient follow-up (Table 4). The percentages of patients reaching the target values of systolic and diastolic blood pressure, defined according to ESC (2018) (8, 9), are presented in Table 5.

Table 3. Mean Values of Systolic and Diastolic Blood Pressure*

Blood pressure (mmHg)		Group 2+0.625 (Mean±SD)	Group 4+1.25 (Mean±SD)	P-value*
Systolic	Baseline visit	156.2±10.6	161.7±14.3	<0.001
	Visit after three months	141.4±10.6	143.0±13.8	0.380
	Visit after six months	132.8±10.8	135.7±10.5	0.650
	Visit after nine months	128.8±7.5	131.8±9.2	0.005
Diastolic	Baseline visit	94.7±5.5	97.3±6.9	<0.001
	Visit after three months	86.5±6.2	86.9±6.7	0.070
	Visit after six months	82.3±5.5	83.3±7.2	0.670
	Visit after nine months	79.6±4.3	81.1±5.5	0.020

*Student t-test.

Table 4. Average Reduction of Systolic and Diastolic Blood Pressure at Baseline and Three Follow up Visits*

Blood pressure (mmHg)		Group 2+0.625 Median with IQR*	Group 4+1.25 Median with IQR*	P-value†
Systolic	Three months vs. baseline	-14.8 (-15.7 to -13.9)	-18.5 (-19.3 to -17.7)	<0.001
	Six months vs. baseline	-23.7 (-25.2 to -22.2)	-26.0 (-26.9 to -25.1)	0.008
	Nine months vs. baseline	-27.9 (-29.4 to -26.5)	-29.9 (-30.8 to -28.9)	0.030
Diastolic	Three months vs. baseline	-8.2 (-8.7 to -7.6)	-10.4 (-10.9 to -9.9)	<0.001
	Six months vs. baseline	-12.3 (-13.4 to -11.0)	-14.0 (-14.4 to -13.4)	0.002
	Nine months vs. baseline	-15.3 (-16.2 to -14.5)	-16.4 (-16.9 to -15.9)	0.038

*Interquartile range. †Analysis of Variance (ANOVA) test followed by Tukey or Games-Howell post-hoc test.

Table 5. Proportion of Patients with Achieved Target Values of Systolic and Diastolic Blood Pressure according to European Society of Cardiology ECS (2018) in Relation to the Treatment Group

Achieved target blood pressure		Group 2+0.625	Group 4+1.25	χ^2 and P-value
Systolic	Visit after three months	107/426 (25.1%)	157/747 (21.0%)	χ^2=2.60; P=0.011
	Visit after six months	192/316 (60.8%)	391/856 (45.7%)	χ^2=21.00; P<0.001
	Visit after nine months	241/295 (81.7%)	538/873 (61.7%)	χ^2=39.60; P<0.001
Diastolic	Visit after three months	134/426 (31.5%)	220/747 (29.5%)	χ^2=0.52; P=0.510
	Visit after six months	199/316 (63.0%)	452/856 (52.8%)	χ^2=9.70; P=0.002
	Visit after nine months	246/295 (83.4%)	596/873 (68.3%)	χ^2=25.10; P<0.001

Independent Predictors of Achieving Systolic and Diastolic Blood Pressure Targets according to the ESC (2018)

In the logistic regression analysis model, we examined the predictors for achieving the target values of systolic and diastolic blood pressure according to the ESC (2018), after three, six and nine months of therapy.

In the 2+0.625 group, age was a negative predictor of reaching target systolic and diastolic blood pressure after six months of therapy. Male gender was a negative predictor of reaching target systolic blood pressure after nine months of therapy, and target diastolic blood pressure after three months of therapy. The presence of diabetes mellitus was a negative predictor of reaching target diastolic blood pressure after three months of therapy (Table 6).

In the 4+1.25 group, age was a negative predictor of reaching target systolic blood pressure at all time points evaluated. Male gender was a negative predictor of reaching target diastolic blood pressure after three and nine months of therapy. The presence of diabetes mellitus was a negative predictor of reaching target systolic blood pressure after three months of therapy, and reaching target diastolic blood pressure after nine months of therapy. The number of additional antihypertensive drugs was a positive predictor of reaching target systolic blood pressure after three months of therapy (Table 6).

Table 6. Independent Predictors of Reaching Target Systolic and Diastolic Blood Pressure Obtained by the Logistic Regression Analysis

Therapy duration	Predictor	B coefficient*	P-value	Odds ratio†
Independent predictors of reaching target systolic blood pressure				
Group 2+0.625				
Three months	None identified	-	-	-
Six months	Age	-0.030	0.013	0.97 (0.95–0.99)
Nine months	Male gender	-0.700	0.040	0.50 (0.27–0.98)
Group 4+1.25				
Three months	Age	-0.030	0.005	0.97 (0.96–0.99)
Three months	Diabetes mellitus	-0.600	0.027	0.57 (0.34–0.94)
Three months	Number‡	0.400	0.034	1.50 (1.03–2.10)
Six months	Age	-0.020	0.001	0.98 (0.96–0.99)
Nine months	Age	-0.020	0.003	0.98 (0.97–0.99)
Independent predictors of reaching target diastolic blood pressure				
Group 2+0.625				
Three months	Male gender	-0.600	0.009	0.55 (0.35–0.86)
Three months	Diabetes mellitus	-0.960	0.040	0.38 (0.15–0.96)
Six months	Age	-0.050	<0.001	0.95 (0.93–0.98)
Nine months	None identified	-	-	-
Group 4+1.25				
Three months	Male gender	-0.380	0.030	0.69 (0.50–0.96)
Six months	None identified	-	-	-
Nine months	Male gender	-0.020	0.008	0.98 (0.97–0.99)
Nine months	Diabetes mellitus	-0.500	0.008	0.61 (0.43–0.88)

*B coefficient showing the change in log odds that occur for a one-unit change in an independent variable when all other independent variables are kept constant; †95% confidence interval; ‡Number of added antihypertensive drugs.

Target values of systolic and diastolic blood pressure achieved in relation to the duration of hypertension

Compared to patients with pre-existing hypertension, a significantly higher number of patients with newly diagnosed hypertension reached the target values of systolic blood pressure after six months in both groups (Table 7). Compared to patients with pre-existing hypertension, a significantly higher number of patients with newly diagnosed hypertension in the 4+1.25 group reached the target values of diastolic blood pressure after three and six months. However, in the 2+0.625 group there was no significant difference in the proportion of patients who reached the target values in relation to the presence of hypertension (Table 7).

Heart Rate

Heart rate dropped significantly during the nine-month therapy in both study groups, although the mean values were within the reference range. The mean heart rate in the 2+0.625 group was significantly lower at the second and third follow up visits compared to the 4+1.25 group (Table 8).

Results of Laboratory Tests after Nine Months of Therapy

The mean values of potassium, sodium, creatinine, urea and glucose remained within the reference intervals, and there was no significant difference between the examined groups. Mean glucose values remained significantly higher in the 4+1.25 group (Table 9). Hypokalemia occurred in 1.0% patients in the 2+0.625 group and 0.5% patients in the 4+1.25 group (Table 9).

Table 7. Proportion of Patients with Pre-existing and Newly Diagnosed Hypertension Who Reached the Target Values of Systolic and Diastolic Blood Pressure in the Period of Three, Six and Nine Months of Follow-up in Relation to the Therapeutic Group

Therapy duration	Group 2+0.625			Group 4+1.25		
	Hypertension (mmHg)			Hypertension (mmHg)		
	Pre-existing	Newly diagnosed	P-value*	Pre-existing	Newly diagnosed	P-value*
Reaching target systolic blood pressure						
Three months	46/196 (23.5%)	60/225 (26.7%)	0.500	104/528 (19.7%)	53/216 (24.5%)	0.170
Six months	75/137 (54.7%)	116/174 (66.7%)	0.035	253/586 (43.2%)	138/267 (51.7%)	0.022
Nine months	100/122 (82.0%)	138/168 (82.1%)	1.000	361/600 (60.2%)	178/270 (65.9%)	0.110
Reaching target diastolic blood pressure						
Three months	54/196 (27.6%)	79/225 (35.1%)	0.110	140/528 (26.5%)	79/216 (36.6%)	0.008
Six months	87/137 (63.5%)	110/174 (63.2%)	1.000	295/586 (50.3%)	157/267 (58.8%)	0.020
Nine months	105/122 (86.1%)	136/168 (81.0%)	0.270	411/600 (68.5%)	185/270 (68.5%)	1.000

*Chi-square test.

Table 8. Heart Rate in Patients in Relation to the Treatment Group during the Nine-month Follow-up. Data Are Presented as Mean±SD*

Heart rate	Group 2+0.625	Group 4+1.25	P-value*
Baseline visit	82.3±12.8	82.0±12.7	0.700
After three months	77.2±9.7	77.3±9.6	0.820
After six months	74.3±8.0	75.4±8.6	0.040
After nine months	72.6±7.3	73.8±8.1	0.010
P-value† (baseline visit vs. nine months)	<0.001	<0.001	

*Student t-test. †Analysis of Variance (ANOVA) test followed by Tukey or Games-Howell post-hoc test.

Table 9. Laboratory Parameters After Nine Months of Follow-up in Relation to Therapeutic Groups. Values are Presented as Mean±SD or Absolute Numbers and Percentages

Parameters (mmol/l)	Group 2+0.625	Group 4+1.25	P-value*
Potassium	4.4±0.4	4.6±0.5	0.500
Sodium	139.9±5.0	139.7±6.2	0.920
Creatinin	91.4±54.9	93.9±57.8	0.550
Urea	6.4±3.9	7.0±5.9	0.130
Glucose	5.5±1.3	5.9±1.4	<0.001
Hypokalemia†	3/295 (1.0%)	4/873 (0.5%)	-

*Student t-test; †<3.5 mmol/l.

Adverse Events

The most common side effects at the first follow up visit were nausea in the 2+0.625 group (1.2%) and cough in the 4+1.25 group (0.4%) (Table 10). The prevalence of side effects during the subsequent visits decreased, and after nine months of therapy, a cough was present in only one patient in the 2+0.625 group and in 2 patients in the 4+1.25 group (Table 10).

Discussion

In this prospective, non-interventional, post marketing study, a fixed combination of perindopril and indapamide was shown to be effective and safe in the treatment of unregulated essential hypertension. Mean blood pressure decreased significantly, and most of the patients reached the blood pressure target after nine months of therapy with a better response to therapy in newly diagnosed patients. Usage of additional antihypertensive therapy decreased over time. Changes in blood pressure were accompanied by decreases in heart rate. Negative predictors of target blood pressure achievement were age, gender and co-existence of diabetes mellitus.

Diabetes and hypertension are considered as "bad companions." Large hypertension outcome trials comparing antihypertensive drugs with a placebo or usual care in patients with diabetes and hypertension have only compared thiazide type

Table 10. Adverse Events in Patients after Three, Six and Nine Months of Therapy in Relation to Treatment Groups

Therapy duration (months)	Adverse event	Group +0.625 (N; %)	Group 4+1.25 (N; %)
Three	Cough	4 (0.9)	3 (0.4)
	Nausea	5 (1.2)	2 (0.3)
	Headache	-	1 (0.1)
	Diarrhea	-	2 (0.3)
	Dizziness	-	1 (0.1)
	Tinnitus	-	2 (0.3)
	BP* oscillation	-	1 (0.1)
Six	Cough	3 (0.9)	1 (0.1)
	Tachycardia	1 (0.3)	-
	Hypotension	-	2 (0.2)
	Constipation	-	1 (0.1)
	Dizziness	-	1 (0.1)
	Tinnitus	-	1 (0.1)
Nine	Cough	1 (0.3)	2 (0.2)
	Hypotension	-	1 (0.1)

*Blood pressure.

diuretics and calcium-channel blockers. These drugs have been shown to reduce cardiovascular disease events and mortality. In the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation) study, fixed combination perindopril and indapamide, as the thiazide type diuretic, significantly reduced all-cause mortality by 14%, cardiovascular disease mortality by 18% and combined macrovascular and microvascular events by 9% and reduced separate macrovascular outcomes by 8% and microvascular outcomes by 9%, although not significantly (10). In patients with diabetes, a combination of a RAS-blocker and a thiazide-type diuretic might be the most reasonable initial antihypertensive regimen (10).

As shown in clinical studies, gender, as another negative predictor of target blood pressure has an influence on the dose-ranging of perindopril+indapamide combination in hypertension with its effects on systolic and pulse pressure. In hypertensive subjects, the low dose combination perindopril+indapamide (2+0,625) mg and (4+1,25) mg was the most effective in reducing blood pressure and avoiding hypokalemia compared to other combinations of perindopril and indapamide doses, and this result was more pronounced in women (11).

When considering age, resistant hypertension is more prevalent in elderly patients. In the Hypertension in the Very Elderly Trial (HYVET), patients in the "late elderly" group (≥ 80 years of age with elevated SBP) were randomized to receive indapamide, with the addition of perindopril if needed, or a placebo. In this study, the patients in the indapamide group had a 30% risk reduction for fatal and non-fatal stroke (12).

The results of our study are consistent with previous studies, and the therapy is in accordance with the European guidelines (ESC/ESH 2018) for the treatment of moderate to severe hypertension, that highly recommend initiation of treatment with a single pill combination containing two drugs (13). These single pill combinations have been found to be effective and to control blood pressure faster, especially when monotherapy is inadequate to achieve the target range of blood pressure (10, 11). The efficacy and safety of perindopril (an ACE inhibitor)+indapamide (a chlorosulphamoyl diuretic) has been proven through many studies. In daily medical practice, a combination of ACE inhibitors and a diuretic is the drug of choice for initial therapy or maintenance therapy. The combination of perindopril and indapamide has synergistic activity, resulting in lower required doses compared to monotherapies (10-12). Analysis of data from nearly 30,000 patients showed that 2/3 of patients (mostly on monotherapy: ACE-inhibitors, calcium channel antagonists and diuretics) do not have controlled blood pressure. In the PRETEND study (N=3,198 patients) 2/3 patients received concomitant therapy (lipid-lowering therapy, antithrombotic and antidiabetic therapy) and perindopril+indapamide was included as the first drug of choice in the treatment of blood pressure, or used as a replacement drug for prior antihypertensive therapy. Therapy with a fixed combination of perindopril+indapamide reduced blood pressure with a significantly improved control rate from 1.1 to 38.7%. The systolic blood pressure control rate improved from 3.1% to 44.15% and diastolic blood pressure control rate improved from 20.5% to 77.5%. The study confirmed the beneficial action of 2 mg perindopril+0.625 mg indapamide in daily clinical practice, where this combination effectively reduced blood pressure rates and pulse pressure in various patients (14). This fixed combination is more effective than monotherapy with 10 mg enalapril in the treatment of hypertension and subclinical organ damage, as well as cardiovascular events. The PICXL study showed that perindopril+indapamide, besides reducing hypertension, has positive effects on hypertrophy of the left ventricular and large blood vessels. Further, perindopril + indapamide reduces systolic and diastolic blood pressure, and reduces the albumin excretion rate (AER) in patients with type 2 diabetes (T2DM) (11, 15).

A multicenter, prospective, observational study showed that the fixed combination of 4 mg perindopril + 1.25 mg indapamide was effective in more than 90% of uncontrolled or newly diagnosed

patients with moderate to severe arterial hypertension, including patients with diabetes. During 90 days of therapy, systolic and diastolic blood pressures were significantly reduced and blood pressure was less than 140/90 mmHg (13).

In a study including 11,140 patients with T2DM (≥55 years), with isolated systolic hypertension, perindopril + indapamide combinations (2 mg+0.625 mg and 4 mg+1.25 mg) per day reduced mortality and major macrovascular and microvascular events, renal complications, and overall coronary diseases (16). In patients with T2DM, it is especially important to reduce cardiovascular and kidney diseases. Extensive data from clinical trials show that perindopril+indapamide therapy reduces mortality and vascular events in patients with T2DM (13, 17, 18). In obese patients, or those with metabolic syndrome, current recommendations for the treatment of hypertension (ESC/ESH, ACC/AHA, ISH) are not specified, but a single pill combination containing two drugs is preferred (3).

In our study, the treatment was well tolerated. The most common adverse reactions were cough and nausea, and all reactions were reduced by the end of the study (ninth month of therapy). Levels of sodium, potassium, creatinine, and urea were within the reference intervals, and there was no significant difference between the examined groups. The perindopril+indapamide single pill combination shows a higher antihypertensive effect with a smaller number of side effects compared to antihypertensive monotherapy. Co-administration of the two agents reduces the incidence of hypokalemia seen with indapamide alone (13, 14, 19). Other studies have also shown that adverse events with the fixed combination perindopril+indapamide (from 2 mg+0.625 mg to 8 mg+2.5 mg) are mild, and that this combination has a favorable safety profile in patients with mild, or moderate to severe hypertension (13, 20). Adverse events, such as a dry cough, headache, high fever, gastroesophageal reflux diseases, giddiness and paronychia, are often not associated with the therapy (13). Perindopril+indapamide therapy does not significantly change lipid parameters and serum electrolytes. Furthermore, there were no significant differences in the changes in carbohydrate metabolism parameters. Many studies have demonstrated the metabolic neutrality of this combination, and showed that it does not induce changes in potassium, creatinine, lipid and glucose profiles (11, 14, 17).

To our knowledge this is the first study to evaluate the use of another antihypertensive drug during perindopril+indapamide therapy. It was found that beta blockers are the most common antihypertensive therapy used along with the investigated fixed dose combination. The use of this concomitant therapy decreased during the study. The results suggest that patients receiving perindopril + indapamide reached the blood pressure target and the need for additional antihypertensive drugs decreased.

Limitations of the Study

The study was not placebo or comparator controlled, and the duration of the follow-up was nine months.

Conclusion

The fixed combination of perindopril and indapamide was effective and safe in the treatment of unregulated essential hypertension. There was a significant drop in systolic and diastolic blood pressure in both groups, while adverse events were mild and their number decreased over time. Newly diagnosed patients had a better therapy response. Age, gender and the existence of diabetes mellitus were identified as negative predictors of target blood pressure achievement.

What Is Already Known on This Topic:
The perindopril and indapamide single pill combination is effective therapy for essential hypertension, and known for fast achievement of target blood pressure values. It is recommended especially when monotherapy is inadequate for achieving the target range of blood pressure.

What This Study Adds:
This is the first prospective study conducted on the fixed combination of perindopril and indapamide in Bosnia and Herzegovina especially focusing on the treatment of unregulated essential hypertension. The results of this study indicate the risk for poor blood pressure control in patients with hypertension and diabetes mellitus. Thus, it is of significant importance for health workers in our country to raise awareness about existing diabetes mellitus as a frequent comorbidity with hypertension.

Authors' Contributions: Conception and design: AŠ, JDžJ, ATA, UG and MM; Acquisition, analysis and interpretation of data: AŠ, UG, ŽP, AB, MTČ and MM; Drafting the article: JDžJ, ATA, UG and MM; Revising it critically for important intellectual content: AŠ, ŽP, AB, MTČ and MM; Approved final version of the manuscript: AŠ, JDžJ, ATA, UG, ŽP, AB, MTČ and MM.

Conflict of Interest: Aziz Šukalo, Jasna Džananović Jaganjac, Amna Tanović, Una Glamočlija, Meliha Mehić disclose the following relationships – they are employees of Bosnalijek d.d., a pharmaceutical company producing perindopril and indapamide -based products. Bosnalijek d.d. had a role in the design of the study, in the collection, analyses, and interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

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Endoscopıc Anatomy of the Lacrımal Sac: A Cadaverıc Study

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Abstract

Objective. To describe the anatomy of the lacrimal sac in relation to the lateral nasal wall by cadaver dissection, and to measure the distances of surgically important landmarks from relevant structures for safer and more efficient surgery. **Method.** A total of 12 endoscopic dacryocystorhinostomy (DCR) were performed on both sides (right and left) of 6 fresh-frozen cadavers. The distances of the lacrimal sac to the posterior edge of the uncinate process, the frontal process of the maxilla, the maxillary ostium, the nasal vestibule, the middle turbinate attachment and the inferior turbinate were measured. In addition, the width and length of the lacrimal sac were measured. **Results.** The mean width and length of the lacrimal sac were 5.6 mm and 11.1 mm, respectively. The lacrimal sac was located at 15.2 mm from the posterior edge of the uncinate process, at 35.5 mm from the nasal vestibule, at 13.5 mm from the maxillary ostium, at 12.2 mm from the frontal process of the maxilla, at 8.7 mm from the middle turbinate attachment, and at 7.3 mm from the inferior turbinate. **Conclusion.** This study provides additional measurements regarding the lacrimal sac and its relationships with nearby landmarks for use in endoscopic dacryocystorhinostomy. The distances of the lacrimal sac to the nasal vestibule, the uncinate process and the frontal process of the maxilla are not as reliable as the middle turbinate attachment for predicting the anatomic localization of the lacrimal sac during DCR.

Key Words: Dacryocystorhinostomy ▪ Endoscopic Endonasal Approach ▪ Lacrimal Pump System ▪ Nasolacrimal duct.

Introduction

Although dacryocystorhinostomy (DCR) has been performed via an external approach in the past, it has been replaced by an endoscopic endonasal approach, with the introduction of endoscopy. One reason for this is that it prevents the cosmetic deformities caused by external methods. Other reasons are that endoscopy allows clinicians to observe the inside of the body by providing a clear and wide viewing angle in the endonasal region, and success is achieved without damaging the lacrimal pump system by predicting the localization of the nasolacrimal duct using landmarks. The endoscopic DCR technique was described in 1989 (1). Many studies have been conducted on endoscopic DCR until today. Most of these studies have emphasized that the most important point to be considered for the success of endoscopic surgery is the DCR incision site. Many landmarks, especially the middle turbinate and maxillary line, have been identified for mucosal incision and osteotomy (2, 3). Although many landmarks have been described for endoscopic DCR, there is not enough information about the relationship of these landmarks to the lacrimal sac.

This study aimed to make endoscopic DCR surgery more reliable and successful by revealing the relationships of these landmarks with the lacrimal sac using measurement data. Unlike other studies, we demonstrate the relationship of the lacrimal sac with many landmarks in the same study, and evaluate the most reliable one.

Materials and Methods

A total of 12 endoscopic DCR procedures were performed on both sides (right and left) of 6 fresh-frozen cadavers. A 0-degree rigid endoscope (0^0, Storz Hopkins, Germany) was used during this procedure. A superior-based mucosal flap was removed approximately 5 mm above and 10 mm in front of the middle turbinate attachment (Figure 1).

Before lifting the flap, information about the localization of the lacrimal sac was obtained through the nasal cavity by placing a light source parallel to the lacrimal punctum (Figure 2). After the mucosal flap was removed (Figure 3), a bone window was opened with the help of a chisel and hammer to reveal the entire lacrimal sac. The distances were measured of the anterior border of the lacrimal sac's mid-height, to the posterior edge of the uncinate process's mid-height, posterior edge of the frontal process of the maxilla's mid-height, anterior edge of the maxillary ostium, and nasal vestibule. The distances were measured between the midline of the lacrimal sac and the middle turbinate attachment, and between the lower border of the lacrimal sac's mid-width and the most medial point of the inferior turbinate (Figure 4). We evaluated the

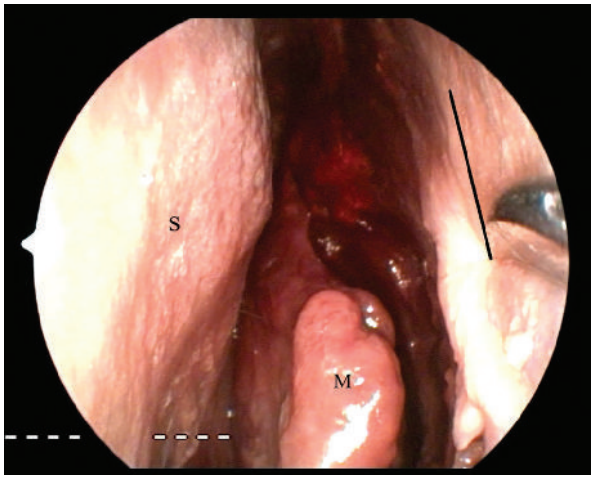

Figure 1. A superior based mucosal flap was prepared with a lancet. The line shows the anterior incision site. S=Septum; M=Middle turbinate.

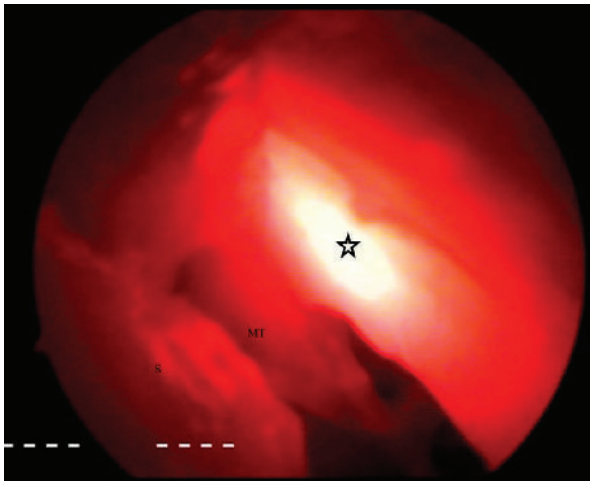

Figure 2. The lacrimal sac was located by placing a light source parallel to the lacrimal punctum. S=Septum; MT=Middle turbinate; Star=Lacrimal sac.

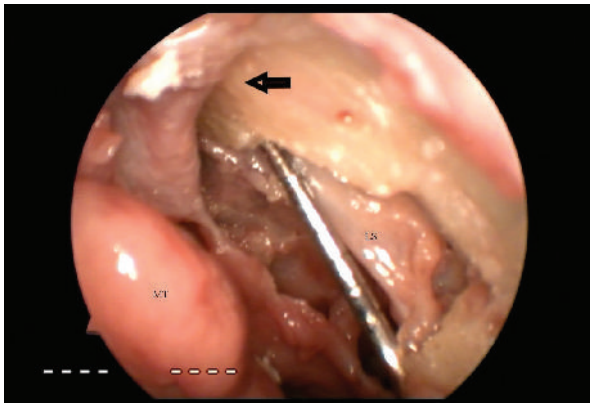

Figure 3. After the mucosal flap was removed. Arrow=Mucosal flap; MT=Middle turbinate; LS=Lacrimal sac.

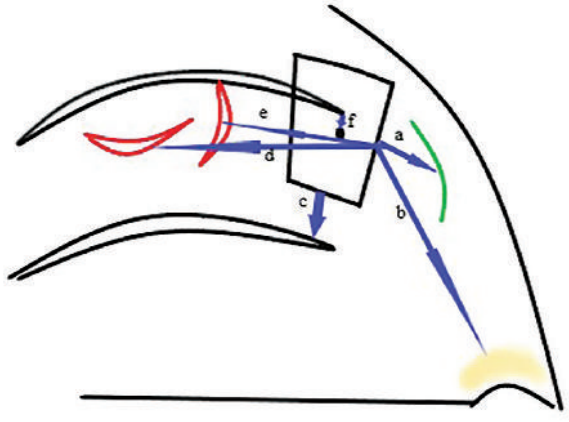

Figure 4. Diagram of the measurements. Distances from the anterior border of the lacrimal sac's mid-height, to (a) posterior edge of the frontal process of the maxilla's mid-height, (b) nasal vestibule, (d) anterior edge of the maxillary ostium, (e) the posterior edge of the uncinate process's mid-height and from the midline of the lacrimal sac to (f) the middle turbinate attachment, and (c) between the lower border of the lacrimal sac's mid-width and the most medial point of the inferior turbinate.

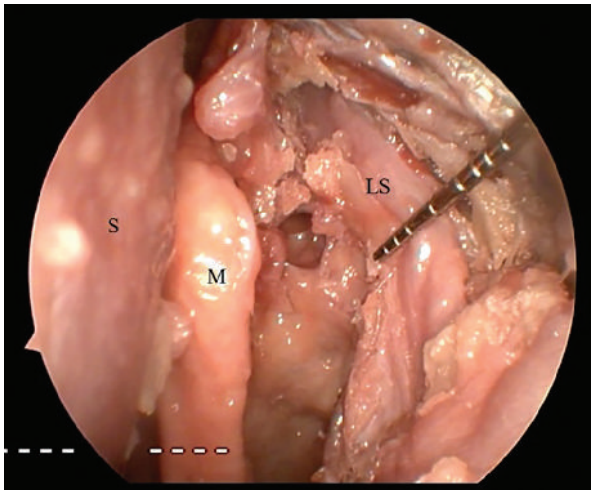

Figure 5. The measurement of the lacrimal sac width was seen on the photograph. LS=Lacrimal sac; S=Septum; M=Middle turbinate.

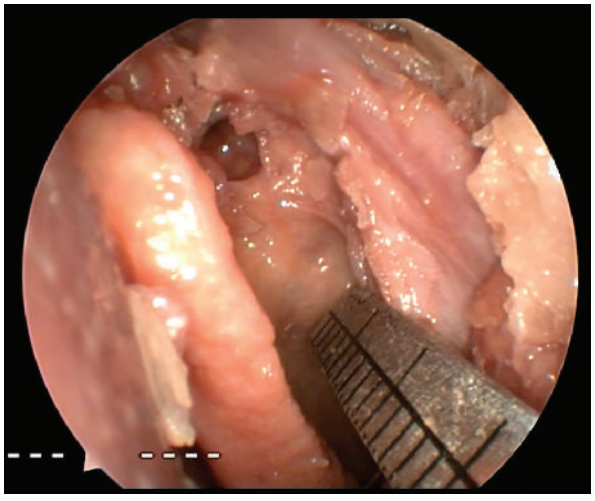
Figure 6. The specified ruler is seen on the photograph.

lower border of the lacrimal sac as upper limit of the inferior turbinate. In addition, the width and length of the lacrimal sac were measured (Figure 5). The distances were measured with a specified ruler that precision was 1 mm (Figure 6).

Ethics Approval

This study was approved by the Ethics Committee of Necmettin Erbakan University Meram Faculty of Medicine (Decision No: 2016/687 and Decision Date: 07/10/2016).

Statistical Analysis

All data were evaluated using SPSS version 20 by descriptive methods. Descriptive analyses were based on range, mean ±SD. The measurements of the right and left sides were compared with paired t-test. A p value of <0.05 was considered statistically significant.

Results

All the cadaveric heads were dissected. All the measurement data are shown in Table 1 and Figure 7. The mean width of the lacrimal sac (LS) was 5.5±3.2 mm (range 4-12) for the right side, 5.8±3.1 mm (range 4-12) for the left side. The mean distance from the LS to the nasal vestibule was 34±1.7 mm (range 32-42) for the right side and 37±2.6 mm (range 34-42) for the left side. The mean distance from the LS to the frontal process of maxilla was 13±4.8 mm (range 9-22) for the right side, and 11.5±3.2 mm (range 7-16) for the left side. The mean distance from the LS to the middle turbinate attachment was 8.5±1.7 mm (range 6-11) for the right side, and 9±2.2 mm (range 7-13) for the left side. The mean distance from the LS to the uncinate process was 15.8±5.7 mm (range 12-27) for the right side, and 14.6±4.9 mm (range 10-23) for the left side. The mean distance from the LS to the maxillary ostium was 15±3 mm (range 13-21) for the right side, and 12.1±2.8 mm (range 7-15) for the left side. The mean distance from the LS to the inferior turbinate was 7.6±4.6 mm (range 12-27) for the right side, and 7±3.1 mm (range 10-23) for the left side. The mean values were calculated separately for the right and left sides. No significant difference ($P>0.05$) was found in any variables between the right and left sides.

Table 1. Measurements between the Lacrimal Sac and the Adjacent Landmarks

Cadaver	Side	NVLS	FPMLS	MTALS	UPLS	MOLS	ITLS	LS width	LS length
1	R	33	10	10	12	15	13	5	10
	L	37	10	10	10	13	13	5	10
2	R	32	14	11	13	13	2	4	11
	L	36	12	8	13	7	7	4	12
3	R	34	9	8	17	14	12	4	10
	L	34	10	13	23	13	6	4	9
4	R	35	13	6	12	13	5	4	12
	L	36	14	7	13	14	6	4	12
5	R	33	10	8	14	14	10	4	8
	L	42	7	9	11	15	4	6	17
6	R	37	22	8	27	21	4	12	13
	L	37	16	7	18	11	6	12	10
Mean±SD	R	34±1.7	13±4.8	8.5±1.7	15.8±5.7	15±3	7.6±4.5	5.5±3.2	10.6±1.7
	L	37±2.6	11.5±3.2	9±2.2	14.6±4.9	12.1±2.8	7±3.1	5.8±3.1	11.6±2.8

Measurements=Millimeter; Adjacent landmarks=Nasal vestibule, Frontal process of maxilla, Middle turbinate attachment, Uncinate process, Maxillary ostium, Inferior turbinate. NVLS=Nasal vestibule-lacrimal sac; FPMLS=Frontal process of maxilla – lacrimal sac; MTALS=Middle turbinate attachment-lacrimal sac; UPLS =Uncinate process - lacrimal sac; MOLS=Maxillary ostium – lacrimal sac; ITLS=Inferior turbinate – lacrimal sac; L=Left sides; R=Right sides; LS=Lacrimal sac.

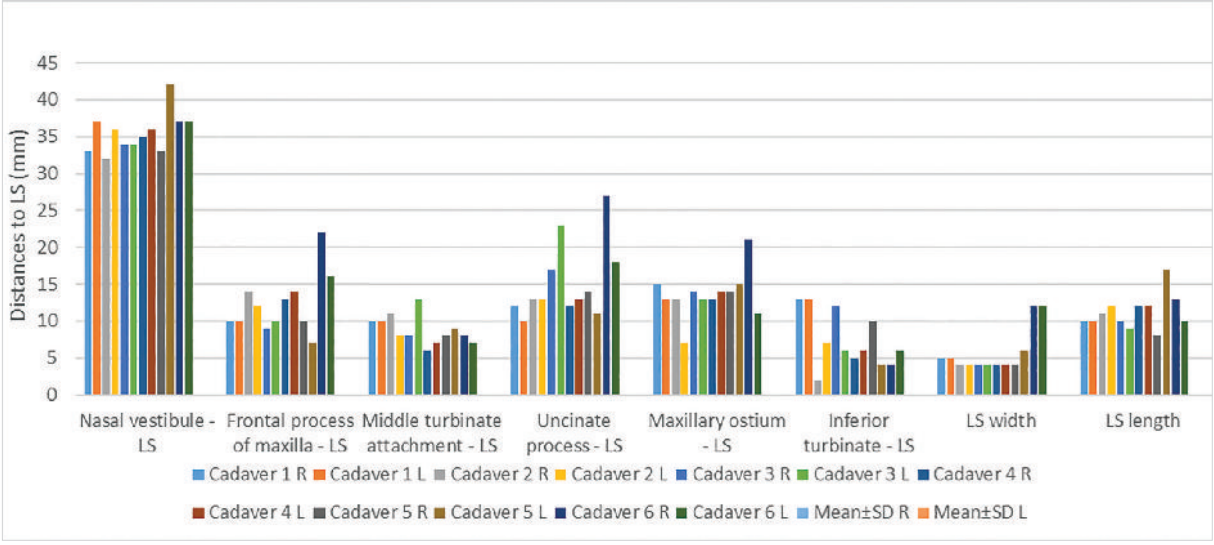

Figure 7. The graphic of the data.

Discussion

We have shown in our study that the distances of the lacrimal sac to the nasal vestibule, the uncinate process and the frontal process of the maxilla are not as reliable as the middle turbinate attachment for predicting the anatomic localization of the lacrimal sac during DCR procedures.

In our study, the nasolacrimal duct was located in front of the middle turbinate attachment in all cases. Previous studies have indicated that the nasolacrimal duct is always located in front of the

middle turbinate attachment if there is no middle turbinate hypertrophy or nasal polyposis (1, 4). In line with this information, we made a mucosal incision approximately 5 mm above and 10 mm in front of the middle turbinate attachment. The mean distance of the midline of the nasolacrimal duct to the middle turbinate attachment was measured as 8.7 mm±2 mm. The incision should be made at least 10 mm in front of the middle turbinate attachment to be careful not to damage the middle turbinate axilla during DCR procedures.

Metson et al. described the maxillary line as a curvilinear eminence along the lateral nasal wall, and it is also known to correspond to the maxilla-lacrimal bone junction (5, 6). Our study showed that the nasolacrimal duct was aligned with the maxillary line. Orhan et al. demonstrated that the maxillary line overlapped the lacrimal sac in 18/20 cadaveric specimens, and that the lacrimal sac was located posterior to the maxillary line in the other two specimens (7). Another study showed that the nasolacrimal duct ostium overlapped the maxillary line in 24 (67%) of 36 cases, was located posterior to the maxillary line in 10 cases and anterior to the maxillary line in 2 cases (8). In the light of these data, if the lacrimal sac cannot be found at the level of the maxillary line, the incision should be widened posteriorly.

There are many studies that evaluate the topography of the lacrimal sac according to the nasal vestibule. A study involving 26 Iranian patients demonstrated that the mean distance of the anterior border of the lacrimal sac to the nasal vestibule was 39 mm (9). Our study determined that the mean distance of the anterior border of the lacrimal sac to the nasal vestibule was 35.5 mm. These differences between studies may depend on gender, age and race (8). Therefore, there is a great need for large-scale studies in our country. Moreover, obtaining information on landmarks would be a guide in all cases.

This study determined that the mean distances of the anterior border of the lacrimal sac to the uncinate process and maxillary ostium were 15.2 mm and 13.5 mm, respectively. Orhan et al. found that the mean distances of the posterior border of the lacrimal sac to the uncinate process and maxillary ostium were 5 mm and 7.2 mm, respectively. We believe that this difference may be due to the fact that we measured the distance from the anterior border of the lacrimal sac, while they measured the distance from the posterior border of the lacrimal sac. In our study, the standard deviation was 5.3 for the distance between the LS and the uncinate process, and this was the highest value in all the landmarks. In our opinion, this is due to anatomical variations of the uncinate process. An investigation of paranasal sinus variations showed that variations existed in the uncinate process, such as pneumatization, medial deflection and lateral deflection (10). Anatomic variations in the uncinate process existed with a reported incidence between 15.9% and 65% in this study. We concluded that the uncinate process is not a reliable landmark for DCR, as variations in the uncinate process are common.

Identifying the lower border of the lacrimal sac has been an important factor affecting the success rate of endoscopic rhinostomy. Although most studies have emphasized that the width of the rhinostomy is an important factor for the success of endoscopic DCR, some studies have indicated that performing a rhinostomy from the lower border of the lacrimal sac leads to failure (2, 11-14). Our study determined that the mean distance of the lacrimal sac to the inferior turbinate was 7.3 mm. Similarly, Orhan et al. found that the mean distance of the lacrimal sac to the inferior turbinate was 8.2 mm. According to these data, osteotomies made 7-8 mm above the inferior turbinate would probably be successful.

Conclusion

External dacryocystorhinostomies are not preferred for common canalicular obstructions due to the widespread use and easy accessibility of endoscopy. As endoscopy maintains its popularity, the need to understand the anatomy of the nasal cavity in endoscopic surgery continues. Therefore, studies are needed to understand nasal anatomy. We obtained data in our study by applying DCR

in accordance with endoscopic technique and believe that these data will guide surgeons during endoscopic DCR. We showed in our study that the distances of the lacrimal sac to the nasal vestibule, the uncinate process and the frontal process of the maxilla are not as reliable as the middle turbinate attachment for predicting the anatomic localization of the lacrimal sac during DCR procedures.

What Is Already Known on This Topic:

Many studies have been conducted to reveal the localization of lacrimal structures. In many cadaver studies, information has been obtained on the lacrimal sac and nasolacrimal duct, and some landmarks have been identified for surgery. The nasal spine, nasal limen, inferior turbinate attachment, maxillary ostium, maxillary line, uncinate process, and middle turbinate attachment are landmarks identified by various studies (6, 7). The maxillary line and the middle turbinate attachment are the most commonly used landmarks. Current literature describes the lacrimal sac commonly anterior to the middle turbinate attachment, but it may also be overlapped by it or posterior to the middle turbinate attachment. Lacrimal sac usually overlaps with maxillary line or situated posterior to maxillary line.

What This Study Adds:

We evaluated all the nearby landmarks of the lacrimal sac such as nasal vestibule, frontal process of the maxilla, uncinate process, the middle turbinate attachment, maxillary ostium and inferior turbinate. Our study showed that the distances of the lacrimal sac to the nasal vestibule, the uncinate process and the frontal process of the maxilla are not as reliable as middle turbinate attachment for predicting the anatomic localization of the lacrimal sac during DCR procedures.

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Validation and the Reliability of the ACIC Questionnaire in the Primary Health Care Setting: a Study from Bosnia and Herzegovina

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Abstract

Objective. The aim of this study was to carry out the cultural adaptation and validation of the Assessment of Chronic Illness Care questionnaire (ACIC) in the Republika Srpska, Bosnia and Herzegovina. **Methods.** A validation study was conducted in two randomly selected primary health care centers in the Republika Srpska, Bosnia and Herzegovina, during March and April 2016. The study participants were all physicians working in family medicine departments during the study. Translation of the ACIC questionnaire version 3.5 was performed following the guidelines of the World Health Organization. The validity and reliability of the questionnaire were tested with face validity, construct validity, and internal consistency. **Results.** The questionnaire was distributed to 66 family physicians. Missing values were negligible, therefore the criteria for factor analysis were met. Exploratory factor analysis confirmed that the questionnaire measured one factor. The Cronbach alpha coefficient (0.970) showed the excellent level of internal consistency of the questionnaire. The intraclass correlation coefficient (0.802) confirmed the good reliability of the questionnaire. **Conclusion.** The ACIC questionnaire can be used to assess the quality of chronic care in family medicine practice in Bosnia and Herzegovina. Further research is needed to explore how changes in healthcare care delivery impact changes in the Chronic Care Model domain.

Key Words: Primary Healthcare ▪ Delivery of Healthcare ▪ Non-Communicable Chronic Diseases.

Introduction

Chronic, non-communicable diseases have been recognized as a significant burden within the European Region, and are one of the four priority areas of the 2020 Health Action Plan (1). In Bosnia and Herzegovina (BH), ischemic heart disease and cerebrovascular diseases are the highest-ranking causes of premature death (2). In order to resolve the problem of epidemics of non-communicable diseases, and tackle outcomes in terms of chronic illness, the Government of Bosnia and Herzegovina has conducted a primary health care reform based on the family medicine (FM) model, over the period of the last 20 years (3-5). Globally, as the gatekeepers, family physicians have been given a crucial role in the treatment and control of most common chronic conditions, such as hypertension, diabetes, chronic obstructive pulmonary disease, and osteoarthritis (6, 7). The FM teams in the Republika Srpska (RS) are obliged to record cardiovascular risk factors, as well as to have registries of patients with hypertension, diabetes, and chronic obstructive pulmonary disease. This is in accordance with the Accreditation Guide for Family Medicine, launched by the RS Agency for Certification, Accreditation and Health Care Improvement.

This Agency conducts the certification and accreditation process of health institutions, monitoring and improving healthcare quality (8, 9). Increased quality of care, better clinical outcomes, patient empowerment, improved multidisciplinary collaboration, and better evaluation of chronic care quality have been assigned as the goals of the Chronic Care Model. The model encompasses six elements: health care organization, delivery system design, clinical information systems, decision-support, self-management support, and community resources (10-12). Evaluation of the Chronic Care Model implementation includes the attitudes and perceptions of patients (Patient Assessment of Chronic Illness Care – PACIC) as well as of health professionals (Assessment of Chronic Illness Care - ACIC). Validation of the Patient Assessment of Chronic Illness Care questionnaire was conducted previously in the same geographic regions (13). The ACIC survey measures to what extent the model is implemented in a specific healthcare system (14-18). Worldwide, previous studies have explored the impact of the Chronic Care Model and health system organization on the Assessment of Chronic Illness Care (19-21), however, it was unknown whether the psychometric properties of the ACIC instrument could be applicable for assessment of chronic care delivery in family practice in BH.

The aim of this study was to describe the cultural adaptation and validation of the Assessment of Chronic Illness Care questionnaire in the Republika Srpska, Bosnia and Herzegovina.

Methods

Study Participants

The cross-sectional study was carried out in two randomly selected primary health care centers, in two cities in the RS, BH (Bijeljina and Prijedor). Primary health care reform in BH introduced the concept of family medicine teams, consisting of a family physician and two nurses. The nursing profession's job description still varies greatly between the health institutions, in terms of care and treatment of chronic diseases, including education on self-management and community linkage. Therefore, we performed validation of the ACIC questionnaire among the physicians working in family medicine departments, employed by the aforementioned primary health care centers. Eligibility criteria were: working in a family medicine department and having a registered group of patients. The minimum number of responses was calculated to be 60, with a population size of 96, with an error margin of 5%, and a confidence interval of 95. The physicians were informed about the research objectives, and were asked to sign an informed consent form to participate in the study. Participants were assured that confidentiality and anonymity would not be breached by the release of any personal information without permission. To avoid coercion, the researchers approached each prospective respondent individually. Physicians who did not provide written informed consent were excluded from the study, and a questionnaire with incomplete answers was excluded from the data analysis.

The study was conducted in accordance with the World Medical Association Declaration of Helsinki, as revised in 2008. Data collection took place in the period from March to April 2016.

Instruments

The Assessment of Chronic Illness Care, version 3.5, was the tool used in the study. The questions are divided into three parts and the Integration of Chronic Care Model Components, according to the six elements of the Model. The first part, Organization of the Healthcare Delivery System, includes the following components: Overall Organizational Leadership in Chronic Illness Care, Organizational Goals for Chronic Care, Improvement Strategy for Chronic Illness Care, Incentives and Regulations for Chronic Illness Care, Senior Leaders, and Benefits. Community Linkages, the second part, includes Linking Patients to Outside Resources, Partnerships with Community Organizations, and Regional Health Plans. The third part of the questionnaire, entitled Practice Level, consists of four parts: Self-Management Support, Decision

Support, Delivery System Design, and Clinical Information Systems.

The final part of the Assessment of Chronic Illness Care questionnaire contains the Integration of Chronic Care Model Components, combining all the elements of the Model. It includes six key components related to patient information on clinical guidelines, information systems and registries, community programs, organizational planning, follow-up appointments with patient assessment and goal planning, as well as chronic care guidelines.

The Assessment of Chronic Illness Care questionnaire is organized so that the highest "score" ("11") for any individual item, subscale, or the overall score (the average of the seven subscale scores) indicates optimal support for chronic illness. The lowest possible score on any given item or subscale is "0", which corresponds to limited support for chronic illness care. The interpretation guidelines are as follows:

Between "0" and "2" = limited support for chronic illness care

Between "3" and "5" = basic support for chronic illness care

Between "6" and "8" = reasonably good support for chronic illness care

Between "9" and "11" = fully developed chronic illness care (18, 19).

Translation and Cultural Adaptation

At the beginning of the study, translation of the ACIC questionnaire was performed following the guidelines of the World Health Organization (22). Two healthcare professionals, fluent in both languages, translated the questionnaire independently from English to Serbian. The translations were reviewed for accuracy, and discrepancies between the translations were resolved by a third bilingual translator, not involved in the previous translation. Backward translation was performed by a fourth bilingual translator, unaware of the questionnaire's objective. The back-translated version was compared with the original source to reach equivalence. After subsequent revision, a consensus was reached by the translators on all questions, and a prefinal version of the ACIC was prepared for preliminary pilot testing.

Five family physicians at each primary health care center were asked to provide their opinion on each questionnaire item's meaning, and consider its applicability for the local care context. No need for any additional modifications of the translation was identified, and the final translated version of the ACIC was produced. The final version of the ACIC was administered to 14 family physicians for whom the questionnaire is intended.

Ethical Approval

The research protocol was approved for each survey by the Ethics Committee of the Primary Health Centre in Prijedor on December 17, 2015 (reference number 01-1545-3/15) and in Bijeljina on December 30, 2015 (reference number 6372/15). All personal data were anonymized,

Statistical Analysis

The validity and reliability of the questionnaire were tested with face validity, construct validity, and internal consistency. Face validity was assessed with the mean, median, standard deviation, Interquartile range, percentage of missing values, the extent of ceiling and floor effects, and normality measures, by the Kolmogorov-Smirnov test. A percentage larger than 20% was associated with floor/ceiling effects (23). Internal consistency was expressed in terms of Cronbach alpha for seven subscales, and the total Assessment of Chronic Illness Care questionnaire and reliability were expressed as the intra-class correlation coefficient. The analysis of construct validity was based on the hypothesis that higher scores would be positively correlated with the implementation of chronic disease clinical guidelines at primary care level, assessed as the percentage of examinations and the percentage of normal results. Spearman's rank-order correlation was used due to the non-normal distribution of the variables. Factor analysis (factors with eigenvalue >1) was applied to

examine the structure of our version of the ACIC questionnaire.

Results

At the time of the study, 96 family physicians were employed in both regions. Twenty-nine family physicians were on vacation or sick-leave, and sixty-six family physicians consented to participate in the study (response rate = 67.7%).

The Demographic Characteristics of the Physicians

The majority of the family physicians were younger than 39 years of age, N=23 (35.3%) and had worked in practice for less than 11 years, N=35 (53.8%). Mostly the physicians were certified (had completed residency training in family medicine, N=30, 46.9%) and were women, N=56 (84.8%). The accreditation process (meeting regulations and standards set by external accreditation bodies for family medicine) had been implemented three years before the study among 27 (41.5%) of the physicians, and 17 (26.2%) of the physicians had not been previously accredited (Table 1). Family physicians have registries and patients' lists for hypertension, diabetes, and chronic obstructive pulmonary disease (Table 1).

Table 1. Demographic Characteristics of Family Physicians and Their Practices

Categorical variables		N (%)
Gender	Male	10 (15.2)
	Female	56 (84.8)
Age (years)	28-39	23 (35.3)
	40-51	20 (30.8)
	52-65	22 (33.9)
Working years	1-12	35 (53.8)
	13-24	12 (18.5)
	25-38	18 (27.7)
Education level	Medical doctor*	12 (18.8)
	Certified family physicians	30 (46.9)
	Professional additional education†	25 (34.4)
Type of work place	Urban	36 (54.5)
	Suburban	11 (16.7)
	Field	19 (28.8)
Accreditation status	No accreditation	17 (26.2)
	Accredited during 3 years	27 (41.5)
	Accredited longer than 3 years	21 (32.3)
Using registries for chronic diseases (with indicators)	Hypertension	56 (86.2)
	Diabetes	56 (86.2)
	Chronic obstructive pulmonary disease	46 (70.8)
Using patients' lists for chronic diseases (without indicators)	Hypertension	60 (93.8)
	Diabetes	59 (93.7)
	Chronic obstructive pulmonary disease	55 (87.3)

*Physicians without formal education in family medicine; †Physicians with other specializations and additional training in family medicine.

The Psychometric Characteristics of the Questionnaire

One questionnaire was incomplete and excluded from the study. Missing values were negligible therefore the criteria for factor analysis were met. During the analysis of the percentage of answers with 0 and 11 points, it was confirmed that none of the items had a floor effect, while many items had a ceiling effect. All items in the parts entitled Organization of the Healthcare Delivery System and Delivery System Design had a ceiling effect, and one item related to the Continuity of Care had this effect reaching over 50% (Table 2). These results suggested non-normal distributions, as confirmed by the Kolgomorov-Smirnov test. Exploratory factor analysis confirmed one component showing that the questionnaire had one dimension, measuring one factor.

The Cronbach alpha coefficient showed an excellent level of internal consistency of the questionnaire, with a value 0.970. Internal consistency for each of the seven subscales was measured by the Cronbach alpha, and values varied from 0.861 to 0.950. The intraclass correlation coefficient for the questionnaire was 0.802 (Table 2).

Table 2. Data Quality of the Assessment of Chronic Illness Care Questionnaire

Assessment	Mean±SD	Missing values (%)	Floor (%)	Ceiling (%)	Cronbach alpha
Total ACIC* score	8.099 (2.145)	-	-	-	0.970
Organization of the HDS†	8.97 (2.112)				0.944
Q1	8.57 (2.562)	1.5	-	31.8	-
Q2	9.05 (2.011)	1.5	-	27.3	-
Q3	8.97 (2.449)	1.5	-	37.9	-
Q4	8.22 (2.870)	1.5	-	30.3	-
Q5	9.45 (2.031)	1.5	-	48.5	-
Q6	9.57 (2.311)	1.5	-	51.5	-
Community linkages	7.45 (2.606)				0.937
Q7	7.26 (3.017)	1.5	-	-	-
Q8	7.48 (2.658)	1.5	-	-	-
Q9	7.62 (2.602)	1.5	-	-	-
Self-Management support	7.83 (2.755)				0.942
Q10	8.05 (3.074)	1.5	-	21.2	-
Q11	7.40 (3.156)	1.5	-	-	-
Q12	7.77 (3.306)	1.5	-	22.7	-
Q13	8.12 (2.308)	1.5	-	-	-
Decision support	7.82 (2.477)				0.861
Q14	9.03 (2.481)	1.5	-	37.9	-
Q15	6.37 (3.781)	1.5	-	-	-
Q16	8.45 (2.450)	1.5	-	27.3	-
Q17	7.45 (2.889)	1.5	-	-	-
Delivery system design	9.12 (1.793)				0.941
Q18	9.03 (2.172)	1.5	-	37.9	-
Q19	9.32 (1.953)	1.5	-	37.9	-
Q20	8.89 (2.306)	1.5	-	31.8	-
Q21	9.15 (2.152)	1.5	-	39.4	-
Q22	9.51 (1.592)	1.5	-	40.9	-
Q23	8.82 (1.991)	1.5	-	27.3	-
Clinical information systems	8.13 (2.298)				0.934
Q24	8.37 (2.589)	1.5	-	30.3	-
Q25	7.92 (2.564)	1.5	-	22.7	-
Q26	7.52 (2.784)	1.5	-	-	-
Q27	7.94 (2.855)	1.5	-	21.2	-
Q28	8.92 (1.971)	1.5	-	30.3	-
Integration of components	7.35 (2.540)				0.950
Q29	7.46 (2.599)	1.5	-	-	-
Q30	7.42 (2.839)	1.5	-	-	-
Q31	6.20 (3.336)	1.5	-	-	-
Q32	6.89 (3.098)	1.5	-	-	-
Q33	8.42 (2.277)	1.5	-	24.2	-
Q34	7.77 (2.760)	1.5	-	21.2	-

*Assessment of Chronic Illness Care; †Healthcare Delivery System.
Total ACIC score: Intraclass correlation coefficient 95%: 0,802; Confidence Interval: 0.739-0.859

Scores of the Questionnaire

The total score was 8.1, indicating good support for chronic illness care. Organization of the Healthcare Delivery System (8.97) and the Community Linkage (7.45) scores indicated advanced support for chronic illness care. Self-Management Support and Decision Support in the third part of the questionnaire had almost the same score (7.83, 7.82) and Delivery System Design had the highest score (9.12). The Clinical Information Systems score was 8.14. The lowest score was evidenced for Integration of Components (7.36).

The total average score of male family physicians was 8.5 (standard deviation, SD 1.5) and of female family physicians 8.0 (SD 2.2). The difference is not statistically significant. Two item scores of male physicians were significantly different, Linking Patients to Outside Resources (P=0.005) and Organizational Planning for Chronic Illness Care (P=0.035).

There was no statistically significant regression of the scores or the following predictors: age and gender, education level, working years, accreditation status, and type of working place.

Discussion

This study aimed to validate the original ACIC questionnaire in the RS, BH, as an instrument to evaluate the level of non-communicable chronic disease care. The Cronbach alpha and intra-class correlation coefficients showed high internal consistency for the total instrument. The internal consistency and reliability of the ACIC questionnaire are in line with the validation study previously carried out in BH, showing high internal consistency and reliability of the PACIC questionnaire (13). These findings are important due to the influence on the information provided by this instrument (24). Exploratory factor analysis found one latent factor (one dimension) that could explain as much of the variability of the initial multidimensional instrument as possible in the context of primary health care in BH. The Integration of Chronic Care Model Component is a variable that reliably measures one (latent) factor.

Bonomi et al. (18) defined the ACIC questionnaire as a tool for identifying areas for improvement of chronic illness care, as well as to evaluate the level of improvement. Initial testing of this questionnaire was done within 108 organizational teams across the United States during quality improvement collaboration focused on chronic illness care. The results of the initial testing showed the best average scores for Organization of the Healthcare Delivery System and Community Linkage, and the lowest scores for Clinical Information System. The results of the final testing showed improvements in Decision Support as well as the Clinical Information System. Therefore, the authors suggested the questionnaire as a "useful quality improvement tool" (18). The good validity and reliability of the BH version of the ACIC suggest its applicability to measure changes in the primary care system of BH.

Family physicians in the current study stated that chronic illness care was well supported, with Delivery System Design scoring best, as previously found in a study by Cramm et al. (19), and Community Linkage and Integration of Components having the lowest average scores. Most of our study respondents work in urban areas, and it has been shown that physicians who work in urban areas, as well as in individual practices, commonly provide the lowest average scores for Community Linkage. The low scores in this domain may be attributed to the family physicians' high work overload or time constraints, but also to the lack of patient motivation for community programs (25).

In contrast to our findings, physicians in the Netherlands perceived good community linkage (19). Bar et al. (26) suggested a need for tighter connection between delivery system design and the community, affecting citizen organizations, non-profit groups, and the healthcare organization (26). The Netherlands has a very well-developed primary care system, continuity of health care services, and different innovative initiatives to increase community engagement. Increasing access to effective programming in the community, through linkages with the relevant agencies was a cost-effective way to improve quality of care (27).

Davy et al. (28) launched a review of results from 77 quantitative and qualitative studies as the relevant international evidence on the effectiveness of Chronic Care Model elements for improving healthcare practices and health outcomes. This review showed the wide range of variations of the model elements and their implementation, depending on the country. The most commonly used elements in the chronic care model were self-management support and delivery system design (28). The scores in these domains may have been better in other countries in comparison to Bosnia and Herzegovina due to differences in primary health care system organization. First, primary care reform in Bosnia and Herzegovina is still not complete, and healthcare delivery calls for improvement (29). Following the trend in Western European countries, governmental institutions could transfer tasks from the medical to the nursing profession by employing a higher number of nurses with a bachelor's degree, well-trained to provide chronic patient care. Second, there is a lack of community engagement programs within the country, which commonly deepen relationships between healthcare providers and the community. Stakeholders have an important role in supporting community initiatives targeted to derive sustainable decisions and social transformation. Third, patients have a central role in providing quality chronic disease care (30), but their path toward self-management and responsibility is not always smooth. Patient empowerment can only be achieved through consistent medical feedback expenditure on social services in long-term care.

This study has several limitations. The results of the study cannot be generalized as the survey included two primary health care centers at a single point of time. Although a family medicine team consists of one FM physician and two nurses, the current study focused only on family physicians; therefore, it is possible that including nurses in the validation process would provide a consensus rating for each item. We did not estimate either the test-retest reliability of the ACIC or responsiveness to changes, and checking the time stability of the questionnaire by re-administering it after a defined period would reflect whether measurement errors could be attributable to differences in participants' responses over time.

Conclusions

The validated Assessment of Chronic Illness Care questionnaire is available now in Bosnia and Herzegovina. It could be applicable in the health care system of the country in order to analyze the current system, to identify areas for improvement, and to evaluate all improvements.

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What Is Already Known on This Topic:

The Chronic Care Model encompasses six elements: health care organization, delivery system design, clinical information systems, decision-support, self-management support, and community resources. The Model implementation includes the attitudes and perceptions of patients as well as health professionals. Previous studies explored health professionals' attitudes using the Assessment of Chronic Illness Care instrument. However, it is unknown whether the psychometric properties of this instrument could be applicable for the assessment of chronic care delivery in family practice in Bosnia and Herzegovina.

What This Study Adds:

The Assessment of Chronic Illness Care instrument, validated in Bosnia and Herzegovina, is a useful tool to assess the quality of care for patients with chronic diseases in primary care.

The tool could be used to strengthen collaboration between patients, nurses and physicians.

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

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

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The Prevalence and Morphometry of the Atlas Vertebra Retrotransverse Foramen

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Abstract

Objective. The current study records the prevalence of the accessory foramen, located posterior to the transverse foramen (TF), the so-called the retrotransverse foramen (RTF), its morphometry, exact location, and coexistence with ossified posterior bridges. Additionally, factors associated with the length of the RTF are investigated. **Materials.** One-hundred and forty-one dried atlas vertebrae were examined. **Results.** Thirty-seven out of the 141 vertebrae (26.2%) had at least one RTF. The RTF was unilateral in 67.6% and bilateral in 32.4%. The mean RTF anteroposterior diameter (length) was 4.2±1.4 mm on the right and 3.8±1.0 mm on the left side. The mean RTF laterolateral diameter (width) was 2.6±1.2 mm on the right and 2.5±0.8 mm on the left side. Both dimensions were symmetrical. The RTF was symmetrically located from the TF, at a mean distance of 4.6±1.1 mm on the right and of 4.5±0.9 mm on the left side. For the given TF-RTF distance, laterality, and presence of posterior bridges, each mm increase in the RTF width was associated with a 0.74 mm increase in the relevant length. **Conclusion.** The estimated prevalence was higher than most of those reported in other studies. However, the between-studies prevalence varies to a significant degree. Hence, a systematic review and meta-analysis should be performed to identify a more precise estimate due to the clinical importance of the RTF.

Key Words: Retrotransverse Foramen ▪ Accessory Foramen ▪ Variation ▪ Atlas Vertebra ▪ Morphometry ▪ Regression Analysis.

Introduction

The atlas, the topmost cervical spine vertebra (C1), provides a stable support for the head, by means of its ring-shaped form. The posterior arch has a vertebral artery (VA) groove for transmission of the homonymous artery and the dorsal ramus of the first cervical spinal nerve. The transverse foramen (TF) transmits the VA, the vertebral vein (VV), and the sympathetic nerves (1). The presence of an accessory foramen posterior to the TF, a so-called retrotransverse foramen (RTF), or posterolateral foramen or canaliculus venosus (2) is noticed as a variant, with a reported prevalence that differs widely between studies in different populations (Table 1). Most of the published studies investigating the RTF have examined dried vertebrae, while a few cadaveric (3-6) and imaging (6-10) studies have provided additional information on the RTF perforating structures, or have described possible clinical implications (headache, migraine, and loss of consciousness relating to certain neck movements) (11). However, knowledge of RTF variants is not only limited to such manifestations, since an accessory foramen may be of paramount surgical importance in upper cervical spine surgery when exposing the C1 posterior arch (12), and when interpreting neck compression syndromes, due to VA entrapment (13).

The present study provides information about the prevalence of RTF, its morphometric characteristics, exact location, and coexisting ossified variants (posterior bridges-PBs) in a dried C1 vertebrae sample of Greek origin. On a secondary basis, an attempt to identify factors associated with the RTF length was performed.

Table 1. Identification of the Presence (Prevalence) and Laterality of the Retrotransverse Foramen (RTF) in Cervical Vertebrae, Among the Published Studies

Authors	Publication year	Continent	Study type	Total sample	Frequency	Prevalence (%)
Le Double et al.	1912	Europe	Dried bone	500	60	12.0
Barbosa Sueiro et al.	1933	Europe	Dried bone	400	33	8.3
Sassu et al.	1965	Europe	Dried bone	66	13	19.7
Sylla et al.	1976	Africa	Dried bone	50	32	64.0
Veleanu. et al.	1977	Europe	Dried bone	71	9	12.7
Taitz et al.	1978	Asia	Dried bone	480	34	7.1
Gupta et al.	1979	Asia	Dried bone	35	2	5.7
De Boeck et al.	1984	Europe	Dried bone	55	7	12.7
De Boeck et al.	1984	Europe	Imaging	14	1	7.1
De Sousa et al.	1989	Europe	Dried bone	200	18	9.0
Le Minor	1997	Europe	Dried bone	500	71	14.2
Wysocki et al.	2003	Europe	Dried bone	100	1	1.0
Jaffar et al.	2004	Europe	Dried bone	29	3	10.3
Bilodi and Gupta	2005	Asia	Dried bone	34	3	8.8
Das et al.	2005	Asia	Dried bone	132	2	1.5
Paraskevas et al.	2005	Europe	Dried bone	115	17	14.8
Chinnappan and Manjunath	2008	Asia	Dried bone	102	9	8.8
Ilie et al.	2008	Europe	Dried bone	75	6	7.6
Karau et al.	2010	Africa	Dried bone	102	16	15.7
Sharma et al.	2010	Asia	Dried bone	200	16	8.0
Kaya et al.	2011	Asia	Dried bone	262	59	22.5
Murlimanju et al.	2011	Asia	Dried bone	363	6	1.7
Aggarwal et al.	2012	Asia	Dried bone	176	11	6.3
Agrawal et al.	2012	Asia	Dried bone	28	1	3.6
Chaudhari et al.	2013	Asia	Dried bone	133	31	23.3
Gupta et al.	2013	Asia	Dried bone	123	23	18.7
Karau et al.	2013	Africa	Dried bone	102	4	3.9
Laxmi et al.	2013	Asia	Dried bone	210	10	4.8
Rathnakar et al.	2013	Asia	Dried bone	140	8	5.7
Katidireddi and Setty	2014	Asia	Dried bone	100	3	3.0
Murugan et al.	2014	Asia	Dried bone	150	19	12.7
Ramachandran et al.	2014	Asia	Dried bone	120	19	15.8
Rekha et al.	2014	Asia	Dried bone	153	10	6.5
Yadav et al.	2014	Asia	Dried bone	120	8	6.7
Akhtar et al.	2015	Asia	Dried bone	174	25	14.4

Authors	Publication year	Continent	Study type	Total sample	Frequency	Prevalence (%)
Apurba et al.	2015	Asia	Dried bone	150	33	22.0
Sultana et al.	2015	Asia	Dried bone	100	1	1.0
Travan et al.	2015	Europe	Dried bone	129	11	8.5
Quiles-Guiñau et al.	2016	Europe	Dried bone	86	2	2.3
Gul et al.	2017	Asia	Dried bone	100	9	9.0
Sanchis et al.	2017	Europe	Dried bone	206	15	7.3
Sanchis et al.	2017	Europe	Imaging	110	4	3.6
Gupta et al.	2019	Asia	Dried bone	161	42	26.1
Natsis et al.	2019	Europe	Dried bone	244	116	47.5
Medeiros et al.	2021	America	Dried bone	44	4	9.1
Xing et al.	2021	Asia	Imaging	427	50	11.7
Ranjan et al.	2022	Asia	Dried bone	170	24	14.1
Unweighted average						11.9
Present study	2022	Europe	Dried bone	141	37	26.2

Materials and Methods

One hundred and forty-one dried C1 vertebrae obtained from the ossuaries of the Anatomy and Surgical Anatomy Department of the Aristotle University of Thessaloniki (AUTh) (100 vertebrae) and from a local Greek cemetery located in the city of Serres (41 vertebrae) were used. All these specimens were of unknown sex and age, and free of fractures or deformities.

Measurements

Prior to measurement, each RTF patency was evaluated using an orthodontic wire of 0.2 mm thickness. Foramina that were not perforated fully (blind - grooves) were considered as absent. All the remaining subjects were visually inspected bilaterally for RTF presence, and the total, bilateral and unilateral frequencies were recorded. On the basis of these frequencies, the prevalence and laterality (bilaterally or unilaterally present foramina in each vertebra) were estimated. Additionally, the coexistence of completely ossified PBs was calculated (Figure 1).

The morphometric characteristics of the anteroposterior diameter (APD) or length and the laterolateral diameter (LLD) or width, as well as the RTF-TF minimum distance (Figure 2) were independently measured by two assessors, using the digital sliding caliper (Mitutoyo ABSOLUTE 500-196-20), accurate to 0.01 mm.

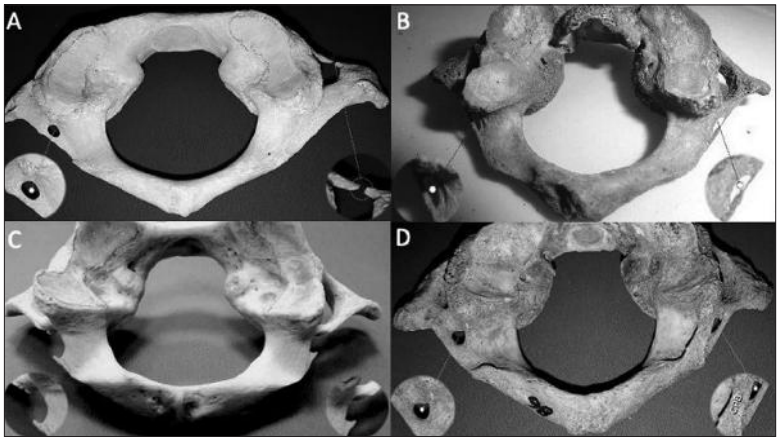

Figure 1. Photograph collection demonstrating the main characteristics of the retrotransverse foramina (RTF). A. unilateral RTF, B. bilateral RTF, C. an unclosed RTF (considered as absent), D. a case of coexistence of a RTF with a complete ossified posterior bridge (CPB). A case of an incomplete transverse foramen (TF) is depicted by an asterisk (*) in frame A.

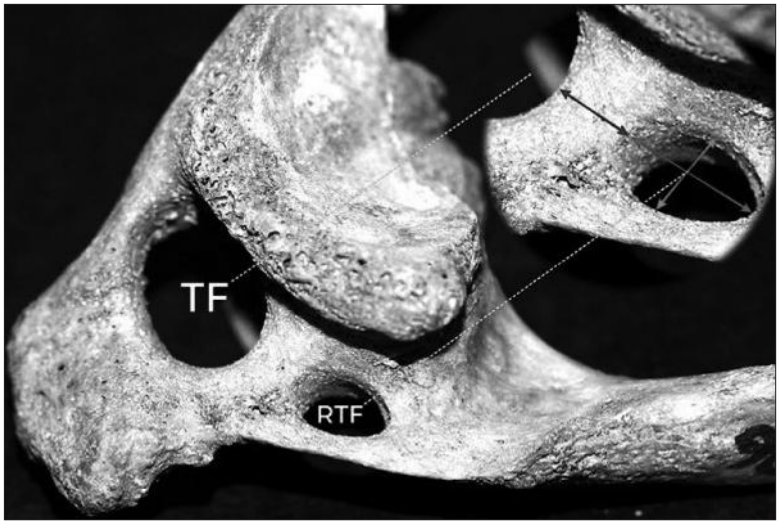

Figure 2. Schematic demonstration of the calculated diameters of the retrotransverse foramen (RTF) (anteroposterior and laterolateral diameters- double arrows) and the minimum distance between the transverse foramen (TF) and the RTF (red double arrow).

Ethics Statement

The vertebrae from the osseous collection of the Anatomy and Surgical Anatomy Department of AUTh belonged to body donors that offered their bodies before death, by signing an informed consent (body donation program). In relation to the rest of the sample (41 vertebrae), the Ethics Committee of the AUTh gave full permission prior to the beginning of the study. The current research was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments.

Statistical Analysis

Statistical analysis was carried out using STATA statistical software (Release 14.0, Stata Corp., TX, USA) for MacOS. The Shapiro–Wilk test, the evaluation of skewness and kurtosis values, as well as the visual interpretation of the produced histograms were used to assess data normality. In the case of a normally distributed quantitative variable, mean and standard deviation (SD) values were used, otherwise the median and the interquartile range (IQR) were recorded. All qualitative variables were expressed in absolute (N) and relative (%) frequencies. For the continuous variables, the presence of interobserver error (differences in measurements between assessors) was estimated by calculating the intraclass correlation coefficient (ICC), and interpreted using Koo and Li's reported guidelines (14). Paired sample Student's t-tests were performed to assess whether the APD, LLD and TRF-TF minimum distance varied by laterality and by the presence of PBs, respectively. Chi-Square tests of independence or Fisher's exact tests were performed to evaluate the potential difference in prevalence and laterality according to the coexistence of a PB. Univariate and multivariate linear regression analyses were performed to assess the relationship between APD and all recorded variables. Unless otherwise stipulated, $P<0.05$ was considered statistically significant.

Results

Retrotransverse Foramen Prevalence

At least one RTF was identified in 37 vertebrae, leading to an estimated prevalence of 26.2%. An RTF was detected unilaterally in 25 vertebrae (67.6%; 17 right, 68.0% and 8 left, 32.0%) and bilaterally in 12 (32.4%) (Table 2). Ossified PBs were identified in 12 atlas vertebrae (32.4%). No significant differences were detected between the side of the location of RTF (laterality) and the presence of PBs (P=0.711) or side of their location (P=0.998).

Table 2. Description of the Frequencies of Main Characteristics and the Estimated Mean Values of the Morphometric Characteristics Investigated

Qualitative variables		Quantitative variables	
Variable	Frequency (%)	Variable	Mean (± SD)
Prevalence	37 (26.2)	APD*	
Laterality		Right side	4.2 (±1.4) mm
Bilateral	12 (32.4)	Left side	3.8 (±1.0) mm
Unilateral	25 (67.6)	Mean	4.1 (±1.3) mm
Right side	17 (68.0)	LLD†	
Left side	8 (32.0)	Right side	2.6 (±1.2) mm
Presence of posterior bridges		Left side	2.5 (±0.8) mm
No	25 (67.6)	Mean	2.6 (±1.1) mm
Yes	12 (32.4)	RTF‡ – TF§ distance	
Right side	9 (75.0)	Right side	4.6 (±1.1) mm
Left side	3 (25.0)	Left side	4.5 (±0.9) mm
-		Mean	4.6 (±1.0) mm

*Anteroposterior diameter; †Laterolateral diameter; ‡Retrotransverse foramina; §Transverse foramina.

The mean values, standard deviations (SD), and the differences between right and left side (including the respected P-values) for each measurement are provided in the text.

Retrotransverse Foramina Morphometry and Minimum Distance from the Transverse Foramina

ICC calculation supported the existence of the excellent reliability of the measurements between observers (ICC=0.921, P>0.05). The morphometric details RTF (APD, LLD and RTF-TF minimum distance) followed the normal distribution (P>0.05). Symmetry was found in all morphometric parameters. Significant differences in the APD and LLD were identified in relation to the presence or absence of PBs (Table 3). The mean values of the APD, LLD and RTF-TF minimum distance were 4.1±1.4 mm, 2.6±1.1 mm, and 4.6±1.0 mm (Table 2). None of these values varied by laterality, the presence of PBs or their location (Table 3).

Table 3. Results of the Student's T-tests for the Evaluation of Differences in Measured Diameters and the Retrotransverse Foramen - Transverse Foramen Distance, Retrotransverse Foramen, Transverse Foramen

Test	Mean1	Mean2	Diff.	P value
Anteroposterior diameter				
Sidewise (right – left)	3.98	3.65	0.33	0.304*
Laterolateral diameter				
Sidewise (right – left)	2.22	2.41	-0.2	0.395*
RTF† – TF‡ distance				
Sidewise (right – left)	4.36	4.21	0.16	0.445*
Two-sample Students' t tests				
Anteroposterior diameter				
By the laterality	3.82	4.24	-0.43	0.362*
By the presence of posterior bridges	3.76	4.82	-1.07	0.018*
By the side of posterior bridges	4.91	4.57	0.34	0.686*
Laterolateral diameter				
By the laterality	2.32	2.83	-0.51	0.182*
By the presence of posterior bridges	2.39	3.23	-0.84	0.025*
By the side of posterior bridges	3.27	3.08	0.19	0.837*
RTF† – TF‡ distance				
By the laterality	4.29	4.77	-0.49	0.168*
By the presence of posterior bridges	4.53	4.78	-0.25	0.485*
By the side of posterior bridges	4.69	5.06	-0.36	0.562*

*Paired samples Students' t-tests; †Retrotransverse foramen; ‡Transverse foramen.

Table 4. The Univariate and Multivariate Linear Regression Analyses for the Investigation of the Association between the Recorded Variables and APD*

Independent variables	Univariate models			Multivariate model		
	B†	95% CI‡	P-value	B†	95% CI‡	P value
LLD§	0.77	0.44–1.09	<0.001	0.74	0.36–1.12	<0.001
RTF‖–TF¶ distance	0.14	-0.31–0.58	0.542	-0.17	-0.56–0.21	0.363
Laterality						
Bilateral**	0	-	-	0	-	-
Unilateral	0.43	-0.51 – 1.36	0.362	0.08	-0.69 – 0.86	0.829
Presence of posterior bridges						
No**	0			0		
Yes	1.07	0.19 – 1.94	0.191	0.49	-0.32 – 1.29	0.227

*APD; †Beta coefficient; ‡95% Confidence Intervals; §Laterolateral dimension; ‖Retrotransverse foramen; ¶Transverse foramen; **Reference category.

Factors Associated with the Accessory Foramina Anteroposterior Diameter

The APD was only found to be significantly associated with the LLD, on the basis of the univariate (B, 0.77, 95% CI, 0.44 – 1.09, P<0.001) and multivariate (B, 0.74, 95% CI, 0.36 – 1.12, P<0.001) regression analyses (Table 4). On the basis of the interpretation of the multiple linear regression output, holding all the other parameters constant (RTF-TF minimum distance, laterality, and PBs presence), for each mm increase in the accessory foramen LLD, a 0.74 mm increase in the relevant ADP was noticed.

Discussion

The Accessory Foramina Prevalence in Dried Bone Studies

The current study highlights a prevalence of RTF of 26.2%. Significant heterogeneity exists among studies conducted in different populations in relation to estimation of RTF prevalence. Most of them suggest that RTF are mostly found with an unweighted prevalence of 11.9% (Table 1). Studies of Africans (15-17) and Europeans (6-8, 18-28) report higher values of maximum prevalence than those of Asian (4, 5, 9, 29-50) and American origin (51). This discrepancy may be related to the existence of intra- and inter-population differences or may be associated with the fact that in most of the studies the crude (prevalence calculated from all the available specimens) rather than the vertebra-adjusted prevalence (specified for each of the cervical vertebrae) is reported. Hence, systematic reviews and meta-analyses should be performed in an attempt to explain the source of heterogeneity, and to obtain more accurate estimates.

The Retrotransverse Foramen Morphometric Characteristics

The current study's findings regarding RTF morphometry can be considered similar to those provided by Mederios et al. (51). However, the diameters reported by Mederios et al. (51) were calculated by evaluating all the available cervical vertebrae. Hence, vertebrae-specific RTF measurements must be conducted to estimate the RTF exact dimensions accurately per vertebra.

Ossified Posterior Bridges, as Coexisted Variants

Ossified PBs were identified in 12 subjects (32.4%) in the present study. A significantly higher prevalence (72.2%) was recorded by Paraskevas et al. (24) and attributed to the redirection of the blood into the retrotransverse vein, possibly due to VV compression into the PB. The high difference between the two studies highlights the existing heterogeneity.

The Retrotransverse Foramina Content

The current study lacks details of the RTF content, as it was exclusively performed on dried C1 vertebrae. A few cadaveric studies have reported data related to the RTF content. These studies have been considered as the gold standard for identification of C1 variants (6). Veleanu et al. (6) and Bodon et al. (3) identified an anastomotic vein perforating the RTF and connecting the venous sinuses above (suboccipital cavernous sinus) and below (VA and vertebral venous plexus) the C1 posterior arch. Therefore, the presence of RTF could be related to modifications in the regional venous circulation associated with the erect posture and bipedal locomotion of humans (2). However, other studies reported neural (17) or arterial (52) components perforating the RTF. Additionally, the same issue remains when assessing imaging studies dealing with this topic. Particularly, even though some authors have reported that the RTF content may be the VA (9) and/or the VV (11, 47) Xing et al. (9) supported that RTF is only perforated by venous components, as they did not find any case of the passage of the VA. Their conclusion contradicts Kaya et al. (39) who related VA duplication with the existence of a RTF.

Clinical Implications

Knowledge of the surgical anatomy of the C1 vertebra and surrounding structures is important for neurosurgeons, orthopaedic surgeons, and radiologists. An unnoticed RTF may be a risk factor for intraoperative bleeding from the venous plexus, covering the C1 lateral mass and obscuring the surgical field (52). The VA may commonly be injured (53) in posterior exposure of the upper cervical spine, especially when using posterior instrumentation. Even minor lesions of the VA may result in severe hemorrhage, or even death. Madawi et al. (54) highlighted a high prevalence (28.3%) of VA injury during posterior upper cervical spine trans-articular fixation for atlantoaxial instability, in cases in which the RTF is used as a landmark, and the entry point for the lateral mass screw will be just medial to the RTF. Thus, preoperative imaging will help determination of the safe trajectory for screw placements in posterior procedures (55) and has become an essential examination before posterior approach surgeries.

Limitations of the Study

The sample size is small and more studies on a larger population should be conducted. Additionally, the lack of computed tomography (CT) measurements and information regarding the specimens' sex, age and ethnicity, as well as the inability to identify the structures perforating the RTF, should be taken into consideration when interpreting this study's results.

Study Strengths

The calculation of measurements by two assessors and the intraobserver error estimation improved the accuracy and precision of the study findings. This study utilized linear regression analysis to associate the recorded variables with the accessory foramina APD (length).

Conclusions

RTF prevalence is estimated at 26.2%. RTF morphometry (diameters) and location (minimum distance from the TF) showed side symmetry. The RTF anteroposterior diameter is significantly associated with the laterolateral measurement.

What Is Already Known on This Topic:
C1 has the highest variability among the cervical vertebrae. Among C1 variants, the frequency of RTF presence has been studied in different populations. The frequencies of RTF presence vary between 2.3% and 47.5%. The RTF has been associated with a high incidence of C1 posterior bridging (72.2%). A few cadaveric studies focus on the RTF content and are considered the gold standard for assessing the presence of C1 variants. A recent study asserted the exclusive passage of veins through the RTF, as they did not find any case of VA passage. An unnoticed RTF may be a risk factor for intraoperative bleeding from the venous plexus, covering the C1 lateral mass and obscuring the surgical field. Preoperative imaging is an essential examination before posterior approach surgeries.

What This Study Adds:

The study provides additional information on the prevalence and morphometry of RTF. Additionally, information is provided on the location of RTF, as well as an analysis of the factors associated with its length (anteroposterior diameter). Characteristic depictions, meticulous tables and a detailed report of the available data focusing on the RTF clinical importance are provided.

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High Flow Nasal Cannula Versus Noninvasive Positive Pressure Ventilation as Initial Respiratory Support in Patients with Chronic Obstructive Pulmonary Disease and Covid-19: Exploratory Analysis in Two Intensive Care Units

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Abstract

Objective. To identify the type of the non-invasive ventilatory treatment for patients diagnosed with chronic obstructive pulmonary disease (COPD), with respiratory status deteriorated by COVID-19 pneumonia, and in need of treatment in the Intensive Care Unit (ICU). **Materials and Methods.** This cross-sectional study was conducted over a one-year period in the medical intensive care units of two hospitals. As the patients' clinical condition deteriorated and the parameters of the arterial blood gas (ABG) analysis worsened, oxygen support was applied via a high flow nasal cannula (HFNC) or by non-invasive positive pressure ventilation (NPPV). According to the control values of the arterial oxygen saturation (SaO_2) and the parameters of ABG, the patients were enabled to be transferred between the two types of non-invasive ventilatory support. The primary outcome was the length of hospital stay, while secondary outcomes were the rate of intubation, the mortality rate, and respiratory support-free days. **Results.** Out of 21 critical patients with COPD and COVID-19, 11 (52.4%) were initially treated with NPPV and 10 (47.6%) with HFNC. The ages (67±9.79 in NPPV group vs. 70.10±10.25 in HFNC group) and severity of illness (SOFA score 5 (3.5) in NPPV group vs. 5 (2.8) in HFNC group) were similar between the two groups. Switching the mode of respiratory support was more common in NPPV (58.3% in survivor group vs. 41.7% in non-survivor group). Patients treated with NPPV compared to HFNC had a nominally longer length of stay (15 (11) vs. 11.5 (4.25)), and higher risk of intubation (66.7% vs. 33.3%) and mortality (66.7% vs. 33.3%), but the comparisons did not reach statistical significance. Survivors had significantly longer Medical Intensive Care Unit and hospital stays, but significantly lower FiO_2 (0.60 vs.1) and higher values of PaO_2/FiO_2 (78(32.4) vs. 56.3(17.8)) than non-survivors. All patients were treated with corticosteroids, and the duration of treatment was similar between groups. **Conclusion.** In critically ill patients with COPD and COVID-19, both HFNC and NPPV were commonly used as the initial mode of ventilation. Switching to a different mode and adverse patient outcomes were more frequent in patients initially treated with NPPV. Survivors had higher values of PaO_2/FiO_2 than non-survivors.

Key Words: COPD ▪ HFNC ▪ NPPV ▪ COVID-19 Pneumonia.

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Introduction

The SARS CoV2 virus usually infects the respiratory system, causing severe pneumonia. Patients with acute respiratory failure (ARF) require intensive care treatment, with a subsequent need for ventilators (1-3). High-flow nasal cannula oxygen therapy (HFNC) and non-invasive positive pressure ventilation (NPPV) are widely used in patients experiencing ARF as alternatives to standard oxygen therapy, to avoid invasive mechanical ventilation (IMV) (4). In addition, awake prone positioning in spontaneously breathing patients is a therapeutic intervention for COVID-19 respiratory failure, and is expected to reduce the treatment failure when combined with HFNC or NPPV (5).

The infection with SARS CoV2 appears to be more severe in patients with chronic obstructive pulmonary disease (COPD), and in smokers, due to tobacco exposure that leads to an alteration in the regulation of an angiotensin-converting enzyme 2 (ACE-2) and its overexpression. The levels of ACE-2 are inversely related to the forced expiratory volume in the first second (FEV1) (6). Preexisting COPD often leads to severe deterioration of symptoms, and a 4–5.9-fold greater risk of developing a severe form of COVID-19, compared to patients without it (7, 8).

Patients who develop a moderate or severe form of COVID-19 requiring hospitalization should be treated with the current pharmacotherapeutic agents, including treatment with corticosteroids (9). HFNC should be preferred over NPPV in acute hypoxemic respiratory failure, noted in severe COPD cases, despite conventional, low-flow oxygen therapy, due to the lower failure rate (10, 11). NPPV can potentially worsen lung injury due to the high transpulmonary pressure and tidal volume (12). The patients on HFNC or NPPV should be monitored closely for deterioration of their clinical status and early intubation, leading to IMV (13, 14). Understanding COPD's pathophysiology leads to the hypothesis that NPPV will reduce the number of ICU days compared to HFNC. The data regarding COPD treatment with severe COVID-19 is insufficient for low- and middle-income countries due, in the first instance, to limitations in the available oxygen resources and apparatus providing high flow oxygenation treatment and noninvasive ventilatory support (15).

Methods

This cross-sectional study, including 21 patients, was conducted in the period from June 2020-December 2021 in two hospital facilities' Medical Intensive Care Units (MICU) in the Western Balkans: the University Clinical Center of Republika Srpska, Banja Luka, and Prim. Dr. Abdulah Nakaš General Hospital, Sarajevo.

The study population comprised patients diagnosed with COVID-19 pneumonia and COPD, without respiratory acidosis, identified on the day of hospital admission according to arterial blood gas analysis ($7.35 \leq pH \leq 7.45$). As their clinical condition deteriorated and the parameters of the arterial blood gas (ABG) analysis worsened, determined by a decrease in the partial arterial pressure of oxygen (PaO_2) ≤60 mmHg (milimeters of mercury), there was an increase in the partial arterial pressure of carbon dioxide ($PaCO_2$) ≥50 mmHg, or arterial oxygen saturation decreased (SaO_2) ≤92%, despite the oxygen support delivery via an oronasal mask with 15l/min flow, patients were admitted to the Intensive Care Unit (ICU). Chest X-ray analysis or computed tomography (CT) chest scan (due to technical limitations) was performed on the day of hospital admission to confirm the diagnosis of pneumonia, and repeatedly with the deterioration of the patient's clinical and respiratory status. According to the parameters of SaO_2 and the parameters of ABG, oxygen support via HFNC or NPPV was applied. Once the HFNC was initiated, the fraction of inspired oxygen (FiO_2) as a percentage (%) and the oxygen flow rate (l/min) were recorded.

Two methods of NPPV were applied: continuous positive airway pressure (CPAP) or bilevel positive airway pressure (BiPAP) with recordings of the values of NPPV. However, the therapeutic effectiveness of these non-invasive modes was not further evaluated individually through the results of the study due to the small sample size.

HFNC treatment was initiated for patients with SpO_2 <88% under oxygen supply (O_2) at 15 L/min and/or PaO_2/FiO_2 <150. HFNC treatment was applied with a properly fitted nasal cannula, and the application was initiated and titrated according to the following: oxygen flow raised from 30 L/min until 60 L/min to accustom the patient; FiO_2 to maintain peripheral oxygen saturation (SpO_2 90-92%); temperature 31–37°C, according to the patient's comfort level.

CPAP ventilatory support was initiated in patients if PaO_2/FiO_2 <200 or PaO_2 <60 mmHg (while on oxygen or HFNC) or if PaO_2/FiO_2 <300 or SpO_2 <88% on O_2 >15 L/min and the patient had BMI >30. BiPAP was initiated in patients if PaO_2/FiO_2 <100 or with respiratory distress under CPAP. The FiO_2 initial value in both centers was 100% with a gradual decrease in value according to the peripheral SpO_2 and the parameters of arterial ABG. Suggested parameters for BiPAP initiation include PEEP 10-12 cmH_20 and a pressure support (PS) set, with the aim of tidal volume (VT) 4–6 ml/kg and FiO_2 aimed at a target of SpO_2 90-92%. In the case of clinical deterioration and respiratory distress, BiPAP was assigned as the first treatment option in patients with hypercapnic respiratory failure (pH<7.3, $PaCO_2$ ≥50mmHg). The criteria for endotracheal intubation were: respiratory arrest, respiratory pause with unconsciousness, severe hemodynamic instability (i.e., systolic blood pressure (SBP) <90 mmHg instead of adequate volume resuscitation), and intolerance to CPAP leading to discontinuation of the device, if after 4 hours of CPAP, PaO_2/FiO_2 was decreasing, with a respiratory rate (RR) ≥30, and PaO_2 <60 mmHg. Once invasive mechanical ventilation was initiated, the most commonly used ventilation mode was synchronized intermittent mandatory ventilation (SIMV). The parameters for the invasive mechanical ventilation should be set up in concordance with a "lung protective" ventilation strategy, but they are not currently under consideration within the framework of this study.

According to the control values of SaO_2, the parameters of ABG and clinical follow-up aimed at improvement of ventilatory status, the patients were enabled to transfer between two types of non-invasive ventilatory support (HFNC and NPPV). The ratio of oxygen saturation (ROX index) was calculated within every 24 hours. Accordingly, HFNC failure was determined if ROX was below 2.85 at 2 hours, below 3.47 at 4 hours; or below 3.85 at 12 hours. CPAP failure was determined if PaO_2/FiO_2 <100 or there was a 20% increase in $PaCO_2$. BiPAP failure was determined by the criteria reached for endotracheal intubation. The termination of the non-invasive ventilatory support by HFNC/NPPV was determined by the improvement in the patient's clinical condition (SpO_2 ≥92%), and by an increase in the values of ABG (PaO_2 ≥60 mmHg or SaO_2 ≥92%), with a gradual decrease in peripheral oxygen supply. Awake proning was performed for the patients on HFNC once ventilatory support was initiated, and in certain cases of invasive mechanical ventilation, according to clinical case-by case decisions.

The following laboratory tests were conducted on the day of admission to the ICU: complete blood cell count (CBC), differential blood cell count, including neutrophil granulocytes (Neu), lymphocytes (Lym), monocytes (Mon), and eosinophil granulocytes (Eos), C reactive protein (CRP), D-dimer, parameters of ABG: partial arterial pressure of oxygen and carbon dioxide (PaO_2, $PaCO_2$ respectively), pH value, bicarbonate level (HCO_3) and base excess (BE) according to the reference range values.

All patients, on the day of admission to the hospital, were assigned the following descriptive parameters: gender, age, smoking status (smoker, non-smoker), and comorbidities (chronic heart failure, arterial hypertension, acute coronary syndrome, obesity, diabetes mellitus type II, and chronic renal insufficiency). The following parameters were calculated on the day of admission to the ICU: body mass index (BMI) and Sequential Organ Failure Assessment (SOFA) score. The SOFA score was calculated for all patients admitted to the ICU to determine the level of organ dysfunction (based on the dysfunction of six organ systems) and mortality risk. Arterial blood gas analysis (ABG) was taken on the day of admission to the hospital and the day of admission to the ICU.

On admission to the ICU, some patients underwent prone positioning. The therapeutic procedures consisted of using corticosteroids in various doses, and duration of treatment. After serious clinical, diagnostic, and laboratory parameter evaluation, some patients were treated with monoclonal antibody-tocilizumab. The primary outcome was the length of hospital stay. The secondary outcomes were: the rate of intubation, the death rate (survivor, non-survivor), and respiratory support-free days.

Statistical Analysis

The data are expressed as the mean and standard deviation for normally distributed, or as the median and interquartile range for not normally distributed continuous variables and counts with percentages for categorical variables. The normality of data distribution was tested using the Kolmogorov-Smirnov test. A comparison of measures for continuous variables was performed by using the Mann– Whitney U and Student's t-tests. As appropriate, a proportion comparison was calculated using the Pearson Chi-square test and Fisher's exact test. The Kaplan Meier test was used for survival analysis. The analyses were performed using IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp. A P value <0.05 was considered significant. We calculated the odds ratio (OR) for intubation between the groups of patients treated with different ventilatory modes.

Results

A total of 21 confirmed COVID-19 pneumonia and COPD patients, of which 13 were men (61.9%), with a mean age of 67.9±9.7 years, were included in the study. Thirteen (61.9%) of the evaluated patients were current smokers. Chronic heart failure (CHF) was identified as the most common comorbidity in 10 patients (47.6%). Once transferred to the MICU, in 11 patients (52.4%), NPPV was the preferred mode of non-invasive ventilatory support. A ventilation mode switch, regardless of the deterioration or improvement of respiratory distress and clinical status, was identified in 12 (57.1%) patients (Table 1). Patients treated with NPPV had higher intubation and mortality rates compared to the patients treated with HFNC, but the difference did not reach statistical significance (Table 2). Moreover, patients on NPPV were switched to another ventilation mode significantly more often than patients on HFNC, as shown in Table 3.

Table 1. Demographic and Clinical Characteristics of COPD* Patients with COVID-19 Treated with NPPV†/HFNC‡

Demographic and clinical characteristics	All patients (N=21)	NPPV† (N=11)	HFNC‡ (N=10)	P value
Age (years) (mean±SD)	67.9±9.7	67±9.79	70.10±10.25	0.516§
Male sex (N; %)	13 (61.9)	8 (61.5)	5 (38.5)	0.201‖
SOFA** (median, IQR)	5 (3)	5 (3.5)	5 (2.8)	0.91 ¶
BMI†† (mean±SD)	27.3±5.4	27.62±6.01	26.33±3.77	0.635§
CHF‡‡ (N; %)	10 (47.6)	7 (70)	3 (30)	0.890‖
Smoker (N; %)	13 (61.9)	10 (76.9)	3 (23.1)	0.477‖
Ventilation mode switch (N; %)	12 (57.1)	11 (91.7)	1 (8.3)	0.018‖
Prone position (N; %)	11 (52.4)	8 (72.7)	3 (27.3)	0.890‖
Vasopressors (N; %)	2 (9.5)	1 (50)	1 (50)	0.481‖
Corticosteroids (N; %)	21 (100)	15 (71.4)	6 (28.6)	-
Tocilizumab (N; %)	5 (23.8)	3 (60)	2 (40)	0.517‖

*Chronic obstructive pulmonary disease; †Non-invasive positive pressure ventilation/‡High flow nasal cannula; §Student's t-test; ‖Pearson's Chi-square test; ¶Mann-Whitney U test; **Sequential Organ Failure Assessment; ††Body Mass Index; ‡‡Chronic Heart Failure

Table 2. Comparison of Intubation and Mortality Rates Between COPD* Patients with COVID-19 Treated with NPPV†/HFNC‡

Comparison of intubation and mortality rates	All patients (N=21)	NPPV† (N=11)	HFNC‡ (N=10)	P value
Hospital stay, days (median, IQR)	14 (8.5)	15 (11)	11.5 (4.25)	0.256§
Intubation (N; %)	12 (57.1)	8 (66.7)	4 (33.3)	0.577‖
Mortality (N; %)	12 (57.1)	8 (66.7)	4 (33.3)	0.577‖

*Chronic obstructive pulmonary disease; †Non-invasive positive pressure ventilation/‡High flow nasal cannula; §Mann-Whitney U test; ‖Pearson's Chi-square test.

Table 3. Clinical Characteristics Between Survivor and Non-survivor COPD* Patients with COVID-19 Treated with NPPV†/HFNC‡

Clinical characteristics between survivor and non-survivor	Survivor (N=9)	Non-survivor (N=12)	P value
Age (years) (mean±SD)	63.6±8.9	71.17±9.4	0.07§
Male sex (N; %)	6 (46.2)	7 (53.8)	0.70‖
Hospital stay, days (median, IQR)	20 (24)	11 (5)	0.01¶
ICU** stay days (mean±SD)	12.3±7.7	6.7±2.6	0.03§
Respiratory support free days (median, IQR)	1 (2)	0	0.004¶
Smoker (N; %)	5 (38.5)	8 (61.5)	0.60‖
††CHF (N; %)	6 (60)	4 (40)	0.13‖
Ejection fraction (%) (mean±SD)	55±9.1	47.3±11	0.28§
Arterial hypertension (N; %)	5 (33.3)	10 (66.7)	0.16‖
Acute coronary syndrome (N; %)	3 (50)	3 (50)	0.67‖
Diabetes mellitus type 2 (N; %)	3 (50)	3 (50)	0.67‖
‡‡BMI (mean±SD)	26.3±3.6	27.9±6.5	0.51§
SaO_2 (%) (mean±SD)	74.3±12.2	80.5±10.5	0.23§
Initial setting Prone position (N; %)	7 (63.6)	4 (36.4)	0.04‖
Initial setting NPPV (N; %)	7 (46.7)	8 (53.3)	0.58‖
Duration of initial setting, days (median, IQR)	5 (6)	3.5 (3.75)	0.08¶
Switch to different setting (N; %)	7 (58.3)	5 (41.7)	0.09‖
Duration of secondary setting, days (median, IQR)	3 (7.5)	0 (1.75)	0.07¶
PaO_2 (mmHg) (median, IQR)	49.3 (19.9)	53.5 (17.2)	0.34¶
FiO_2 (decimal) (median, IQR)	0.60 (0.35)	1 (0.08)	0.01¶
PaO_2/FiO_2 (mmHg/decimal) (median, IQR)	78 (32.4)	56.3 (17.8)	0.03¶
Platelets ($x10^9$/L) (mean±SD)	253.9±69.5	204.67±96.2	0.21§
Glasgow comma score – reduced consciousness (N; %)	1 (20)	4 (80)	0.24‖
Billirubin (umol/L) (median, IQR)	7.8 (8)	12.2 (6.7)	0.57¶
Mean arterial pressure (mmHg) (mean±SD)	101.4±12.3	87.7±16.1	0.04§
Vasopressor (N; %)	1 (50)	1 (50)	0.83‖
Creatinine (umol/L) (median, IQR)	86 (14)	91 (36.2)	0.32¶
§§SOFA score (median, IQR)	5 (3.5)	5 (2.8)	0.91¶
Leukocytes ($x10^9$/L) (median, IQR)	8.8 (5.8)	12.3 (9.2)	0.20¶
Hemoglobin (g/L) (mean±SD)	133.7±17.7	144±19.5	0.23§
Hematocrit (1) (mean±SD)	0.41±0.07	0.43±0.05	0.37§
Neutrophils (%) (mean±SD)	79.8±12.2	87.8±9.5	0.11§
Lymphocytes (%) (median, IQR)	13 (21.8)	4.7 (11.8)	0.19¶

Table 3 (Continued)

Clinical characteristics between survivor and non-survivor	Survivor (N=9)	Non-survivor (N=12)	P value
C-reactive protein (mg/L) (median, IQR)	44.7 (69.7)	60.7 (140.1)	0.12¶
D-dimer (g/L) (median, IQR)	1.5 (9.3)	2.4 (8.2)	0.48¶
$PaCO_2$ (mmHg) (median, IQR)	32.03(45.75)	42.0 (23.25)	0.32¶
HCO_3 (mmol/L) (mean±SD)	24.6±7.4	27.5±5.5	0.37§
pH (median, IQR)	7.45 (0.05)	7.42 (0.08)	0.47¶
BE (mmol/L) (median, IQR)	1 (6.5)	2.9 (5.8)	0.39¶
Duration of corticosteroid therapy, days (median, IQR)	14 (4.5)	11.5 (5)	0.11¶
Tocilizumab (N; %)	3 (60)	2 (40)	0.38‖

*Chronic obstructive pulmonary disease; †Non-invasive positive pressure ventilation/‡High flow nasal cannula; §Student's t-test; ‖Pearson's Chi-square test; ¶Mann-Whitney U test; **Intensive care unit, ††Chronic heart failure, ‡‡Body mass indeks, §§Sequential Organ Failure Assessment.

We calculated the odds ratio (OR) for intubation between the groups of patients treated with different ventilatory modes, and found that it was 1.75 times more likely in patients on NPPV compared to HFNC (OR=1.75, 95% CI 0.242–12.642). The relative risk (RR) for intubation in patients on NPPV was 1.4 (95% CI 0.399–4.907). Table 3 compares the clinical characteristics of survivors and non-survivors, showing that survivors had significantly longer MICU and hospital stay compared to non-survivors. In addition, a considerably higher number of patients in the survival group were placed in the prone position, and these patients had significantly lower FiO_2 and higher values of PaO_2/FiO_2 compared to non-survivors. PaO_2/FiO_2 and FiO_2 values in Table 3 refer to mean calculations from initiation of the specific noninvasive ventilation mode up to half an hour of measurement. All patients were treated with corticosteroids, and the duration of treatment was similar between the groups. Tocilizumab was used somewhat more often in the survivors' group.

Although arterial hypertension was more frequent in the non-survivor group (66.7%), with a decrease in the mean value of the ejection fraction (47.3±11), it did not reach statistical significance in comparison to survivors. Diabetes mellitus type II showed the same occurrence rate (50%) between survivors and non-survivors.

Survival analysis was presented using a Kaplan-Meier curve. The survival rate in patients on HFNC was slightly higher, but the difference between the groups did not reach statistical significance. In the curve, a slightly higher intubation rate is seen in patients on NPPV, but this difference also did not reach statistical significance (Figure 1).

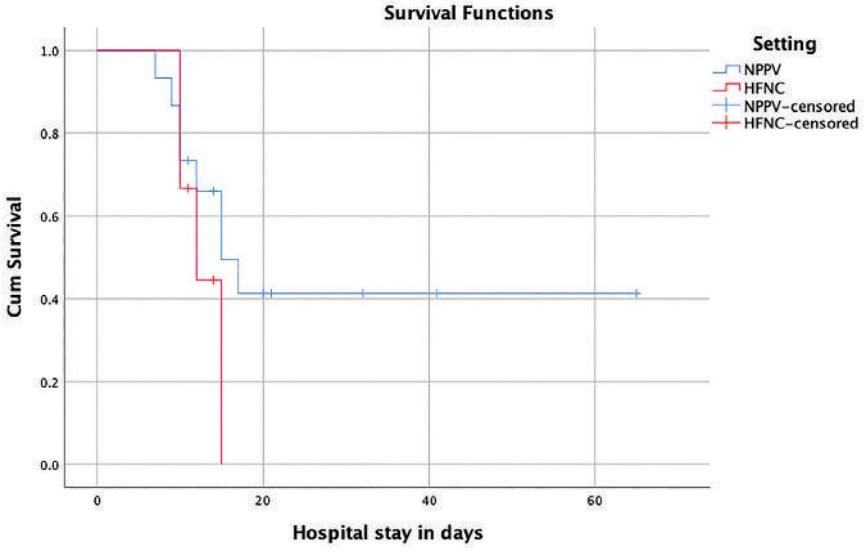

Figure 1. Kaplan Meier curve: test of equality of survival distribution in Chronic obstructive pulmonary disease of patients with COVID -19 treated with *Non-invasive positive pressure ventilation and †High flow nasal cannula.

Discussion

According to the results of our study, even though NPPV was the preferred mode of non-invasive ventilatory support, patients on NPPV were switched to another ventilation mode significantly more often than patients on HFNC. A significantly higher number of COPD patients in the survival group had significantly higher values of PaO_2/FiO_2 ratio and lower FiO_2 values compared to non-survivors, which is in concordance with the favorable clinical outcome. Our results correlate with the study by Grasseli et al., who evaluated risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. They identified several independent risk factors associated with mortality, including: older age, male sex, a high fraction of inspired oxygen (FiO_2) and low PaO_2/FiO_2 ratio on ICU admission, and a history of chronic obstructive pulmonary disease (16). Patients treated with NPPV in our study had prolonged overall hospital stay due to the more severe clinical course of the disease. The duration of ICU stay days was also prolonged for survivors in our study compared to non-survivors. The switch to another mode of ventilatory setting in our study occurred more frequently in the survivor group, most probably due to the successful treatment.

In half of the patients in our study, treatment was initiated with NPPV due to their deteriorated clinical condition, resulting in survival for 46.7% of them. In contrast, the results of the study by Sun J. et al., accounting for 82 COPD patients, identified treatment failure in 39.5% of patients treated with NIV (17). No significant differences were found for 28-day mortality in the same study (15.4% in the HFNC group and 14% in the NIV group, P=0.824). However, in our study, mortality and intubation rates were twice as high for NPPV than HFNC (66.7% and 33.3%, respectively), but the difference did not reach statistical significance. According to the previous findings it is indicative that patients with NPPV were in a worse condition than patients with HFNC at the time of the treatment initiation, as well as that the patients with BIPAP were in worse condition than those with CPAP.

The values of $PaCO_2$ in our study were lower in the survivor group compared to the non-survivors, but the difference did not reach statistical significance. Moreover, meta-analyses involving 525 COPD patients with hypercapnic respiratory failure indicated that HFNC could significantly reduce $PaCO_2$ levels and the length of hospital stay, without greatly influencing PaO_2 level, the incidence of tracheal intubation, and mortality rate compared to NIV (18). A randomized, controlled trial by Li et al. evaluated COPD patients with acute compensated hypercapnic respiratory failure, and identified that HFNC improved the prognosis compared to conventional oxygen therapy, with a reduction in $PaCO_2$, but also identified the value of $PaCO_2$ higher than 59 mmHg as an independent risk factor for treatment failure after 24 hours (19). The pH value in arterial blood gas analysis was decreased in non-survivors compared to the survivor group, yet it did not reach the level of acidosis or statistical significance. However, a randomized controlled trial by Cortegiani et al. assessed the potential of HFNC compared to non-invasive ventilatory support (NIV) in the reduction of $PaCO_2$ in patients with hypercapnic ARF with mild-to-moderate respiratory acidosis, and determined the benefit of treatment with HFNC, especially in cases of COPD exacerbations, as an alternative to NIV (20).

Acute coronary syndrome was equal in appearance (a total of three cases in survivors as well as in the non-survivor group) without reaching statistical significance between the groups in our study, but arterial hypertension was more common among non-survivors. The study by Sheikh et al. associated patients with COVID-19 pneumonia suffering from COPD with more cardiovascular events and extended hospital stays (21). According to their results, the presence of COPD was associated with 1.74 higher odds of ICU admission and 1.47 higher odds of death.

The study of Chen et al. evaluated the predictors of the severity of COVID-19 in patients suffering

from underlying chronic airway disease, and identified: elevated neutrophil counts (P=0.001), decreased lymphocyte counts (P<0.001), eosinopenia (P<0.001), elevated D-dimer levels (P=0.001), increased LDH (P<0.001), elevated blood urea nitrogen (P<0.001), and increased inflammation markers, including CRP (P<0.001) as potential indicators of disease progression and treatment effectiveness (22). These results correlate with the findings of our study, where in the non-survivor group neutrophil count, D-dimer values, and C reactive protein levels were much higher compared to the survivor group, along with the decrease in lymphocyte count, but that difference did not reach statistical significance.

Limitations of the Study

The main limitations of our study are related to the small number of subjects. Another limitation relates to the finding that patients with NPPV were in a worse condition than patients with HFNC when choosing the initial treatment. There are also possible confounding factors that might have affected the observed small differences between the study arms, such as baseline lung function and severity of preexisting COPD, treatment adherence among patients before onset of COVID-19, the time between symptom onset and medical care, that have not been evaluated in this study. Moreover, further investigations, implying more accurate results and proper treatment directions, should be conducted in the future.

Conclusion

In this exploratory study, noninvasive ventilation and high flow oxygen were commonly used as initial respiratory support for COVID-19 respiratory failure in patients with COPD. Switching between the modes was common. Patients initially treated with high flow oxygen had overall better outcomes but the comparisons did not reach statistical significance.

What Is Already Known on This Topic:

Patients diagnosed with COPD and COVID-19 can be evaluated for several ventilatory support strategies, depending on the type of respiratory failure (hypoxaemic or hypercapnic or acute on chronic respiratory failure) including oxygen supplementation, HFNC or noninvasive ventilatory support (CPAP, BiPAP) (23). Insufficiently controlled hypoxaemia in such patients demands application of noninvasive ventilatory support, with HFNC as the first line of treatment in patients with COVID-19 and acute hypoxaemic respiratory failure (11). The benefits of HFNC in COPD and COVID-19 patients are related to the reduction in hypercapnia and the work of breathing (24). However according to the available data, in patients with COPD with acute (or chronic) hypercapnic respiratory failure, NPPV is determined as the first line of treatment, regardless of the previously stated benefits of HFNC (25). COPD and COVID-19 burden in developing countries has been recently questioned. According to the available data COVID-19 case-fatality rates are relatively higher in countries with higher COPD prevalence (26). The strong correlation between COPD prevalence and COVID-19-related mortality in developing nations could be related to differences in level of comorbidities among patients, or to inequalities in distribution of healthcare resources (15).

What This Study Adds:

Since this study accounted for a significantly small number of COPD patients with severe hypoxaemic respiratory failure due to COVID-19, no optimal treatment strategy using either NPPV or HFNC was identified. However, since the survival rate in our study was higher on HFNC and the intubation rate was higher on NPPV, it led us to undertake a further obligation to identify the optimal timing for HFNC application to prevent further clinical deterioration and pending intubation. This study was conducted in the lack of oxygen resources and noninvasive positive pressure ventilators within the environment of the middle-income country, providing the insight in the most appropriate approach to COPD patients with COVID-19 requiring the intensive care treatment.

Authors' Contributions: Conception and design: AM and PK; Acquisition, analysis and interpretation of data: TK, GB, AJ and NI; Drafting the article: AM; Revising it critically for important intellectual content: AM, EB, AF; Approved final version of the manuscript: PK.

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Case Report
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A Remarkably Rare Position of a Cutaneous Ciliated Cyst in a 16 Month-old Female: A Case Report

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Abstract
Objective. The aim of the work was to show a Cutaneous Ciliated Cyst (CCC) in an unusual location in a 16-month-old girl. **Case Report.** We present the case of a 16-month otherwise healthy girl presented to our hospital, with a report of a palpable mass in the left suprascapular region. Physical examination revealed a soft-textured, fluctuating, mobile and painless entity, with no further indications of local inflammation. The mass was totally excised, under general anesthesia, for both diagnostic and therapeutic purposes. According to the histopathological findings, the cystic lesion was covered by a pseudostratified ciliary epithelium, resembling the epithelium of a normal fallopian tube, surrounded by a smooth muscle layer. Immunohistochemical studies identified the cyst epithelium as having cytokeratin (CKAE1/AE3) expression, despite the negative immunostaining findings on Estrogen and Progesterone Receptors. **Conclusion.** Our case report concerns a CCC in an unusual position, in the suprascapular area. After a thorough review of the international literature, we concluded that this is the second published case regarding this specific location. To our knowledge our patient is the youngest ever diagnosed with CCC.

Key Words: Cutaneous Mullerian Cyst ▪ Unusual Position ▪ Paediatric Patient ▪ Müllerian Duct Remnants.

Introduction

The entity was first described by Hess, in 1890 and the term Cutaneous ciliated cyst (CCC) was established by Farmer and Helwig, describing a unique type of cyst arising on the lower extremities of young women (1). CCCs, also known as cutaneous Müllerian cysts, are cystic lesions, mostly found on the lower extremities of females. They are covered by a pseudostratified ciliary epithelium, resembling that of fallopian tubes. Usually the wrong impression is established of them being epidermoid or dermoid cysts.

We present a case of a CCC located in the suprascapular area. The unusual location, together with the young age of our patient were the stimuli for writing this study. There is no case of CCC described in the literature concerning such a young patient.

Case Presentation

A 16-month-old girl was referred to our Paediatric Surgery Clinic due to a painless lesion in her left suprascapular region. This growth was detected during the first month of her life. From then, it had remained asymptomatic and stable in its dimensions. There was no history of previous trauma or other remarkable medical problems. Clinical examination revealed an almond-sized mass, which was mobile, fluctuating, and had a soft texture on palpation. No macroscopic signs of local inflammation were detected, while there was no attachment to the subcutaneous fat. Ultrasonography showed a well-demarcated round mass, measuring 1.3 cm × 0.8 cm, with internal hyperechogenic regions.The formation was totally excised under general anesthesia (Figure 1).

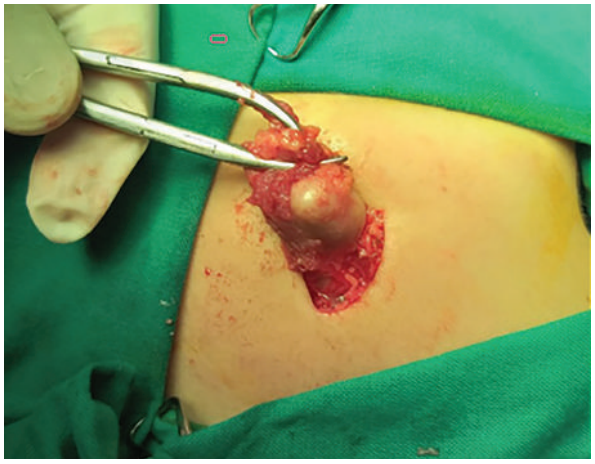

Figure 1. The gross appearance of the lesion, indicating the excised unilocular cyst.

The patient had an uneventful postoperative course and left our hospital the next morning. Microscopic investigation of the excised specimen identified a pseudostratified cilia columnar epithelium, clearly resembling a fallopian tube. A surrounding smooth muscle layer was also detected (Figure 2).

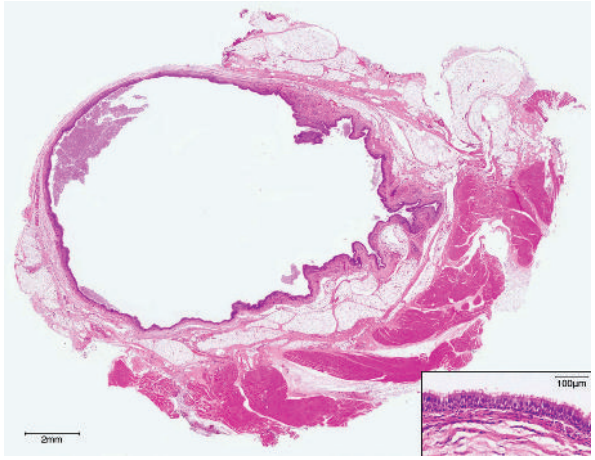

Figure 2. The pseudostratified columnar ciliary epithelium resembling the tubal epithelium (H&E stain). *Detail: The cilias are depicted on the surface of the cells.

The cytoplasmic membrane of the cyst had a positive reaction to Cytokeratin immunocytochemistry (CKAE1/AE3), but intranuclear staining for Estrogen and Progesterone receptors was not shown. The young patient remained asymptomatic during the one-year follow-up.

Discussion

A cutaneous Mullerian cyst is an extremely rare, benign entity, frequently located on the lower extremities of young women after puberty, during the second and third decade of life (1, 2). However, there are some published cases worldwide concerning men (2, 3). Different sites of location have also been reported, including the abdominal wall, the inguinal region, the umbilicus and the scalp (3). As far as our case is concerned, this formation was located in the patient's suprascapular area, which is an extremely rare position, as it is only the second case published worldwide in the literature, concerning such a young pediatric patient (3). These entities usually appear in a specific age group of the population. It is crucial to mention that our patient does not belong to this specific category (2, 3). Fabien-Dupuis et al. reviewed 60 patients with CCC, from 1890 to 2015. In only one 15-year-old patient (1/60) was the cutaneous Mullerian cyst located between the two scapular regions (1). Consequently, our case report is the second one referring to that uncommon location.

In the recent review by Yon Hee Kim et al. 31 patients were recorded, including both sexes, using the PubMed search. The youngest was 7 years old (4). This statement demonstrates that our 16-month girl is the youngest to be diagnosed with CCC described in the literature. CCCs immunohistochemically most often have identical epithelium to fallopian tubes, as they are covered by a cuboid or columnar pseudostratified epithelium (5). To the best of our knowledge, all case reports, published in the literature concerning women, have ER and PR positivity for nuclear cells (2). What makes our case special, is its negativity for these immunostains. The differential diagnosis for CCC also includes an unusual cystic entity known as the Bronchogenic Cyst (BC). The main difference lies in the thoracic location of the BC, apart from its predominance in male patients. The presence of smooth muscle, seromucous glands and, rarely, cartilage in the cystic wall are the main characteristics of a BC during histopathological examination (6). Concerning our patient, the data

excluded the diagnosis of BC. The CCC's epithelium had an obvious resemblance to fallopian tubes. Their nuclear positivity for antibodies of ER and PR, together with the epithelium, led to the hypothesis of Müllerian heterotopia (2). On the basis of that theory, the cysts derive from remnant cells of the paramesonephric duct during the embryological period, and especially between the 6th and 7th week of gestation (3). Cells that are not capable of being integrated with the mesoderm may migrate to the lower extremities.

Consequently, during menarche hormonal stimulation plays a role in the growth of those cystic formations (2-5). A second theory, concerning the etiological background of this entity, supports the cilia metaplasia of the eccrine glands. This concept was developed due to the published cases of CCCs appearing in men. Apart from that, more cases with ER and PR negativity for nuclear cells have been published over time, meaning the Müllerian duct hypothesis is void (3, 7). Finally, therefore, we believe that cutaneous ciliated cysts should be divided into two subgroups: cutaneous Müllerian cysts and ciliated cutaneous eccrine cysts. The cases presenting with ER and PR positivity belong to the first subgroup, in contrast to those that do not have positive immunostaining findings for Estrogen and Progesterone receptors. The first subgroup includes the majority of cases, specifically involving young women between the ages of 15 and 30 years. The cystic formations are usually located on the lower extremities, and their nuclear cells meet the above mentioned positivity for ER and PR. On the other hand, cases of uncommon locations, and also those found in male patients with ER/PR negativity, belong to the second subgroup of cutaneous ciliated cysts (2, 5, 7).

Conclusion

Consequently, our case refers to an eccrine cyst found in an exceptionally rare position. Our patient is the youngest reported in the literature. Surgical resection of the cystic mass is the only therapeutic solution. There was no technical difficulty in removing this entity (2, 3).

What Is Already Known on This Topic:
Cutaneous Ciliated Cysts(CCCs) are rare, cystic entities usually located on the lower extremities of young reproductive female adults. CCCs may also be found on the abdominal wall, in the inguinal region, the umbilicus and the scalp. The etiology is not fully known, but there are two dominant theories. The first is that they are ectopic Mullerian residues, while the second theory supports the ciliated metaplasia of eccrine glands.

What This Case Adds:
This case report presents an uncommon location of a Cutaneous Ciliated Cyst found in the suprascapular area of a 16-month girl. After a thorough review of the literature using Pubmed search, we found that our patient is the youngest presented from 1890 up to the beginning of this year. So, our case is novel, because the patient does not belong to the usual age group, and also in terms of the rare location.

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Case Report
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A Hypertrophic Anterior Scalene Muscle and the Passage of a Subclavian Artery Through its Fibres: The Location of Possible Entrapment

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Abstract

Objective. The presence of cervical ribs, 1st rib anomalies, cervical muscle hypertrophy and repetitive motion are possible aetiologies of subclavian artery (SCA) entrapment and/or compression. Thoracic outlet syndrome of the arterial type may appear with symptoms of hand pain due to the aneurismal part of the compressed SCA. The current cadaveric case describes a hypertrophic right-sided anterior scalene muscle (ASM) and the possible entrapment of the right SCA (RSCA) passing through its fibres. Furthermore, the branching pattern of the entrapped vessel is analysed. **Case Report.** A hypertrophic ASM was identified in the right infraclavicular area of a male Greek donated cadaver (70 years of age). The RSCA passed through the ASM belly, and some deeply situated fibres extended posteriorly to the RSCA. The ASM compressed the RSCA against the superior part of the 1st rib. **Conclusion.** Knowledge of such variants may be important in the diagnosis of upper limb muscle atrophy or neurosensory loss.

Key Words: Thoracic Outlet Syndrome ▪ Anterior Scalene Muscle ▪ Variant ▪ Compression ▪ Subclavian Artery.

Introduction

The presence of some anatomical variants, such as cervical ribs, 1st rib anomalies, hypertrophy of the cervical muscles in combination with repetitive motion, as well as fibrocartilaginous bands (1-3) may cause subclavian artery (SCA) entrapment and/or compression (2, 4-8). In addition, the atypical passage of the SCA through the anterior scalene muscle (ASM) or posterior to it (9, 10) may cause compression on the SCA.

Thoracic outlet syndrome (TOS), an extensively studied entity, includes a constellation of disorders that arise from the compression of the brachial plexus, and/or the subclavian and axillary vessels within the thoracic outlet, due to the narrow apertures and compartments created by the first rib inferiorly, the surrounding musculature and the clavicle, as well as the anterior and middle scalene muscles (11, 12). TOS sites of compression are the interscalene triangle, followed by the costoclavicular triangle or the subcoracoid and pectoralis minor space (1, 13). The arterial form of the syndrome includes hand pain due to the aneurismal part of the compressed SCA. It is also characterized by chronic and repetitive SCA compression, which may lead to arterial wall damage, aneurysm, embolization, and thrombosis (5, 7, 8, 14).

The current cadaveric case describes an unusual case of hypertrophic right sided ASM and the possible entrapment of the right SCA (RSCA) due to its course through the ASM fibres. Furthermore, it analyses the branching pattern of the entrapped vessel.

Case Presentation

During a routine dissection at the Anatomy Department of the Medical School of the National and Kapodistrian University of Athens, the unusual course of the RSCA through the ASM fibres was identified in a 70-year-old male cadaver, derived from a body donation programme after signed informed consent. The cause of death was cardiac arrest, with no other identified pathologies. No further details of the clinical file of the subject existed. The cadaver presented a right hypertrophic ASM and the RSCA passed through the ASM belly and some deeply situated fibres extended posteriorly to the RSCA, the so-called ASM posterior fibres (PF) (Figures 1, 2).

The RSCA branching pattern sequence was as follow: right vertebral artery (RVA), right thyrocervical trunk (RTCT) at the same level of origin as the right internal thoracic artery (RITA) and the right costocervical trunk (RCCT). The RTCT gave off the right inferior thyroid artery (RiTA) and the ascending and the transverse cervical arteries. The right suprascapular artery (RSSA) was not a branch of the RTCT and originated from the distal part of the RSCA. The RCCT gave off the deep cervical and the supreme intercostal arteries. The novel variant pattern is classified as a subtype of the Type Y of Hada et al. (10). The ASM compressed the RSCA against the superior part of the first rib. Since the RSCA passed through the anterior and posterior fibres (AF-PF) of the ASM, the compression was not caused solely by the hypertrophic muscle, but also by muscle contraction. The contralateral side had no such variant.

Figure 1. **A.** The right subclavian artery (RSCA), the branch of the brachiocephalic trunk (BCT) is depicted, between the anterior scalene muscle (anterior and posterior) fibres (AF and PF), IJV- internal jugular vein, and T-trachea. The BCT formed a common trunk (CT) with the left common carotid artery (LCCA). The inclination of the trachea (T) at the left side is evident, RSSA- right suprascapular artery, RSSN-right suprascapular nerve, **B.** The RSCA branching pattern (right vertebral artery-RVA, right thyrocervical trunk-RTCT at the same level of origin with the right internal thoracic artery-RITA, RTA-right inferior thyroid artery, RRLN- right recurrent laryngeal nerve passing anterior to the RTCT. **C.** The same level of origin of the RTCT and the RITA. The RRLN passed anterior to the RSCA branches. AF and PF created a musculoaponeurotic tunnel through which the RSCA coursed.

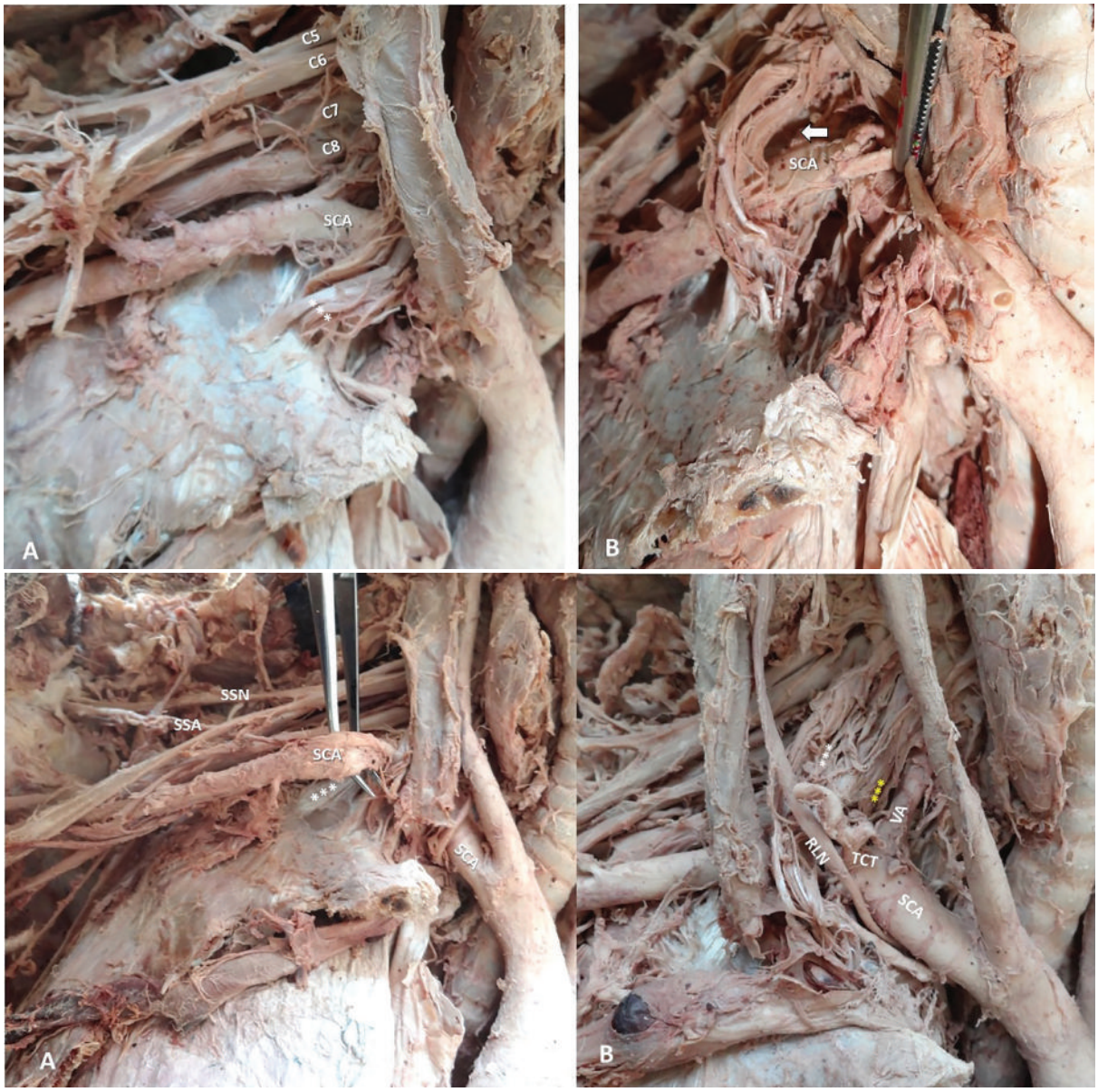

Figure 2. **A.** ***The anterior scalene muscle's anterior fibres (AF) insertion into the 1st rib. A few millimeters below the right suprascapular artery originated (RSSA) and passed between the upper and middle trunks (UT and MT) of the brachial plexus,. accompanied by the right suprascapular nerve and vein (RSSN and RSSV). The area of the internal jugular vein (IJV) and right subscapular vein (RSCV) anastomosis into the venous angle (black arrow) area of the thoracic duct, LT- lower trunk. **B.** Posterior fibres (PF) appear posterior to the right vertebral artery (RVA) origin. The passage of the RSCA through the muscular tunnel and RSCA compression, RRLN-right recurrent laryngeal nerve, RTCT-right thyrocervical trunk, BCT-brachiocephalic trunk.

Discussion

The case is presented of a possible arterial TOS, due to a hypertrophic ASM and the atypical passage of the RSCA through its fibres. The special feature of this case is the variant course of the RSCA through the hypertrophic ASM fibres. Therefore, there were concurrent compression factors, one from the hypertrophic ASM and one from the ASM contractions upon movement. Several rare anomalies have

been discovered and classified in the Roos system, and include cervical ribs, additional tendons, and accessory muscles (12). Several scalene muscle variants found in TOS have also been reported, such as ASM hypertrophy, the passage of the brachial plexus through the ASM, and excessively anterior and middle scalene muscle insertion into the first rib, anomalous fibrous bands within the thoracic outlet, and others (15). A TOS of arterial type may be caused by the variant presented here, although no further details exist from the personal record of the subject.

Several authors have reported 13 isolated cases in their cadaveric studies, with an incidence ranging from 0.2-1.8% (9, 16). Uemura et al. (9) declared that the ASM position in relation to the SCA significantly affects the artery's ramification patterns. Thus, the branching pattern sequence of the current study, not classified into a type, presents similarities to Type Y of Hada et al. (10) and may be a new subtype of it. The developmental mechanism of this type remains unknown.

On the basis of the published literature and the current finding, it is evident that there is the need for a unified classification of SCA pattern types based on the ASM location (anterior to the SCA, posterior to the SCA and passing through its fibres). Larger studies following this protocol could further record common, uncommon and unique variants, and classify them according to the severity of their symptoms.

Conclusion

The current cadaveric report highlights the value of the in-depth knowledge of anatomical variants that may compress the brachial plexus and/or subclavian artery. This knowledge may help clinicians to diagnose neurogenic and arterial TOS. Cases of hypertrophic ASM may act as a compression factor, through the muscular tunnel created surrounding the SCA and its branching pattern.

What Is Already Known on This Topic:

The entrapment and compression of the subclavian artery by a variant form of the anterior scalene muscle may cause arterial thoracic outlet syndrome. The syndrome is characterized by chronic and repetitive artery compression which leads to arterial wall damage, aneurysm, embolization, and thrombosis. It is generally associated with cervical ribs and soft tissue anomalies, such as anterior scalene muscle hypertrophy. Other aetiological factors could be due to first rib anomalies, clavicle fracture, and the presence of fibrocartilaginous bands.

What This Study Adds:

The current cadaveric report presents the rare presence of a unilateral hypertrophic anterior scalene muscle, and the relationship of its fibres with the subclavian artery. Specifically, some muscle fibres create a muscular tunnel, surrounding and compressing the subclavian artery, thus causing a rare form of the arterial type of thoracic outlet syndrome. The subclavian artery is compressed during contractions of the anterior scalene muscle. Special emphasis should be given to the probable presence of variants or accessory muscles inserted into the 1st rib.

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Review Article
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Tumor-Type Agnostic, Targeted Therapies: BRAF Inhibitors Join the Group

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Abstract
In the present review, we briefly discuss the breakthrough advances in precision medicine using a tumor-agnostic approach and focus on BRAF treatment modalities, the mechanisms of resistance and the diagnostic approach in cancers with *BRAF* mutations. Tumor-type agnostic drug therapies work across cancer types and present a significant novel shift in precision cancer medicine. They are the consequence of carefully designed clinical trials that showed the value of tumor biomarkers, not just in diagnosis but in therapy guidance. Six tumor-agnostic drugs (with seven indications) have been approved through October 2022 by FDA. The first tumor-agnostic treatment modality was pembrolizumab for MSI-H/dMMR solid tumors, approved in 2017. This was followed by approvals of larotrectinib and entrectinib for cancers with *NTRK* fusions without a known acquired resistance mutation. In 2020, pembrolizumab was approved for all TMB-high solid cancers, while a PD-L1 inhibitor dostarlimab-gxly was approved for dMMR solid cancers in 2021. A combination of BRAF/MEK inhibitors (dabrafenib/trametinib) was approved as a tumor-agnostic therapy in June 2022 for all histologic types of solid metastatic cancers harboring $BRAF^{V600E}$ mutations. In September 2022, RET inhibitor selpercatinib was approved for solid cancers with *RET* gene fusions.
Conclusion. Precision cancer medicine has substantially improved cancer diagnostics and treatment. Tissue type-agnostic drug therapies present a novel shift in precision cancer medicine. This approach rapidly expands to provide treatments for patients with different cancers harboring the same molecular alteration.

Key Words: Precision Medicine ▪ Targeted Therapy ▪ BRAF ▪ BRAF Inhibitors ▪ Molecular Diagnostics.

Introduction

Precision Medicine and Tumor-Agnostic Approach

Precision (or personalized) medicine in oncology represents a novel approach to cancer treatment. It implies using the right anticancer drug for the right patient at the right time. In contrast to the traditional oncologic treatment, this innovative approach considers individual differences in patients' genes, environment and lifestyle. Precision medicine was coined in 2011 by the USA's National Research Council's (NRC) report "Towards Precision Medicine" (1). In 2015-2020, 290 different precision and matched clinical trials were conducted (2), resulting in the approval of numerous targeted treatment modalities for various solid and hematologic malignancies; the list is provided here (3).

Much of the progress in precision medicine is due to rapid advances in high-throughput genomic sequencing technologies (e.g., next-generation sequencing/NGS) that enabled clinical implementation of assays. These assays can rapidly interrogate cancers for various molecular genomic alterations and targetable biomarkers and allow for more appropriate clinical decision-making and patient outcomes (4).

The precision medicine approach has led to a substantially higher proportion of responding cancer patients, with markedly improved clinical outcomes compared with traditional clinical trials involving unselected patients (5). In particular, clinical trials based on comprehensive molecular profiling may provide "customized multidrug regimens" with a substantial positive impact on the outcome of hard-to-treat and refractory cancers (6). Tissue/tumor type-agnostic drug therapies present a significant, albeit gradual, shift in precision cancer medicine. It is a consequence of carefully designed clinical trials showing the value of tumor biomarkers, not just in diagnosis but also in therapy guidance. Advances in molecular-genetic testing capabilities coupled with understanding complex molecular pathways interactions have led to the stratification of histologically diverse malignancies into biomarker/pathway-similar tumors. Three essential criteria should be fulfilled for tumor agnostic treatment: (1): Cancers must be enriched for at least one genomic alteration; (2) Such an alteration should be predictive of response to a matched therapy, (3) and the genomic alterations should be found across the cancers (7). Tissue type-agnostic drugs are usually assessed in "basket trials" in which small patient cohorts with diverse cancers are treated with the same targeted therapy (8).

Consequently, most basket trials are prospective phase II clinical trials designed to assess durable and objective therapeutic responses to a targeted treatment across different histologic cancer subtypes (9). Up to 2019, 49 basket trials were completed and their results were published (10). Our literature search revealed 76 different basket trials registered in the database ClinicalTrials.gov, most of which are related to cancer treatment (11).

The Food and Drug Administration (FDA) approved six different agnostic-based drugs (seven indications) in oncology from the period 2017 – October 2022 (12) (summarized in Table 1). The first drug approved in 2017 in a tissue-agnostic manner was pembrolizumab for the treatment of unresectable or metastatic solid tumors that have been identified as a microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) (13, 14). Three years later, FDA approved pembrolizumab for adult and pediatric patients with advanced and/or metastatic solid tumors exhibiting a high tumor mutational burden (TMB) (defined as ≥10 mutations/Mb) (15, 16). In 2018, larotrectinib was approved for pediatric and adult tumors harboring neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions without a known acquired resistance mutation (17, 18), while another NTRK inhibitor, entrectinib, was approved in August 2019 for a similar indication (Table 1) (19, 20). In 2021, FDA granted accelerated approval for the PD-L1 inhibitor dostarlimab-gxly for adult patients having dMMR advanced or recurrent solid cancers (21, 22). FDA also approved the VENTANA MMR RxDx assay as a companion diagnostic (CDx) test to select patients with dMMR solid cancers for treatment with dostarlimab-gxly. In June 2022, FDA granted accelerated approval to dabrafenib in combination with trametinib for the treatment of adult and pediatric patients ≥6 years of age with unresectable or metastatic solid tumors with *BRAF* ^{V600E} mutations who have progressed following prior treatment and have no satisfactory alternative treatment options (Table 1) (23). This approval was based on marked therapeutic responses to targeted BRAF/MEK inhibition of various solid malignancies with *BRAF* ^{V600E} mutations, including low-grade gliomas, biliary, gynecological and gastrointestinal cancers (24-26).

Highly potent RET inhibitors were developed, targeting the *RET* oncogene that encodes a receptor-type tyrosine kinase. *RET* (rearranged during transfection) acts as an essential oncogene in several cancers, including medullary thyroid, non-small cell lung (NSCLC), pancreatic, breast, and ovarian carcinomas (27). *RET* is usually rearranged via mutations or gene fusions (28). FDA has already approved the RET-inhibitor selpercatanib for RET-positive (fused or mutated) NSCLC, medullary thyroid and differentiated thyroid carcinomas (29), while another RET inhibitor pralsetinib was approved in 2020 for metastatic RET-fused NSCLC (30). In September 2022, FDA granted accelerated approval for selpercatanib for treating

Table 1. Overview of the Agnostic-Based Approved Targeted Treatments in Oncology

Name of the drug(s)	Year of approval	Mechanism of action	Indications
Pembrolizumab	2017	PD-1 inhibition	Adult and pediatric patients With solid cancers harboring MSI-H/dMMR status
Larotrectinib	2018	pan-TRK (NTRK1-3) inhibition	Adult and pediatric patients with NTRK1-3-fused solid cancers
Entrectinib	2019	NTRK1-3, ALK, and ROS1 inhibition	Adult and pediatric patients with NTRK1-3-fused solid cancers
Pembrolizumab	2020	PD-1 inhibition	Adult and pediatric patients with TMB-H solid cancers*
Dostarlimab-gxly	2021	PD-1-PD-L1/PD-L2 inhibition	Adult patients with dMMR recurrent or advanced solid cancers
Dabrafenib and trametinib	2022	BRAF and MEK (MAP2K1) inhibition	Metastatic solid cancers with *BRAF*^{V600E} mutations
Selpercatinib	2022	RET kinase inhibition	Adult patients with locally advanced or metastatic solid cancers with *RET* gene fusions

*TMB-H defined as ≥10 mutations/Mb; PD-1=Programmed cell death protein 1; NTRK1-3=Neurotrophic Tyrosine Receptor Kinase 1-3; MSI-H=Microsatellite instability-high; dMMR=Deficient mismatch repair; TMB-H=Tumor mutational burden high.

locally advanced or metastatic solid cancers harboring *RET* gene fusions. The tissue type-agnostic approval was based on the LIBRETTO-001 basket trial enrolling 45 patients with colorectal, breast, pancreatic, salivary gland, ovarian, small intestine, and cholangiocarcinomas, cancer of unknown primary, soft tissue sarcoma, and bronchial carcinoid (31). The basket trial revealed that selpercatinib exhibited clinically impactful activity in the *RET* fusion-positive tumor-agnostic patients, with a safety profile similar to the one previously reported for selpercatinib (31).

Herein, we review the distribution of *BRAF* mutations and other genomic alterations across tumor types, methods of detection and potential pitfalls and caveats associated with biomarkers testing.

BRAF and Precision Medicine

BRAF Gene

The *BRAF* gene (B-Raf proto-oncogene, serine/threonine kinase), located on chromosome 7q34, is a constitutive part of the mitogen-activated protein kinase (MAPK/ERK) signaling pathway involved in cancer initiation and progression via cell survival and proliferation (Figure 1) (32). The *BRAF* gene encodes a cytoplasmic protein with serine-threonine kinase activity. BRAF is usually activated via surface ligand binding to receptors with tyrosine kinase activity, such as Epidermal Growth Factor Receptor 1 (EGFR/HER1) or Human Epidermal Growth Factor Receptor 2 (HER2/ERBB2), followed by the activation of RAS-family GTPases. This chain of reactions leads to the dimerization of BRAF with BRAF or CRAF and activation of downstream components of the MAPK/ERK pathway MEK1/2 and ERK1/2 (Figure 1). The activation of the MAPK pathway upregulates various transcription factors involved in cellular survival, proliferation, and growth (32).

BRAF Mutations and Other Genomic Alterations

BRAF is frequently mutated in human cancer, with an estimated frequency of ~3-7% (33-37). Since 2002 when Davies et al. described *BRAF* mutations in a subset of human neoplasms (33), numerous studies explored *BRAF* status in various solid tumors (melanoma, carcinomas, brain tumors) and hematological malignancies (e.g., hairy cell leukemia, multiple myeloma, systemic histiocytoses) (35, 38-41). *BRAF* mutations have also been described in various soft tissue tumors, including malignant peripheral nerve sheath tumors (~10%), Ewing sarcomas (3%), and gastrointestinal stromal

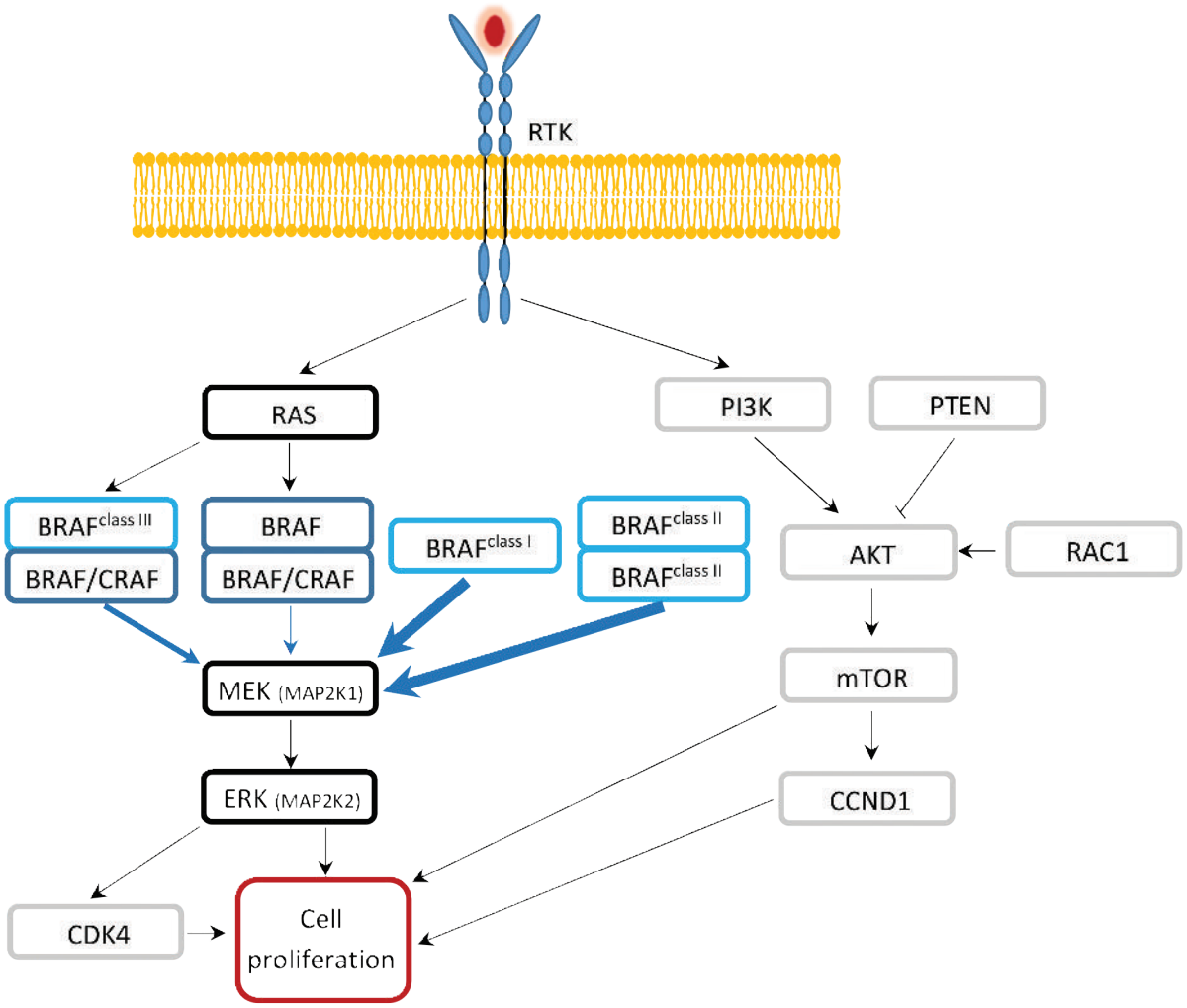

Figure 1. Schematic of MAPK signal (black/dark blue) and related (gray) pathways. Wild type *BRAF* (in dark blue) acts as a dimer (with BRAF or CRAF) to activate MEK in response to activation of RAS, eventually leading to cell proliferation. *BRAF* mutations (light blue) may act in a RAS-independent manner as monomers (Class I) or dimers (Class II), or in a RAS-dependent manner as a dimer (with wild type *BRAF/CRAF*). Mutant *BRAF* (in light blue) appears to be a stronger activator of MEK than wild type, with Class III less strong than classes I and II. BRAF inhibitor resistance may involve mutations at several of the genes encoding the proteins shown (see text).

tumors (7%) [reviewed in (42)]. *BRAFV600E* mutations were also detected in rare, poorly differentiated sarcomas with spindle cell morphology (43).

Tumors with the highest *BRAF* mutation rate (~50-80%) include malignant melanoma, papillary thyroid carcinoma, pilocytic astrocytoma and low-grade serous carcinoma of the ovary (35). However, in other tumors, the frequency of *BRAF* gene mutations is usually seen in the minority of cases (<5%) (44-47). *BRAF* mutations have also been described in several benign tumors, such as melanocytic nevi, metanephric adenomas, and pituitary adenomas, as well as in low-grade neoplasms, such as Erdheim-Chester disease and Langerhans cell histiocytosis (48-51) or locally aggressive neoplasms such as ameloblastomas and craniopharyngiomas (52-55). The data from molecular studies indicate that *BRAF* mutations alone cannot initiate malignant transformation and are usually preceded by the inactivation of

tumor suppressor genes (e.g., *CDKN2A, PTEN* and *BAP1*), *TERT* promoter mutations or inactivation of genes involved in DNA repair (48, 56-58).

Based on their effects on the MAPK pathway, three classes of *BRAF* gene mutations have been described: Class 1, associated with kinase activity (e.g., BRAF V600E, V600K/D/R/M mutations); Class 2 (e.g., K601E, K601N, K601T, L597Q, L597V, L485F, G469A, G469V, G469R, G464V, G464E, and fusions), with constitutively active dimers (codons 601, 597, 469, and 464) (Figure 1); These mutations are resistant to vemurafenib but may be sensitive to MEK inhibitors. Class 3 (D287H, V459L, G466V, G466E, G466A, S467L, G469E, N581S, N581I, D594N, D594G, D594A, D594H, F595L, G596D, and G596R), with low-to nil kinase activity/RAS-dependent mutations, frequently affect exons 11 and 15 and these mutations are commonly observed in non-small cell lung and colorectal carcinomas (48, 59); these mutations are sensitive to MEK inhibitors. Class 1 mutations are usually mutually exclusive with other driver mutations (e.g., *EGFR, KRAS, ALK*). The majority (~80-90%) of *BRAF* mutations are class 1 missense V600E mutations (35, 60). V600E mutation is caused by the transversion of T to A nucleotide 1799 (T1799A), resulting in a substitution of valine (V) for glutamic acid (E) at position 600. The remaining (15-20%) of *BRAF* mutations include V600K, V600R, V600M, V600D and non-V600 mutations (e.g., K601, D594N, G469). Some of these mutations may also be amenable to treatment with BRAF and/or MEK inhibitors (e.g., V600K). However, the efficacy appears to be lower compared to the sensitivity of V600E mutations (61). In contrast, some other mutations (e.g., G469 mutations) are predictors of resistance to anti-BRAF therapies but sensitivity to EGFR inhibitors (57, 62, 63).

In addition, rare *BRAF* gene fusions have been described in various cancer subtypes (frequency 0.3%), particularly in Spitzoid melanomas, pilocytic astrocytomas, papillary thyroid carcinomas, acinar pancreatic carcinomas, gastric carcinomas, serous ovarian carcinomas (both low- and high-grade), salivary gland carcinomas, and histiocytic neoplasms (pediatric and adult xanthogranulomas) (64-73). *BRAF* gene fusions and point mutations have recently been found in a subset of adult and pediatric soft tissue tumors with spindle cell morphology and infantile fibrosarcoma-like growth pattern (74-76). Antonescu also described a poorly differentiated sarcoma with a *BRAF* gene rearrangement; the neoplasm exhibited a whorling growth pattern with the spindle cells within a fibrotic stroma (77). Various *BRAF* gene fusions have also been described in other sarcoma morphologies (78-80). *BRAF*-fused cancers confer resistance to BRAF and EGFR inhibitors but may be sensitive to MEK or pan-RAF inhibitors (65, 81-88).

Not all cancers with *BRAF* mutations are responsive to BRAF inhibitors. Thus, in colorectal carcinoma, there is a strong interplay between *BRAF* and *EGFR,* and BRAF inhibitors alone are ineffective due to the activation of the EGFR pathway. However, a combined treatment with BRAF, MEK and EGFR inhibitors may overcome the potential resistance and induce a much better therapeutic response (89). In contrast, BRAF inhibitors effectively inhibit melanoma cells due to the low expression of EGFR receptor in these cells (89).

BRAF mutations have been associated with a more aggressive clinical course and poor outcomes in cancer patients (90-93). *BRAF* mutations are also strong predictors of response to anti-BRAF treatment modalities, such as BRAF (vemurafenib, dabrafenib and encorafenib) and MEK inhibitors (trametinib, cobimetinib, binimetinib) alone or in combination (45, 94). BRAF inhibitors and five combinations of a BRAF inhibitor plus an additional agent(s) to manage cancers such as melanoma, non-small cell lung cancer, anaplastic thyroid cancer, colorectal cancer, and Erdheim-Chester disease have been approved (Table 2). To date, each regimen is effective only in patients with tumors harboring *BRAF* V600 mutations, and the benefit duration is often short-lived. Further limitations preventing optimal management of *BRAF* mutant cancers are that treatments of non-V600 BRAF mutations have been less profound. Combined therapy is likely

Table 2. Overview of the Cancers with Approved Anti-BRAF Treatment Modalities

Tumor type (indication)	Drug(s)/Combinations	Predictive testing
Malignant melanoma	BRAF/MEK inhibitors /vemurafenib, dabrafenib, encorafenib/trametinib, cobimetinib, binimetinib/	*BRAF* mutational status
Colorectal carcinoma	BRAF/MEK/EGFR inhibitors (encorafenib/binimetinib/ cetuximab)	*KRAS, NRAS* and *BRAF* mutational status
Non-small cell lung carcinoma	BRAF/MEK inhibitors (dabrafenib/trametinib)	*BRAF* mutational status
Anaplastic thyroid carcinoma	BRAF/MEK inhibitors (dabrafenib/trametinib)	*BRAF* mutational status
Erdheim-Chester Disease	BRAF inhibitors (vemurafenib)	*BRAF* mutational status
Solid tumors	BRAF/MEK inhibitors (dabrafenib/trametinib)	$BRAF^{V600E}$ mutations

necessary to overcome resistance mechanisms, but multi-drug treatment options are often too toxic (95). The combination of a BRAF inhibitor and a MEK inhibitor (which acts by inhibiting kinases further downstream of BRAF in the MAPK pathway) substantially inhibits MAPK signaling with a more potent and durable inhibition of MAPK/ERK signaling and delayed acquired resistance (96-98). Dual MAPK pathway inhibition is a standard treatment option for *BRAF*-mutated melanoma (94, 99). Multiple studies also revealed the therapeutic benefit of vemurafenib in patients with several non-melanoma, *BRAF*-mutated cancer types such as NSCLC, Erdheim-Chester disease, Langerhans' cell histiocytosis, and hairy cell leukemia (39, 44, 100).

Resistance to BRAF/MEK Inhibitors

The resistance to BRAF/MEK inhibitors is an emerging problem associated with various genetic and/or epigenetic alterations within the two major signaling pathways, RAF/MEK/ERK and PIK3CA/PTEN/AKT (Figure 1) (35, 101-104). While the intrinsic resistance to BRAF/MEK inhibitors is relatively rare, the acquired resistance (following the treatment) is widespread and nearly inevitable. In particular, mutations of *KRAS, NRAS, MAP2K1,* and *MAP2K2,* along with *BRAF* amplifications (MAPK reactivation or "addiction"), contribute to the resistance to BRAF inhibitors [(reviewed in (57, 104)]. Another potential resistance mechanism is a *BRAFV600E* splice variant that promotes RAF dimerization (105). Mutations within the PIK3CA/PTEN signaling pathway involving *PIK3CA, PTEN, AKT1, PIK3R1, PIK3R2,* and *AKT3* genes are also involved in the resistance to BRAF inhibitors. Genetic alterations of *RAC1, CDK4, CCND1*, and *c-MET* genes also contribute to the resistance to anti-BRAF treatment modalities. Recently, androgen receptor (AR) expression has been described as a potential resistance mechanism in preclinical (animal) models with significantly reduced anticancer activity of BRAF/MEK inhibitors in male mice compared with female mice (106). The study also revealed significantly higher AR expression in melanomas affecting male mice than female mice. The preclinical observations were further translated and confirmed in a clinical cohort of melanoma patients treated with BRAF/MEK inhibitors (106). Further studies should confirm whether androgen suppression could be combined with BRAF/MEK inhibitors in melanoma patients. In NSCLC, the most common causes of resistance to BRAF/MEK inhibitors are mutations of *MEK1, PTEN, NRAS*, and *KRAS* genes (107).

Epigenetic or transcriptome-based changes were speculated to be the likely drivers of the resistance to BRAF inhibitors among ~40% of melanomas that progressed on the treatment and lacked any identifiable genetic abnormality to explain such resistance (104). Among these resistance mechanisms, DNA methylation, post-translational

histone modifications, and various miRNAs appear to play prominent roles (108).

Diagnostic Approaches for BRAF Mutations

For treatment purposes, a routine determination of *BRAF* status is the standard of care (99, 109-111). *BRAF* analysis is usually performed on formalin-fixed paraffin-embedded tissue (FFPE) samples (either primary or metastatic). If FFPE of the primary or metastatic cancer is unavailable, a blood sample or liquid biopsy using circulating tumor DNA (ctDNA) may be an alternative (Guardant 360, Table 3). Although ctDNA presents an essential innovation in cancer diagnostics and management (e.g., diagnosis and molecular profiling of advanced non-small cell lung cancer or the monitoring of BRAF status in melanoma patients during the targeted treatment with BRAF/MEK inhibitors) (34703985), it has certain limitations, including lower sensitivity (47-84%) compared with the PCR-based assays performed on FFPE (112-116).

BRAF analysis is usually performed using various DNA-based molecular assays. The FDA has also approved several diagnostic assays for detecting *BRAF* mutations as CDx tests or authorized assays (summarized in Table 3). Various laboratory-developed assays have also been developed and routinely utilized for *BRAF* gene testing in patients with melanoma and other cancers with approved anti-BRAF treatment modalities (Table 2) (57).

Among the DNA/RNA-based assays, Sanger sequencing, pyrosequencing, mutation-specific Polymerase Chain Reaction (PCR) and mutation-specific real-time PCR, digital PCR (dPCR), High-Resolution Melting curve analysis (HRM), Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS; Sequenom), and many Next-Generation Sequencing (NGS) based assays are available (117). Each of these assays has its characteristics and performances but shares very high sensitivity and specificity (~85-100%) in detecting genomic alterations, including *BRAF* gene mutations (117). Some of these assays were also approved by FDA as CDx tests (summarized in Table 3).

The Cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.) was the first FDA-approved CDx for BRAF assessment. This test was used in the clinical trial that led to the approval of vemurafenib by FDA and later by the European Medicines Agency (EMA) (118). The Cobas 4800 BRAF V600 Mutation Test was approved for the vemurafenib/cobimetinib combination, while another RT-PCR-based assay approved for dabrafenib/trametinib combination is the THxID-BRAF kit (bioMerieux Inc.) (Table 3). The therascreen BRAF V600E RGQ PCR Kit (QIAGEN GmbH) is the third RT-PCR-based assay approved by FDA as a CDx. It assesses *BRAFV600E* mutations in patients with colorectal cancer for the potential treatment with encorafenib in combination with cetuximab (a monoclonal antibody against EGFR).

NGS refers to large-scale (high-throughput) DNA and RNA sequencing technology that allows for querying the whole genome, the exons within all known genes (whole exome), or only exons of selected genes (target panel). The use of NGS revolutionized cancer genomic profiling and has become a cornerstone diagnostic tool in precision medicine management (119, 120). It is a highly efficient and precise assay (sensitivity of 98% and specificity of 100%) that enables comprehensive cancer genomic profiling. It is, therefore, a reliable and affordable tool for detecting various genomic alterations, including those affecting the *BRAF* gene (117). Several NGS-based assays achieved either CDx status or were authorized by FDA. These include CDx assays FoundationOne CDx (by Foundation Medicine, Inc.), and Oncomine Dx Target Test (by Life Technologies Corporation), and FDA-authorized assays MSK-IMPACT (by Memorial Sloan Kettering Center), and Guardant360 CDx (by Guardant Health, Inc.) (Table 3). These assays include gene panels of various sizes (from 55 to 505 genes) and also provide additional valuable information about other predictive biomarkers (e.g., tumor mutational burden or microsatellite instability status) (See Table 3

Table 3. The List of FDA-Approved Companion Diagnostic and Authorized Tests/Assays for *BRAF* Testing [Adopted and Modified From (4)].

Test (Manufacturer)	Indication(s)	Diagnostic method
CDx tests/assays		
Cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.)	Malignant melanoma (covering V600E and V600K mutations, respectively)	PCR-based assay
FoundationOne CDx (Foundation Medicine, Inc.)	NSCLC and melanoma (covering V600E and V600 mutations, respectively)	NGS based assay
Oncomine Dx Target Test (Life Technologies Corporation)	NSCLC (covering V600E mutations)	NGS based assay
The therascreen BRAF V600E RGQ PCR Kit (QIAGEN GmbH)	Colorectal cancer (covering V600E mutations)	Real-time PCR
The THxID-BRAF kit (bioMerieux Inc.)	Malignant melanoma (covering V600E and V600K mutations)	Real-time PCR
FDA-authorized tests/assays		
MSK-IMPACT (Memorial Sloan Kettering/ MSK/)	Melanoma and other cancers with *BRAF* and other mutations (the panel of 505 genes)	NGS based assay
Guardant360 CDx (Guardant Health, Inc.)	NSCLC, CRC (*BRAF* and 54 additional targetable genes)	NGS assay based on liquid biopsy

PCR=Polymerase chain reaction; CDx=Companion diagnostics; NGS=Next-generation sequencing; NSCLC=Non-small cell lung carcinoma; CRC=Colorectal carcinoma.

with the list of FDA-approved CDx assays based on NGS technology).

The VE1 antibody is the only immunohistochemical assay currently available for BRAF protein testing and detection (57) but has not received regulatory approval as a CDx despite its widespread availability. BRAF V600E-specific antibody VE1 has a good concordance with detecting the *BRAFV600E* mutation by some genetic tests (34). A meta-analysis based on 21 studies covering 1687 melanoma cases confirmed an excellent diagnostic utility of the VE1 antibody for detecting *BRAFV600E* mutation, with a sensitivity of 0.96 and specificity of 1.00 (121). Similar performance of the VE1 antibody was reported in colorectal (122-124), thyroid carcinomas (125-128), hairy cell leukemias (129, 130) (Figure 2A-B), and low-grade serous ovarian neoplasms (131).

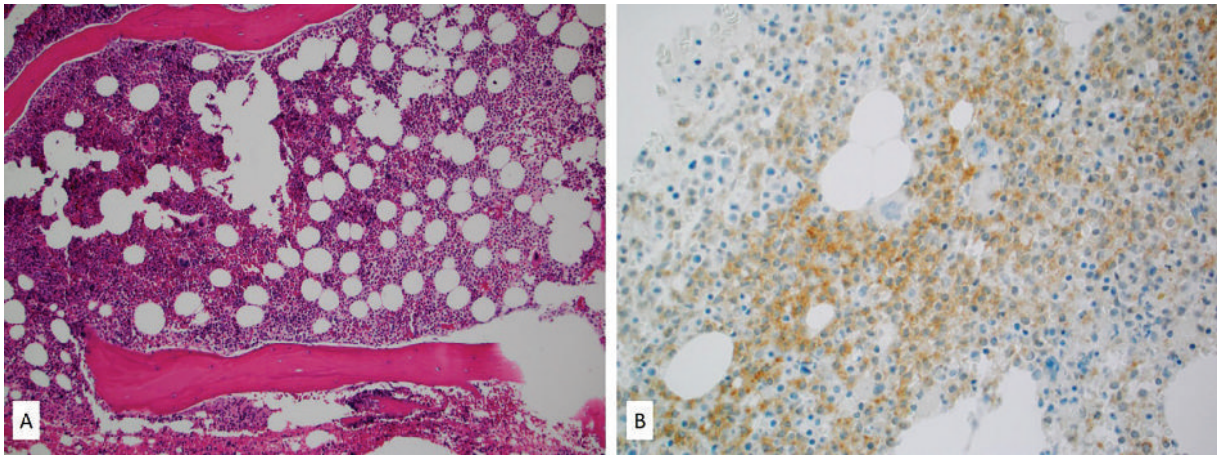

Figure 2A-B. (A): Hematoxylin and Eosin (H&E) slide of a case of hairy cell leukemia with a diffuse bone marrow infiltration (10x magnification); neoplastic cells harbored *BRAFV600E* mutation, which was confirmed immunohistochemically using VE1 antibody (40x magnification).

Our previous study, based on a cohort of diverse cancers, confirmed that the VE1 antibody is 100% sensitive and 91% specific for *BRAFV600E* protein and may serve as a good screening tool, especially in tumor types with a high proportion of *BRAFV600E* mutation (e.g., thyroid carcinoma, colorectal carcinoma, melanoma) (132). However, VE1 IHC screening in tumor types with a higher proportion of non-*BRAFV600* mutation may not be feasible with a high proportion of false-negative results (133-135). For instance, lung adenocarcinomas may have a higher proportion of false negative results due to the finding of the D594V mutation. Rare actionable mutations (e.g., V600K) may also be missed using VE1 IHC alone (132, 136). The discrepancies between VE1 IHC and PCR assays have also been described in the kidney's *BRAFV600E* -mutated metanephric adenomas, pituitary adenomas, and Langerhans cell histiocytosis (51, 137, 138).

Although Martins-de-Barros et al. in the systematic review with a meta-analysis, reported an excellent diagnostic utility of VE1 IHC in ameloblastomas (139), several studies reported its low diagnostic value in maxillary ameloblastomas that are predominantly affected by non-*BRAFV600E*-mutations (52, 140).

Taken together, VE1 IHC appears to be an excellent screening assay, particularly for the detection of *BRAFV600E* mutations, but further confirmation with molecular (PCR)-based methods is still required for the targeted treatment with BRAF and/or MEK inhibitors.

Conclusions

Precision cancer medicine has substantially improved cancer diagnostics and treatment. Tissue type-agnostic drug therapies present a novel shift in precision cancer medicine. It is a consequence of carefully designed clinical trials showing the value of tumor biomarkers, not just in diagnosis but in therapy guidance. Six different tumor-agnostic treatment modalities have been approved for cancer treatment since 2017 when pembrolizumab was approved for MSI-H/dMMR solid tumors regardless of their histotype. In June 2022, a combination of BRAF/MEK inhibitors (dabrafenib/trametinib) was approved in a tumor-agnostic fashion for all solid metastatic cancers harboring $BRAF^{V600E}$ mutations. *BRAF* mutations affect ~3-7% of all cancers, with the highest prevalence in melanoma, papillary thyroid carcinoma, pilocytic astrocytoma, and low-grade serous ovarian carcinoma. However, a low prevalence (≤5%) of *BRAF* mutations has been described in ~50 cancer subtypes. BRAF inhibitors alone or combined with MEK inhibitors have been approved and substantially improved the treatment of several cancers, including malignant melanoma, non-small cell lung cancer, anaplastic thyroid cancer, colorectal cancer, and Erdheim-Chester disease. The diagnosis of *BRAF* mutations remains a cornerstone of anti-BRAF treatment(s), and several highly sensitive and specific diagnostic assays were approved as CDx tests. Resistance to treatment represents an emerging issue among BRAF cancers, mainly when BRAF inhibitors are administered alone. Apart from mutations within MAPK/MEK and PIK3CA signaling pathways, novel and potentially targetable resistance causes have been recently described (androgen receptor overexpression). Further efforts are needed to translate these findings into clinical practice and improve the outcome of patients with *BRAF*-mutated cancers.

Conflict of Interest: Gargi D. Basu and David W. Hall are full-time employees and stockholders of Exact Sciences. Zoran Gatalica is a part-time employee of Exact Sciences. Semir Vranic declares no conflict of interest.

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The Superficial Temporal Artery: Anatomy and Clinical Significance in the Era of Facial Surgery and Aesthetic Medicine

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Abstract

Objective. The aim of this review was to present the vascular pattern of the STA, as well as anatomical variations, and to accentuate the areas which should be taken into consideration during manipulations along the course of the artery. **Background.** The STA may be encountered during several aesthetic procedures on the face, and iatrogenic trauma could be an incriminating factor of diverse sequelae. The constant increase in demand for facial aesthetic procedures has rendered it imperative to maximize safety and patient satisfaction. **Methods.** We conducted a narrative literature review using the electronic databases of PubMed and Google Scholar, retrieving studies concerning the anatomy and variations of the STA. Moreover, we identified registered clinical cases presenting complications which involved the artery. **Discussion.** The anatomic morphology of the STA is described and classification systems summarized, on the basis of the studies retrieved. In addition, the STA is related to defined landmarks, and specific danger zones are emphasized. Finally, the clinical significance of the artery is reflected in registered cases of adverse events following specific aesthetic surgeries. **Conclusion.** Thorough knowledge of the anatomical variability of the STA, and awareness of the danger zones involved in aesthetic procedures, combined with intraoperative vigilance could increase safety and minimize the advent of relevant sequelae.

Key Words: Superficial Temporal Artery ▪ Anatomy ▪ Variations ▪ Aesthetic Surgery ▪ Temporal Rejuvenation.

Introduction

The superficial temporal artery (STA) constitutes a terminal branch of the external carotid artery, which surfaces posteriorly to the mandible in the parotid gland, and crosses the zygomatic process of the temporal bone (1-3). Large areas of the scalp and face derive their blood supply from the STA.

The substantial overall increase in demand for cosmetic facial surgeries, as well as minimally invasive procedures, engender the need to minimize the risk of inadvertent complications and maximize safety (4, 5). This spike in demand has been attributed to an increasing preoccupation with virtual platforms and engagement with social media, especially during the COVID-19 pandemic (4, 6). Knowledge of the localization of the STA and a thorough understanding of its topographical anatomy and clinically relevant arterial variations are crucial for improving safety in surgical and aesthetic procedures involving the vascularized region.

The purpose of this article is to present anatomical variations of the STA tree, assisting the surgeon in delineating a morphological map of the artery and the potential danger zones that could be encountered during facial surgical and minimally invasive procedures. The clinical significance of the artery may be appreciated in the light of the many different complications involving the STA during facial aesthetic procedures, as described in the literature. An assiduous knowledge of the anatomical variability of the artery enables the surgeon to prevent foreseeable complications, and maximize efficacy and safety.

Methods

We conducted a narrative literature review using the electronic databases of PubMed and Google Scholar, retrieving studies related to the anatomy and variations of the STA, published up to July 2022. In addition, articles regarding clinical cases in the field of cosmetic surgery presenting complications involving the STA were included. Associated articles cited in the reference lists of the identified studies were also reviewed. The following search terms were employed: superficial temporal artery OR STA AND anatomy OR variations OR plastic surgery OR cosmetic surgery OR aesthetic surgery OR variants OR pseudoaneurysm OR arteriovenous fistula OR bleeding OR rhytidectomy OR facelift OR fat-grafting OR otoplasty OR hair transplantation OR filler, temporal AND augmentation OR rejuvenation OR filler OR lifting OR hyaluronic acid. All evaluated articles were written in English.

The studies were categorized according to type, and data concerning the ethnicity of the population, the morphology and identified anatomical variations of the arteries were collected. With respect to the pertinent anatomy, a number of danger zones were suggested by different authors, aiming at facilitating safer dissections.

In the second part of the review, the clinical relevance of the STA in the field of aesthetic surgery was studied by identifying cases associated with complications arising from manipulation in areas vascularized by the STA and its variants. Cases involving the development of pseudoaneurysm or arteriovenous fistula of the STA following punch autograft hair transplantation were excluded, given the fact that this specific technique has been relinquished due to the overall drawbacks of the procedure.

Results

A total of 19 studies that could provide appropriate data were included in our review. The morphological tree of the STA was mainly described in 4 studies and respective classifications were formed, whereas variations concerning additional, atrophic or absent branches were displayed in an additional 5 studies. Differences in the ethnicity of the populations studied were related to deviations in the branching patterns. Interestingly, a larger number of cases of African ethnicity showed the presence of double parietal or double frontal branches, in comparison to other ethnicities. Cases of trifurcation were also reported. Consequently, the ethnic background of a patient should be taken into consideration, as multiple vessels could be encountered during dissection. Ethnic differences were also observed in the levels of the division of the STA in relation to the zygomatic arch, which was the point of interest in 13 studies (Table 1). A greater percentage of arteries bifurcated on the level of the zygomatic arch among the Caucasian population compared to African and Asian ethnicities, which were associated with bifurcation points above the zygomatic arch. Such differences should be contemplated during the planning of procedures such as rhytidectomy.

Moreover, the majority of the anatomical patterns of the artery was inconsistent between the two sides of the same person. The anatomical pattern of the frontal branch of the superficial temporal artery (FBSTA) was determined in 3 studies introducing classification systems with regard to its branching structure, whereas 10 studies presented a topographical mapping of the STA in relation to defined landmarks.

In the context of understanding the implication of the artery in adverse events following aesthetic surgery, we identified 15 relevant clinical cases (Table 2). The formation of an arteriovenous fistula of the superficial temporal vessels or a pseudoaneurysm as well as bleeding were the most common complications following surgery, especially after rhytidectomy and malarplasty. Vascular complications concerning the ophthalmic as well as the cerebral arteries were, however, often encountered after autologous fat-grafting. Surgeons should reflect on the possibility of these sequelae, and delve into the anatomy and variations of the vessels, considering the suggested danger zones as well, before engaging on manipulations in the frontal and temporal region.

Table 1. The Bifurcation Point of the STA*

Author and year	Ethnicity	The zygomatic arch		
		Above (%)	Over (%)	Below (%)
Pinar Y, 2006 (2)	Caucasian	74.1	22.2	3.7
Stock A, 1980 (3)	American	60	32	8
Jean-Philippe H, 2021 (7)	Caucasian	61.5	26.9	1
Koziej M, 2019 (8)	Caucasian	75.6	9.7	14.7
Medved F, 2015 (9)	Caucasian	60	26	3
Mwachaka P, 2010 (10)	African	80	13.3	6.7
Marano S, 1985 (11)	American	96	4	-
Chen T, 1999 (12)	Asian	86.5	3.8	9.6
Kim B, 2013 (13)	Asian	82.6	10.1	7.2
Kuruoglu E, 2014 (14)	Caucasian	40	58	2
Tayfur V, 2010 (15)	Caucasian	62	38	-
Cobb M, 2016 (16)	American	96	4	-
Rusu M, 2021 (17)	Caucasian	87	11.6	1.4

*The superficial temporal artery.

Table 2. Complications Involving the STA Following Aesthetic Surgeries

Author and year	Procedure	Complication
Kim Y, 2010 (18)	Intraoral mandibular angle ostectomy	Bleeding
Owsley T, 2009 (19)	Otoplasty	Bleeding
Grazer F, 1992 (20)	Facelift	Late bleeding
Goldwyn R, 1990 (21)	Facelift	Late bleeding
Lin K, 2004 (22)	Facelift	Pseudoaneurysm
Wang D, 2014 (23)	Autologous fat-grafting	Cerebral infarction, embolism of internal and external carotid arteries
Shen X, 2016 (24)	Autologous fat-grafting	Cerebral infarction, embolism of external and internal carotid arteries
Thaunat O, 2004 (25)	Autologous fat-grafting	Cerebral infarction, embolism of anterior cerebral artery
Kominami S, 2012 (26)	Facelift	Arteriovenous fistula
Hua C, 2018 (27)	Rhinoplasty, nasal tip-grafting with concha cartilage	Arteriovenous fistula
Wang X, 2018 (28)	Autologous fat-grafting	Cerebral infarction, embolism of STA
Hu J, 2011 (29)	Autologous fat-grafting	Cerebral infarction, middle cerebral artery embolism
Lu L, 2013 (30)	Autologous fat-grafting	Chorioretinal infarction
Chen Y, 2014 (31)	Autologous fat-grafting	Opthalmic artery embolism
Kim J, 2015 (32)	Reduction malarplasty	Arteriovenous fistula

Discussion

The superficial temporal artery (STA) courses inside the superficial temporal fascia, which is an extension of the galea and the muscular aponeurotic system of the face, and usually bifurcates into two branches: an anterior frontal branch and a posterior parietal branch (7, 33, 34). Apart from the main branches, the STA gives rise to the transverse facial, zygomatico-orbital, auricular and middle temporal arteries (8). As it approaches the temporal crest, the STA traverses layer 2 (the

subcutaneous plane) reaching the occipitofrontalis muscle in the forehead (33).

Branching patterns of the superficial temporal artery

The morphological tree of the STA and its main branches have been described on the basis of cadaver studies and radiological examinations, and respective classifications have been configurated by various authors (Tables 3 and 4). Medved et al. introduced a classification system of the anatomical variations of the STA, depending on the terminal branching pattern. According to this classification system, five types of STA were recognized, with eleven variations (9). Specifically, type A referred to the classic pattern where the main trunk divides into a frontal and a parietal branch, whereas type B concerned additional branches, either frontal (B1) or parietal (B2), or both branches

Table 3. Retrieved Studies and Their Characteristics

Author and year	Ethnicity	Type of study	Arteries (N)
Pinar Y, 2006 (2)	Turkey	Cadaver	27
Stock A, 1980 (3)	USA	Cadaver	15
Stock A, 1980 (3)	USA	Radiological	25
Jean-Philippe H, 2021 (7)	Belgium	Radiological	114
Koziej M, 2019 (8)	Poland	Radiological	419
Medved F, 2015 (9)	Germany	Radiological	93
Mwachaka P, 2010 (10)	Kenya	Cadaver	60
Marano S, 1985 (11)	USA	Cadaver	50
Chen T, 1999 (12)	China	Cadaver	52
Kim B, 2013 (13)	Korea	Radiological	70
Kuruoglu E, 2014 (14)	Turkey	Radiological	53
Tayfur V, 2010 (15)	Turkey	Cadaver	26
Cobb M, 2016 (16)	USA	Radiological	25
Rusu M, 2021 (17)	Romania	Radiological	86
Manoli T, 2016 (35)	Germany	Radiological	76
Lee J, 2015 (36)	Korea	Cadaver	64
Hong W, 2020 (37)	China	Radiological	107
Kleintjes W, 2007 (38)	South Africa	Cadaver	60
Imanishi N, 2002 (39)	Japan	Cadaver	30
Lei T, 2005 (40)	China	Cadaver	30

Table 4. Anatomical Variations of the STA*

Author	Classic (%)	DF/PB† (%)	Trifurcation (%)	AF/PB‡ (%)	AF/PB§ (%)
Jean-Philippe H, 2021 (7)	73.7	26.3	-	-	-
Koziej M, 2019 (8)	11.4	1.4	-	11.2	76
Medved F, 2015 (9)	39.8	8.6	-	10.7	22.6
Mwachaka P, 2010 (10)	53.3	40	6.7	-	-
Marano S, 1985 (11)	66	12	-	8	8
Rusu M, 2021 (17)	80.2	-	-	19.8	-
Manoli T, 2016 (35)	40.8	9.2	-	9.2	31.6

*The superficial temporal artery; †Double frontal/parietal branch; ‡Absent frontal/parietal branch; §Atrophic frontal/parietal branch.

(B3). In type C, atrophic branches were noted either as frontal (C2) or parietal (C1), or both (C3). Subsequently, type D lacked bifurcation, while the STA followed a frontal (D1) or a parietal course (D2). Finally, type E was characterized by the presence of an additional superior auricular artery emanating from the main trunk (E1) or the parietal branch (E2) (9).

On the basis of this classification, Manoli et al. concluded that the anatomical morphology of the STA in the same person is random. Interestingly, in only 26% of the studied cases was the branching pattern consistent on both sides (35). Mwachaka et al. examined the anatomy of 30 cadavers of Kenyan ethnicity, and reported 4 branching patterns. The classical bifurcation was observed in 53.3% of cases, while double frontal branches were reported in 26.7% of the cadavers, and double parietal branches in 13.3% of the cadavers. Remarkably, two cases of trifurcation were identified (10). Compared to other relevant studies, deviations in the branching pattern were related to ethnic differences. Marano et al. proposed a classification in 1985 depending not only on the number of branches but also their diameter, as well as the diameter of their common trunk, rendering those with diameter <1mm atrophic, and the position of the bifurcation in relation to the zygoma. No additional superior auricular artery was reported at the time (11).

The temporal region constitutes a target for rejuvenation techniques, rendering the FBSTA vulnerable to trauma. Lee et al. classified the FBSTA into two categories depending on the number of branches and their course in relation to the frontal belly of the occipitofrontalis muscle. In type I, a single frontal branch reached a single point on the lateral aspect of the frontal belly (Ia) or issued two branches before traversing the frontal belly (Ib). On the other hand, type II arteries consisted of a double frontal branch, which either coursed above the borders of the orbicularis oculi muscle (IIa) or were covered by the muscle (IIb). Remarkably, the type I structural pattern was found in 96.9% of cases (36). Hong et al. published a study of 66 cadavers, differentiating the FBSTA into two types depending on their course over the temporal crest. 89.7% of the arteries turned abruptly by almost 90° upon traversing the temporal crest, whereas 10.3% of them supplied the superior portion of the forehead, turning by a lesser degree (52.3°) (37).

According to Kleintjes et al., the FBSTA reaches the forehead at various transverse levels along a vertical line traversing the lateral orbital rim. In this region, the FBSTA usually issues an ascending branch and a transverse branch, described as the ascending frontal artery and the transverse frontal artery, respectively (38). An anastomosis was often reported between the oblique branch of the supraorbital artery and either the transverse frontal artery or the FBSTA. Notably, the frontal branches anastomose on the galea on the forehead, as well as with the supraorbital and supratrochlear arteries (2, 33). Additionally, perforating branches originating from the FBSTA were observed in a circular area, with a radius of approximately 9 mm situated 40.5 mm laterally from the midline, and 53.6 mm superior to the supraorbital rim (41).

Traditionally, the STA divides into its terminal branches at a point about 5 cm above the zygomatic process of the temporal bone. According to a meta-analysis conducted by Koziej et al., 79.1 % of STAs bifurcated above the zygomatic arch, 11.1% of cases on the arch and 6.7% of them below the arch (42). Morphological differences in the anatomic distribution of the STA with regard to the zygomatic arch were ascertained in a cadaver study of the Chinese population in comparison to corresponding studies in Caucasian adults. Chen et al. observed a classical bifurcation pattern above the zygomatic arch in 86.5% of cases, while 3.8% of the STAs issued their terminal branches on the zygomatic arch and 9.6% below that level (12). A similar distribution was demonstrated in a cadaver study of African ethnicity by Mwachaka et al. (80%, 13.3%, 4%), as well as in a radiological study of Korean patients by Kim et al. (82.6%, 10.1%, 7.2%) (10, 13). Studies on Caucasian patients showed differences in this distribution. Specifically, Stock et al. found a bifurcation above the zygomatic arch in 60% of cases, over the arch in 32% and below it in 8% of cases (3). Similar results were published by Kuruoglu et al. (58%, 40%, 2%), Tayfur et al.

(62%, 38%) and Pinar et al. (74.1%, 22.2%, 3.7%), Hardy et al. (61.5%, 26.9%, 11.54%) (2, 7, 14, 15). According to Imanishi et al. the terminating points of the STA are mainly located in the posterior fourth of the area between the external canthus and the root of the helix, or near this region (39).

Cobb et al. studied the course of the STA around the zygomatic arch, discovering a characteristic "C shaped half-buttonhole configuration" when the artery is traced over the zygomatic arch (96% of STAs studied). In two cases the STA was located posterior to the condylar process of the mandible (16). Furthermore, a radiological study of 43 patients in 2021 showed that the STA was documented to be retrocondylar in 65.1% of cases. The rest of the arteries were located laterally to the mandibular condyle (laterocondylar). The same study demonstrated the existence of kinking and coiling of the STA in 88.4% of the patients (17). Surgeons need to be aware of these structural variability to avoid injury during lateral approaches.

A transverse facial artery was discovered in all specimens in a cadaver study published by Pinar et al., whereas 22.2% issued no zygomatico-orbital artery (ZOA). Interestingly, the zygomatico-orbital artery emanated from the FBSTA in cases where the bifurcation point of the STA was over the zygomatic arch (2). According to a recent study by Park et al., the ZOA was identified in 85.2% of cases, branching from the FBSTA and coursing within ±1cm along a line demarcated by the tragus and the superciliary arch. Occasionally, the ZOA bifurcates from the middle temporal or parietal branch of the STA. Due to its connection to the supraorbital artery and the lacrimal artery, the course of the ZOA is considered a danger zone during filler treatment (43).

Landmarks for Identifying Its Course

The location of the STA has been defined according to distinct anatomical landmarks, specifically the tragus or bony external auditory canal, the zygomatic arch and the lateral canthus (Table 5). The distance measured between the STA and the tragus was 16.68+-0.35 mm in a study by Pinar et al. (2). On the eye-tragus-line, the location of the artery was measured at 15.55+-4.5 mm in front of the tragus (7). Furthermore, the STA was found to be located 12.31+-12.83 mm away from the zygomatic arch in a study published by Hardy et al. Regarding the level of its course, the STA was found to run within a depth of approximately 2.31 mm, while the bifurcation point was identified within a depth of 3.25 mm (7, 44). Remarkably, differences were reported in other studies which were also attributed to ethnic variability (7, 10, 13).

Chen et al. suggested that the distance of the incision from the external auditory canal during facelift procedures should be shorter than 1 cm, in

Table 5. Distance of STA* to Specific Landmarks (in cm)

Author and year	Tragus	Bifurcation to ZA†	EA‡	BEAC§
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Pinar Y, 2006 (2)	1.68±0.03	-	-	-
Stock A, 1980 (3)	-	-	1.39±0.29	0.94±0.38
Jean-Philippe H, 2021 (7)	-	1.23±1.28	-	-
Koziej M, 2019 (8)	-	2.38±1.14 (center)		
Mwachaka P, 2010 (10)	4.41±1.85	5.08±2.09 (center)	-	-
Chen T, 1999 (12)	-	-	1.22±0.79	1.14±0.32
Kim B, 2013 (13)	-	2.17±1.58 (superior margin)	-	-
Kuruoglu E, 2014 (14)	-	1.41±0.77 (superior margin)	-	-
Tayfur V, 2010 (15)	-	1.2-2.3 (superior margin)	-	-
Lee J, 2015 (36)	1.72±0.82	-	-	-

*The superficial temporal artery; †The zygomatic arch; ‡Ear attachment; Bony external auditory canal.

order to avoid lesion of the STA (12). The position of the temporal branches of the facial nerve can be predicted depending on the location of the FBSTA with regard to the superior orbital rim, and thus protected during a rhytidectomy procedure (40). Traditionally, the temporal branches can be traced 1-2 cm anteriorly and inferiorly to the FBSTA, however a minority of cases present a course just inferior to the FBSTA (45). Nevertheless, the galea separates the artery from the temporal branch, as the latter is located in the subgaleal fat pad (46).

Location of Safe and Danger Zones

The use of fillers has become a significant part of our armamentarium in the treatment of patients seeking rejuvenation (47). However, an increased incidence of adverse events has been noted alongside the widespread use of filler treatment, while a turn from 2-dimensional management of wrinkles to 3-dimensional treatment of the entirety of the face gives rise to the amplified risk of vascular compromise (47). Precise injection techniques using anatomical landmarks, as well as detailed knowledge of the target regional anatomy, are of outmost importance in order to maximize safety (48, 49). Various studies have elucidated areas of interest along the course of the STA, emphasizing the need to be aware of them during surgical procedures and aesthetic manipulations (8). These areas are referred to as danger zones. Koziej et al. detected a triangular artery-free region, using the zygomatic bone as an anatomical landmark. The vertical and horizontal sides are formed by the zygomatic bone, and the superior, oblique side consists of the FBSTA. The artery-free zone extends 25.7 mm on the vertical plane and 31.4 mm on the horizontal plane (8). However, a large number of perforators of the middle temporal vessels can be encountered in the anterior half of the lower temporal compartment, that is, the area of the loose areolar tissue layer below the inferior temporal septum, as well as the temporal branches of the facial nerve and the sentinel vein (50). Therefore, it is crucial to ensure that manipulations are limited to the level above the temporoparietal fascia (8). Another safe zone lies in the preauricular region that, however, extends nearly 5.6 mm in front of the external auditory meatus. Moreover, a wide arterial zone is situated 34.7-74.5 mm superior to the external auditory meatus, where the parietal branch of the STA runs. Another danger zone lies superior to the supraorbital rim, as the FBSTA courses about 47 mm superior to this landmark, accompanied by the temporal branches of the facial nerve, running inferior and parallel to it (8, 51). The FBSTA runs within the temporoparietal fascia, transitioning to the subcutaneous plane as it approaches the border of the occipitofrontalis muscle (51). According to Lee et al. the area located 2.5 cm lateral and 3 cm superior to the intersection point between the vertical plane through the lateral epicanthus and the uppermost eyebrow point comprises a danger zone, where the FBSTA crosses the lateral border of the occipitofrontalis muscle. A straightforward clinical way of detecting this area is to position the thumb between these two planes, and avoid the part extending from the tip of the thumb to the first interphalangeal joint (36).

Anatomical Considerations Related to the Use of Fillers

The temporal region has a complex vascular anatomy owing to the multitude of vessels coursing through its different layers, as well as the robust arterial and venous connections to the internal carotid artery and the cavernous sinus (44, 52). The branches of the STA and the zygomatico-orbital artery run within the laminae of the superficial temporal fascia, while the middle zygomatico-temporal vein is detected within the deep fat of the temple, between the superficial temporal fascia and the deep temporal fascia (52). Thus, it is suggested that filler treatment should take place subcutaneously or deep in the supraperiosteal plane (49). Interestingly, a histological study by Chundury et al. showed that even when hyaluronic acid is injected within the subcutaneous tissues, a certain amount of it could unintentionally be placed at deeper levels, as well as within the perivascular tissues (53).

The transverse facial artery runs 2 cm superiorly to the zygomatic arch, and parallel to it. The middle temporal artery emanates from the STA above the superior border of the zygomatic arch, and runs towards the superior border of the orbital rim at a depth of 4.01mm. Its cutaneous branch courses subcutaneously. However, a muscular branch enters the temporalis muscle at a depth of 6.31mm (44). Vascular complications may also arise if filler is injected into the middle temporal vein, which is identified within the superficial temporal fat pad approximately 2 cm superiorly to the zygomatic arch (47, 53). Due to its large diameter, deep injections should be avoided in this area and rather be performed several centimeters superiorly to the zygomatic arch (51, 52). The anterior deep temporal artery is identified in the anterior portion of the temporalis muscle, while the posterior deep temporal artery runs in its middle aspect at a depth of 14.13 mm, and communicates with the middle temporal artery at the center of the temporal area (44, 52).

According to De Maio et al. a safe access point for temple volumization is located 1 cm lateral to the temporal crest and 1 cm superior to the lateral orbital rim, as the middle temporal artery and the deep temporal artery course more posteriorly. The filler should be injected in the supraperiosteal plane (48). Cotofana et al. suggest six injection techniques targeting volumization and lifting in the temporal region subdermally, in the supraperiosteal and interfascial plane. However, the muscular branch of the middle temporal artery, the sentinel vein and branches of the facial nerve could be encountered in the latter (44, 52). Huang et al. suggest injecting in the upper temporal compartment and the lower temporal compartment of the loose areolar tissue layer, entering medially to the junction of the hairline and the temporal crest, and avoiding the anterior half of the lower temporal compartment where the sentinel vein, perforators of the middle temporal artery and temporal branches of the facial nerve are identified (50). Deep injections in the lower or posterior temporal fossa superiorly to the zygomatic arch should be avoided (48).

Complications Involving the Artery after Aesthetic Surgery

Inadvertent injury to the vessels could result in hemorrhage or in the gradual development of a pseudoaneurysm, whose rupture could be the cause of massive bleeding (18). Excessive use of epinephrine locally can also lead to hematoma due to the rebound effect as the vasoconstricting effect dissipates (19, 20). Bleeding has occurred in cases of otoplasty, rhytidectomy and mandibular angle osteotomy. Another source of vascular complications could be the impingement of the artery by a needle (36, 54). The formation of a pseudoaneurysm following partial transection of the artery, or needle penetration during instillation of local anesthetic, has also been reported (21, 22). A pseudoaneurysm of the STA constituted a complication following rhytidectomy and thread-lifting. Likewise, an arteriovenous fistula could form as a result of surgical maneuvers. This complication was observed in cases of rhytidectomy, reduction malarplasty and retrieval of a cartilage graft as part of a rhinoplasty procedure. The propensity for injury of the STA may be explained by the lack of a protective tissue cushion between it and the bones. Finally, embolism and ischemic lesions of the ophthalmic as well as the cerebral arteries were reported after autologous fat-grafting. As a result of an inflow of fat particles, vascular obstruction could occur, coupled with devastating consequences, such as vision loss and cerebral infarction (36). The presence of anastomosis between the STA, the ocular artery and the facial artery, and the abundant arterial connections between the internal and external carotid systems explain the embolic events occurring by the intravasation and retrograde flow of fat. Cases of massive cerebral infarction due to fat embolism of the internal and external carotid artery have been reported (23, 24, 55).

Conclusion

Thorough knowledge of the anatomical variability of the STA, especially in the lateral forehead region is indispensable for aesthetic surgeons, in

line with the basic principle "first, do no harm". Furthermore, awareness of the danger zones involved in each procedure, combined with intraoperative vigilance, can assist surgeons, as well as injectors, in improving safety and minimizing the occurrence of unwanted sequelae. This review aims to improve the surgeon's understanding of the anatomy and clinical relevance of the STA, and highlight the areas that need to be safeguarded, hence, contributing to the overall improvement of safety during surgery and aesthetic treatment.

What Is Already Known on This Topic:

The anatomical variability of the superficial temporal artery has been elucidated in various cadaveric as well as radiological studies. Moreover, the course of the artery has been described in relation to defined landmarks, rendering its preservation and protection possible during surgical procedures involving relevant areas of the face. Due to the complexity of the overall vascular anatomy of the temporal region traversed by the STA and the rising popularity of aesthetic procedures in the region, for the sake of rejuvenation, several authors have presented specific danger zones in order to provide surgeons with anatomical pathways to avoid injury and relevant sequelae following aesthetic manipulations in the temporal region.

What This Study Adds:

The aim of this review was to collect the available data concerning the anatomical particularities of the superficial temporal artery, its relationship to defined adjacent landmarks, as well as the location of danger zones involving relevant regions of the face, which could be encountered during aesthetic manipulations and surgical procedures of the face. Multiple terminal branches and diverse levels of division of the artery could have an impact on the invasive outcome as inadvertent trauma or compression of the artery could occur. Surgeons need to be aware of the traditional course of the artery as well as possible deviations, and proceed with vigilance. When in doubt, preoperative confirmation of the course of the artery can be undertaken radiologically so that the surgeon can anticipate a familiar path during the dissection or the use of minimally invasive facial techniques, and avoid damage to the vessels.

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Narrative Review
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Anatomical Variations of Vascular Anatomy in Meckel's Diverticulum

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Abstract

Objective. The objective of the current study was to describe the anatomical variations of vessels observed in patients with Meckel's Diverticulum. **Methods.** A narrative review of the literature was undertaken by means of the PubMed database, using the terms: "Meckel's Diverticulum AND vessels", "Meckel's Diverticulum AND anatomical variation" and "Meckel's Diverticulum variation". Classical anatomical textbooks were also used for normal anatomy. Additional articles provided useful information in relation to the aim of this review. Hence, the articles that met the inclusion criteria were included in this review, and the collected data were categorized into a single table. **Results.** The majority of studies indicated the presence of an abnormal vitelline artery. Other angiographic findings concerned variations of the ileal and the iliac arteries. However, the literature revealed the presence of vascular variations without the existence of Meckel's Diverticulum, whereas a remnant of the vitelline vein may be present, but it is very rare. **Conclusion.** The detection of vascular variations accompanying Meckel's Diverticulum is not always easy and requires the correct choice of imaging method to prevent misdiagnosis.

Key Word: Meckel's Diverticulum ▪ Anatomical Variation ▪ Vascular Variation ▪ Vitelline Artery ▪ Vitelline Vein.

Introduction

Meckel's Diverticulum (MD) was described for the first time in 1589 by Fabricius Hildanus and is thought to be the most frequent congenital gastrointestinal malformation (1-4). It is caused by the persistence of the omphalomesenteric duct, and it is a true diverticulum as it contains all the three layers of the intestinal wall: the mucosa, muscularis propria and adventitia (5). A mnemonic technique is used since MD is considered to follow "the rule of twos": it is present in about 2% of the general population, more frequent in males with a predominance 2:1, it appears in the first two years of life, it contains two types of mucosal, it is symptomatic in 2% of the affected population, its length is two inches (5 cm), and it is found two feet away from the ileocecal valve (1). The diagnosis is often made incidentally in patients undergoing abdominal exploration surgery, most commonly for acute appendicitis, or on postmortem dissection for forensic medicine cases (5). MD can be asymptomatic, but it may also appear with complications such as: hemorrhage, inflammation, obstruction, ulceration, perforation, intussusception, volvulus, and neoplasms (5-7). The vascular variations that may accompany MD can be found during imaging, such as angiography.

The aim of this study was to review the anatomical variations of the vessels in patients with MD.

Methods

An advanced executive literature search was conducted in PubMed, Google Scholar and other available scientific websites and medical journals, using the following terms: "Meckel's Diverticulum AND vessels", "Meckel's Diverticulum AND anatomical variation" and "Meckel's Diverticulum variation". The resulting literature was carefully screened by

a single investigator. Only studies in English and referring to humans were included. No additional search filters, such as text availability, article type and publication date, were applied. Using the snowballing technique, further references taken from the initial articles with useful information relating to the aim of the review were also screened and taken into consideration. The extracted data were classified in a table according to the type of vascular variation and the presence of MD.

Results

The search of the literature retrieved articles with useful information that are described in Table 1. Most studies suggested the presence of the vitelline artery. Pandey et al. in their study presented a case report of a young male diagnosed with MD accompanied by a blood vessel touching the intestine within the mesodiverticulum band, which was suggested to be a remnant of the vitelline artery (2). Along the same lines, Okazaki et al. reported a young male with a bleeding MD accompanied by a vitelline artery (8). Moreover, Miyoshi et al. also claimed that they noticed the presence of a right vitelline artery (9). Okazaki et al. observed in five patients with MD that the vitelline artery appeared, derived from the distal ileal artery, with increased length and no branches (5). The study by Mitchell

Table 1. Eligible Studies That Correlate Meckel Diverticulum with Vascular Anatomical Variations

Researchers	Year	Type of study	Gender of patients	Variation	Presence of MD	N (%)
Pandey et al. (2)	2016	CR*	Male	Remnant of the vitelline artery	Yes	1
Okazaki et al. (8)	1992	CR*	Male	Remnant of the vitelline artery	Yes	1
Miyoshi et al. (9)	1984	CR*	Male	Remnant of the right vitelline artery	Yes	1
Okazaki et al. (5)	1993	RS† 5 patients	4 males 1 female	Remnant of the vitelline artery: enlogated without branches	Yes	5 (100)
				Irregular tortuous vessels		5 (100)
				Capillary straining		2/5 (40)
Mitchell et al. (10)	1997	RS† 16 patients' angiograms	13 males 3 females	Remnant of the vitelline artery	Yes	11/16 (69)
				The vitellointestinal artery not depicted		5/16 (25)
				Vascular blush		4/16 (25)
				Early venous return		4/16 (25)
				Arterial deformities		2/16 (12.5)
				Vitellointestinal artery without MD	No	4/16 (25)
Takeda et al. (11)	1977	CR*	Female	Abnormal ileal artery	Yes	1
Hall TJ (12)	1975	CR*	Male	Abnormal artery from the superior mesenteric artery	Yes	1
Geelhoed et al. (13)	1986	CR*	Male	Enlargement and elongation of the right iliac artery	Yes	1
Kitsuki et al. (4)	1992	CR*	Female	Enlargement and elongation of the embryonic artery	Yes	1
Sakai et al. (14)	2006	CR*	Male	Abnormal vitello-intestinal artery aneurysm	Yes	1
Watanabe et al. (15)	2011	CR*	Male	Preduodenal portal vein	Yes	1
Mwila et al. (17)	2022	CR*	Female	Remnant of the vitelline artery	No	1
Date et al. (18)	2018	CR*	Male	Remnant of the vitelline artery	No	1
Jalil et al. (19)	2012	CR*	Male	Remnant of the vitelline artery	No	1
Sprangenberg (20)	1819	CR*	NA‡	Remnant of the vitelline vein	NA‡	1
Buchnan et al. (21)	1940	CR*	Male	Remnant of the vitelline vein	NA‡	1
Kleinhaus et al. (22)	1974	CR*	Male	Remnant of the vitelline vein	NA‡	1

* Case report; † Retrospective study; ‡ Not applicable.

et al. (10) is of great interest. The researchers studied the angiograms of 16 patients who had undergone resection of MD, and they observed that 11 patients (69%) had a persistent vitellointestinal artery, nine of them with MD. In these patients the ileal arteries had branches. Other abnormal angiography findings included vascular blush, early venous return, and arterial deformities.

In addition, an abnormal ileal artery was found in a case of a bleeding MD, and in another case an abnormal artery, derived from the superior mesenteric arterial trunk, confirmed the diagnosis of MD (11, 12). Geelhoed et al. concluded with a diagnosis of MD after angiography showed an enlargement and elongation of the right iliac artery, at various points in the abdomen and erythematous mucosa (13). This patient had no sites of bleeding. Another case referred to a pregnant woman who had undergone surgery for resection of symptomatic MD, and the preoperative imaging revealed an artery, increased in length and width, with no branches, that is the embryonic artery (4). It is worth noting a case report referring to the case of an abnormal vitello-intestinal artery aneurysm in an asymptomatic case of MD (14). Last but not least, Watanabe et al. described a clinical case of MD accompanied by a preduodenal portal vein (PDPV) (15). The PDPV is an embryonic variation that is the result of the remnant of the ventral and caudal anastomosis of the vitelline veins (15). The majority of these cases are recorded in children, and the patients are either asymptomatic or present with high bowel obstruction (15).

However, some researchers have recorded the presence of a residual of the vitelline artery without MD (16). Mwila et al. described a case report of a 40-year-old woman with the remnants of a vitelline artery which led to obstruction of the small intestine (17). The patient presented with a fibrous ileal-mesenteric band not attached to MD. Along the same lines, Date et al. and Jalil et al. recorded a remnant of the vitelline artery without MD, where the former noticed simultaneous appendicitis (18, 19). The remnant of the vitelline vein is infrequent and, according to Miyoshi et al., that variation has been described in only three cases in the literature (9, 20-22).

Discussion

The MD is thought to be formed from a residue of the proximal portion of the yolk stalk. It is formed when the omphalomesenteric duct, which connects the midgut with the yolk stalk, does not turn back in the seventh week of pregnancy, as by that time the omphalomesenteric duct, the yolk sac and the vitelline arteries have involuted (1). The persistence of that connection leads to the formation of MD (6). In other words, the origin of MDs is considered to be failure of obliteration of the proximal portion of the vitelline duct (23, 24). Its inflammation can be misdiagnosed as appendicitis because it is close to the appendix in location (25). The remnants of the vitelline duct are depicted in Figure 1.

In terms of embryology, MD consists of three anatomical parts: the omphalomesenteric duct, two vitelline arteries and one vitelline vein (2, 17, 18). The left vitelline artery degenerates, whereas the right remains as the superior mesenteric artery, and is the main blood supply for the MD (2). Nevertheless, the left vitelline artery may persist. Rutherford referred to three types of remnants of vitelline arteries: the right vitelline artery as the

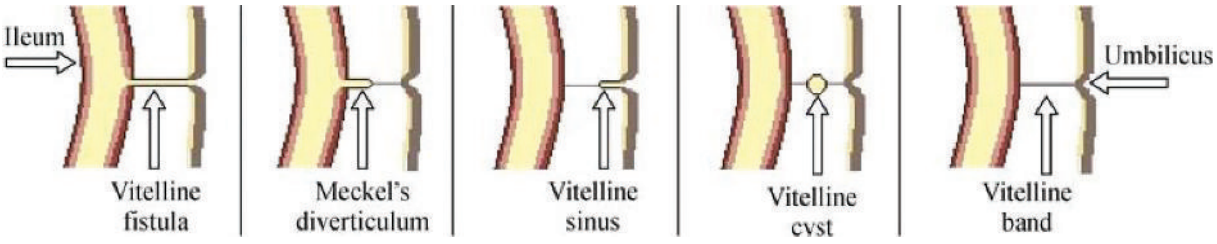

Figure 1. Diagrams showing vitelline duct remnants (24).

terminal part of the superior mesenteric artery, the second type which originates near the ileocecal artery as a branch from the superior mesenteric artery, and the third, which includes the left vitelline artery that arises from the aorta (26). The remnant of the vitelline vein is extremely rare, and derives from the back wall of the umbilicus (9). Variations of the vitelline circulation are not usual, and they are estimated to appear in 8-15% of patients with MD. Their identification is crucial for the diagnosis of MD and they are associated with increased probability of a bleeding MD (2, 4, 5). It should also be underlined that vascular variations may occur without the presence of MD (2, 19). Vascular remnants present as fibrous bands covering the peritoneum, and they expand from the ileal branch of the superior mesenteric artery to the MD, or to the umbilicus (2, 19). When there is no MD, discrimination between the remnant of the omphalomesenteric duct and the vascular remnant is not always easy. A vascular remnant is suspected when the band expands from the intestine to the mesenteric. Its recognition is of paramount importance as it can prevent hemorrhage after surgery. Researchers have emphasized that such bands should be considered to be vascular remnants until proven otherwise (19). According to Okazaki et al., the features of vitelline arteries include their increased length, the absence of branches to the ileal artery from which they are derived, and enlarged tortuous vessels (5).

The mucosae of MD is mainly ileal. It should be mentioned that ectopic mucosa can usually be recorded on histological examination, and gastric mucosa is the most common (2, 6, 19). Other less common types of mucosa include pancreatic and duodenal. Heterotopic mucosa is associated with the presence of symptoms, so it has clinical significance: for instance, acid from the gastric mucosa cells can result in ileal ulceration, hemorrhage and inflammation (2, 18). Furthermore, malignant neoplasms have been recorded that derive from the remnant omphalomesenteric duct (18). For instance, Martre et al. presented a case report of a gastrointestinal stromal tumor (GIST) on MD (3). Moreover, hemorrhage is the most frequent complication, especially in children, and in adults it can lead to chronic anemia due to the chronic blood loss from the gastrointestinal trunk (10, 12, 14).

The omphalomesenteric artery supplies the MD and is represented on angiography as a lone branch of the superior mesenteric artery (15). It should be underlined that arteriography is helpful for the diagnosis of bleeding MD and, more specifically, superselective vitelline arteriography enables the depiction of the vitelline artery (5, 10, 13, 27). When hemorrhage is taking place, arteriography is the best imaging choice. MD can be distinguished by extravasation of contrast medium (5). When bleeding stops, the diagnosis of MD can be made by observation of abnormal vessels, high density due to the heterotopic gastric mucosa, or the mesodiverticular band artery (11). It should not be forgotten that the inability to illustrate the vitelline artery does not exclude the diagnosis of MD (10). Furthermore, Oglevie et al. emphasized the importance of taking findings from other imaging methods into account, for instance, endoscopy and barium series, in order to minimize the possibility of a wrong diagnosis (28).

Limitations of the Study

Concerning the limitations of our study, it should be mentioned that most of the research that exists relies on case reports/small case series. Finally, it is equally important to highlight that aneurysms and vascular anomalies in general, even though they are not common in the general population, are of high clinical significance. A potential rupture of an aneurysm can be life-threatening, and the mortality rate is estimated to be 8.5% (14). The management of patients with a vitelline artery aneurysm and asymptomatic MD can be treated by a minimally invasive surgical procedure, if complications occur (14).

Conclusion

Meckel's diverticulum is present in 2% of the general population, and the remnants of vitelline vessels are even more infrequent. It should be noted

that the correct diagnosis of vitelline vascular remnants is challenging, especially when comorbidities such as appendicitis are present. Fortunately, there are many imaging procedures in the diagnostic armamentarium, and the careful examination and interpretation of the findings can usually lead to a diagnosis, with high accuracy. Taking all the above into consideration, it is obvious that surgeons should be aware of the existence of anatomical vascular variations and suspect them in patients with atypical abdominal symptoms, in order not to miss the diagnosis.

What Is Already Known on This Topic:

Meckel's Diverticulum (MD), even though it is present in only 2% of the general public, is still one of the most common congenital anatomical variations, but the vascular anatomical variations that may accompany it are even more rare. The clinical picture may be confusing, leading to misdiagnosis or failure to recognize the anatomical variations. There are a variety of imaging methods that can be helpful for accurate diagnosis. However, it should be underlined that surgeons should be aware of the probability of the presence of such variations, especially in patients with atypical symptoms. In this way the serious complications of such vascular variations can be prevented, thereby saving patients' lives.

What This Study Adds:

This review sums up the current literature, confirming that recognition of the anatomical vascular variations that in some cases accompany MD, is of high importance. This is because correct and early diagnosis can lead to the appropriate treatment, preventing potentially life-threatening complications.

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

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Historical Article

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Dr. Maša Živanović: A Pioneer in Health Care for Women and Children in Bosnia and Herzegovina

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Abstract
The aim of the article is to present, primarily to the medical world and also the general public, the personality and work of Maša Živanović (1890–1960), a pioneer in the health care of children and mothers in Bosnia and Herzegovina (BH), a health educator and one of the leaders of the Yugoslav Women's Rights Movement in the period between the two world wars. She was born in Croatia (then part of the Austro-Hungarian Empire) as Maria Skopszyński, in a family of Polish-Czech origin. After studying at the Temporary Women's Lyceum in Zagreb and passing the matriculation exam at the boy's High School (1909), she obtained the title of Doctor of Medicine in Vienna (1916). Her activity in the Women's Rights Movement has so far generally attracted more attention from researchers than her medical work. However, this work was very important because the general and health education of women, expectant mothers and mothers, after the two World Wars was very poor in BH, and the rates of child morbidity and mortality were high. Maša Živanović spent almost her entire working life in Sarajevo. For 30 years, she was the head of the Dispensary for Mothers and the Children, later the Institute for Maternal and Child Health Care, into which the previous institution grew in 1931. She was among the first followers of the new concept of "comprehensive paediatrics", which included social care for children, disease prevention and treatment of the sick. She successfully connected the medical mission with the mission of a women's rights activist, also trying to act as a health educator through articles published in the Women's Movement magazine (Ženski pokret). For a time, she was the president of the Society for the Education of Woman and Protection of her Rights, i.e. the Women's Movement, and a delegate at conferences of international feminist organizations. **Conclusion**. Maša Živanović was a physician, a pioneer in the health care of children and mothers in BH, a long-time director of the Institute for Health Care of Mothers and Children in Sarajevo, and one of the leaders of the Yugoslav Women's Rights Movement.

Key Words: Maša Živanović ▪ Mother and child Healthcare ▪ Women's Rights Movement ▪ Bosnia and Herzegovina.

Introduction

The name of Dr. Maša Živanović can be found most often in the literature on the history of the Women's Rights Movement in the former Yugoslavia. The long-term activity and the prominent place she occupied in that Movement seem to have overshadowed her professional activity in the field of child and maternal health protection, in which she was one of the pioneers in Bosnia and Herzegovina as well as Yugoslavia, and to which she dedicated her professional career. Only one *In Memoriam* was published when she passed away, in the magazine *Nova* žena. At the beginning of her emotional text, the author, Dr. Smiljana Kršić, expressed her regrets that the news about the death of Dr. Maša had gone almost unnoticed among the citizens of Sarajevo, assuming that the cause of this was her withdrawal from public life. Along with a short biography, Kršić emphasized Maša's social and cultural work (1).

That Maša Živanović (Picture 1) was not forgotten by her colleagues and the current authorities was evident on April 28, 1961, when a memorial plaque was ceremonially unveiled on the building of the Dispensary for Women and Children

Picture 1. Maša in the mid-1930s. Archives of Maša Živanović, with permission of the family.

Picture 2. A memorial plaque was unveiled on April 28, 1961, at the building of the Dispensary for Women and Children in Sarajevo, Skerlićeva 1 (today Josip Vancaš Street). Archives of Maša Živanović, with permission of the family.

in Skerlićeva Street in Sarajevo (2). It read: "We proudly remember the great and modest pioneer of women's and children's health care in Bosnia and Herzegovina, a tireless fighter for women's rights and a bright figure of the long-time head of this institution, Primarius Dr. Maša Živanović" (Picture 2). Today, after more than 60 years, there is no longer a Dispensary for Women and Children in the building, there is no memorial plaque, and no trace of what happened to it. There are only the memories of rare elderly residents of Sarajevo and a photo of the memorial plaque preserved in the family archive of the descendants of Dr. Maša Živanović.

In 1966, Milan Glibonjski, a teacher, journalist and prominent advocate of the idea of Yugoslav unification in the first decades of the 20th century, wrote down his memories, dedicating them "To the shadow of the late Dr. Maša Živanović, former supervising physician at Osijek hospital." He described an event from the end of the First World War in which Maša played an important role. In September 1918, it was already clear that the Austro-Hungarian Empire was losing the war, but it still ruled its territory. In the garrison in Osijek, Yugoslav-oriented officers were preparing a rebellion, and one second lieutenant, a Croat, publicly insulted the city commander, a Hungarian lieutenant colonel. Therefore, it was necessary to save his life. Glibonjski, who was a member of a secret revolutionary organization linked to the leaders of the Serb-Croatian coalition, asked Maša for help. Putting her own life in danger, she agreed to provide a shelter in the hospital for the young officer. Thanks to Maša's courage and nobility, his life was saved (3).

In the Second World War, Maša participated as a volunteer, joining the National Resistance Movement (Yugoslav Army) on August 23, 1944. For the next nine months, she performed medical duties in military medical units (4). Her participation, however, was not recorded in Vera

Gavrilović's famous work *Women Doctors in the Wars in Yugoslavia from 1876 to 1945* (1976) (5).

Maša's name is mentioned in the books *Women Heroes* (1967) (6) and *Women of Yugoslavia in the Workers' Movement and Women's Organizations, 1918–1941* (1978) (7). Short biographical notes were published in the book *Recorded: Women and Public Life of Bosnia and Herzegovina in the 20th Century* (2014) (8), as well as in the article *Kornelija Rakić: A Woman Doctor for Women and Children in Serbia and Bosnia and Herzegovina* (2021) (9).

More comprehensive biographies of Maša Živanović have only appeared in recent years, six decades after her death. Her biography, given alongside the biography of her husband, Dr. Teodor Živanović, was published in 2018 by the historian Goran Miloradović (10). The following year, an extensive study by Sonja Dujmović was published, in which the author devoted equal attention to the study of and insight into Maša's personality, and her professional and social activities, as well as her role in Bosnian society (11). Two years later, in the *Proceedings of the International Scientific Conference on the Women's Movement Magazine*, Maša's biography, largely based on her notes and memories preserved in the family archive, was published by her great-granddaughter, Maša Miloradović (12). More recent are contributions at scientific meetings and articles in which Maša's name was mentioned in the context of the activities of the *Little Entente of Women* (13) as well as in the context of the personal, friendly ties she maintained with Milica Bogdanović, her former teacher from the Zagreb Lyceum (14, 15, 16).

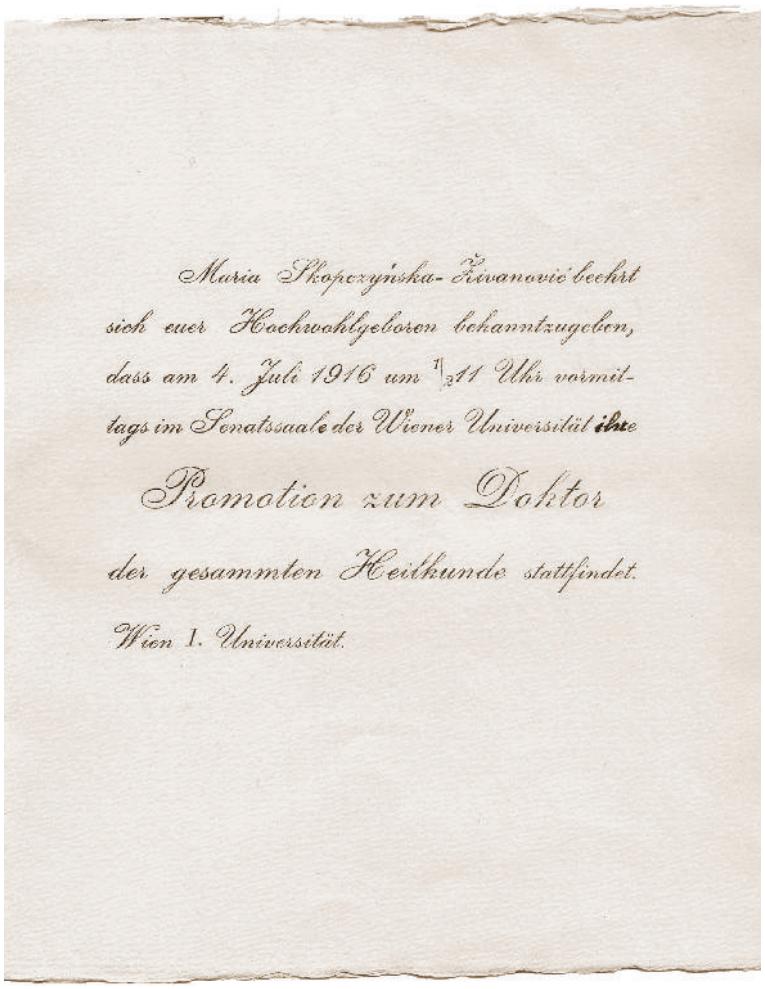
Picture 3. Brod na Savi "Public High school." With permission of the Museum of Brodsko Posavlje, Slavonski Brod, Croatia.

Picture 4. Final (8th) grade of Higher Primary School in Brod na Savi (Slavonski Brod) with teachers, in 1906. Maša is in the middle row, third from the right. Archives of Maša Živanović, with permission of the family.

Since we were unable to find an article about Dr. Maša Živanović in medical journals and publications on the history of medicine, the aim of this article is to present her personality, work and achievements primarily in relation to the medical world, but also the general public.

Živanović's Short Biography

Maša Živanović was born on December 14, 1890 in Delnice (then the Triune Kingdom of Croatia, Slavonia and Dalmatia, as part of the Lands of the Crown of St. Stephen in the Austro-Hungarian Empire), now Croatia, as Maria Skopszyński (12).



Maria Skopczyńska-Živanović beehrt sich euer Hochwohlgeboren bekanntzugeben, dass am 4. Juli 1916 um 11/2 11 Uhr vormittags im Senatssaale der Wiener Universität ihre

Promotion zum Doktor

der gesammten Heilkunde stattfindet.

Wien I. Universität.

Picture 5. Invitation to the graduation ceremony at the Faculty of Medicine in Vienna. Archives of Maša Živanović, with permission of the family.

Picture 6. Maša Živanović in the hospital in Osijek 1916-1917. With permission of Archive of Maša Živanović (Family owned).

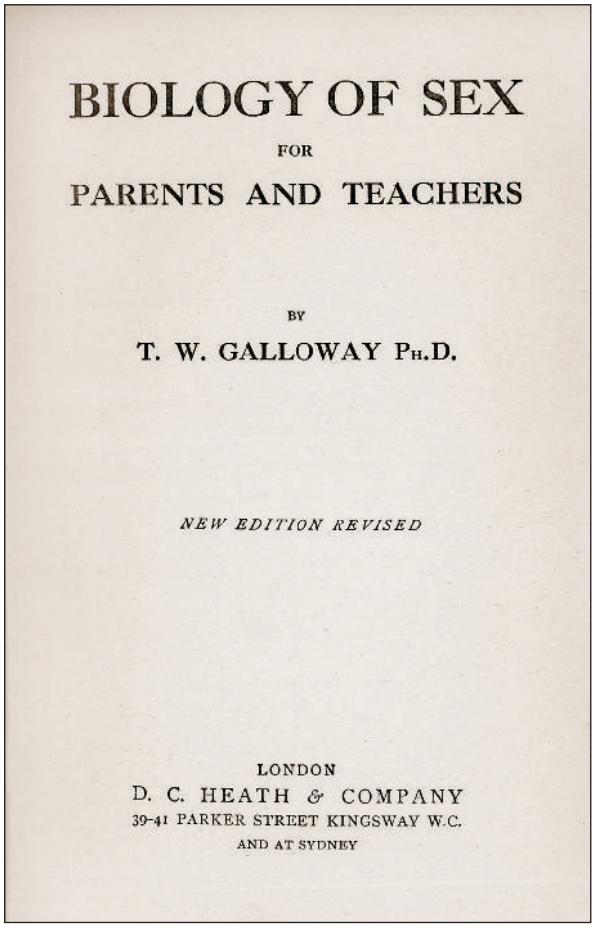
Her father, Teofil Skopcsinsky, was Polish,[1] and her mother, Otilija, a Czech, née Polak. She graduated from teacher training school but did not work as a teacher. She was a housewife and a caring mother who educated and raised her children from their earliest childhood.

In addition to Maria, who was the oldest child, Teofil and Otilija had five more children. Despite their father's limited clerical income, all the children received a solid education (12). Maša began her education in Gospić in 1897, where her father was employed as a civil engineer in the area of the district, but as early as 1900, the family moved to Brod na Savi (Picture 3), today Slavonski Brod, Croatia, where Maša continued her education and graduated from the Public High School in 1906 (Picture 3) (17).

In the autumn of the same year, Maša's mother took her to Zagreb, where Maša continued her education at the Temporary Lyceum for Girls, which she successfully completed in 1909, along with passing the matriculation exam at the Boys' High School in Zagreb, in order to gain the right to enrol in the Faculty of Medicine (4). Many years later, in July 1950, Maša wrote that her departure to Zagreb to study was accompanied by the "general disapproval of all relatives and acquaintances" who "predicted a terrible future, corruption, and misfortunes" (18).

She began her studies of medicine at the Faculty of Medicine in Vienna in the winter semester of 1909. She graduated as Doctor of General Medicine from the same faculty on July 4, 1916 (4) (Picture 5).

Picture 7. Sarajevo at the time when Maša Živanović started there. Published with kind permission of the Bosniak Institute - Adil Zulfikarpasic Foundation.



Picture 8. A book cover from Dr. Maša Živanović›s library. With the permission of the Museum of Science and Technology–Belgrade.

During her studies in Vienna, Maria Skopszyński met Teodor Živanović from Zagreb, who was also a medical student and her future husband. During their studies, they completed one semester together at the Universities of Lausanne and Innsbruck. Just before the beginning of the First World War in 1914, Maria and Teodor were married in Zagreb (19). In the new environment, she changed her family nickname Mici (Mitzi) to Maša and she signed her name in that way from then on in her private and official life, and she freely chose to replace her undecided nationality with the nationality of the Serbian people. After the outbreak of the First World War in 1914, Teodor and other students at the Vienna Medical Faculty were mobilised into the Austro-Hungarian army and sent to the front, and Maša successfully continued her medical studies after the birth of their first child.

After her graduation at the end of the same month, Maša started work as an assistant at the National Maternity Hospital in Zagreb, where she remained until February 1917 (20). From March to October of the same year, she was employed at the Municipal Hospital in Slavonski Brod (21), and from November 1917 to November 1918, she worked at the Foundation Hospital in Osijek (22). It is interesting to note that the work of Dr. Maša was rated very highly in these institutions, and her humane work with the patients and the creation of deep trust and gratitude towards her were emphasized. In recommendations for her future employment, the high rating of her knowledge, diligence, conscientiousness, and ability for independent medical work were emphasised (20, 22).

In March 1919, she went to Sarajevo (Picture 7), where she began her brilliant career in the field of social care for children, and disease prevention and treatment. She stayed in Sarajevo until a few months before her death in 1960, except for the period of the Second World War, when she was forced to flee to Serbia with her family.

Throughout her education, Dr. Maša Živanović was consistently ranked among the top students. She spoke German, French and English, and as a doctor educated abroad, she used foreign medical literature (Picture 8). To acquire new paediatric knowledge, she collaborated with world medical institutions, and she was equally interested in the protection of women's reproductive health. In the 1930s, she corresponded with Dr. Hannah M. Stone (1894–1941),[2] a well-known campaigner for women's reproductive rights and an associate of Margaret Sanger (1879–1966), a founder of the movement to legalise birth control.[3] As a doctor, she established cooperation with top paediatricians in Yugoslavia at that time and beyond, with whom she exchanged experiences and opinions, and thus gained new insights (23).

A few months before her death, already seriously ill, she moved from Sarajevo to Belgrade for treatment, where her older daughter lived at the time. She died on August 12, 1960, and was buried at the New Cemetery in Belgrade. Her last wish, to be cremated, could only be fulfilled a few years later, because the first crematoria in Belgrade and Yugoslavia were not opened until 1964. Her younger daughter lived with her family in Sarajevo until 1967.

Živanović's Professional Activities in Sarajevo

Dr. Maša Živanović spent the majority of her career in Sarajevo. She belonged to a group of doctors who worked in the periods immediately after the First and Second World Wars. Those times were characterized by the poor general and health education of the people, their poor health and sanitary conditions, poor nutrition, especially of the rural population, the frequent occurrence of various infectious diseases that had the characteristics of epidemics, the insufficient number of primary health care institutions, and a shortage of health care personnel. The effect of this situation on the health of the child population was reflected in the occurrence of various infectious or non-infectious diseases, which resulted in a high rate of infant mortality in those periods.

From March 1919 to March 1922, she worked as a children's doctor in the Municipal Health Service, and from July 1923 to May 1924, as a doctor in the Pasteur Institute (4). In the mid-1920s, in addition to her regular duties, she also managed the work of the Sarajevo School for Nurses, which began operations in 1923. The school was a boarding school, located in a separate building in the grounds of the hospital. The classes, which were held in the form of six-month courses in those years, were attended by an average of about six participants (24).

After her education with the help of state scholarships in Switzerland and France in 1920 and in Austria, Czechoslovakia, France, England, and Belgium in 1924, where she was acquainted with the organisation and work of children's institutions, she was appointed a doctor and head of the newly founded institution known as the Dispensary for Mothers and Children in Sarajevo. This institution, known under the abbreviated name the "Children's Dispensary", was founded by the Inspectorate of the Ministry of Public Health in Sarajevo with the intention of "socially and hygienically" protecting children's health, not only after birth but while the child was still in the mother's womb (25).

Picture 9. The Dispensary for Mothers and Children on Musala Street in Sarajevo, c. 1925.

The patients of the Children's Dispensary were pregnant women, mothers in labour, infants, and children of preschool age. In the first year, one doctor and two visiting nurses were employed, who worked alternately one day in the Dispensary and the other day making home visits. The work took place mainly in Sarajevo, although visits to small nearby towns (Pale, Ilidža, Nišići, Vareš) were not rare (25).

Special attention was paid to the work of the visiting nurses, who were required to visit all registered newborns and children who had already been examined in the dispensary. It was necessary to get in touch with the mothers as soon as possible after the birth and warn them not to use previously acquired bad habits in terms of child care, that is, to familiarise the mothers with the procedures of proper care of the newborn, and invite them to come to the dispensary with the child as soon as they recovered and not to only come when the child was in pain. Visiting nurses were supposed to demonstrate practically the procedures for caring for a newborn, write a social map of the family, record their observations, and hand it all over to the doctor at the end of the week. They were also required to submit monthly reports, on the basis of which, among other things, the further programme of work of the dispensary was drawn up.

When visiting a woman in labour at home, the visiting nurses carried a brush for washing their hands, a towel, soap, alcohol, scissors, educational leaflets, two thermometers, tincture of iodine, dermatol, a sterile bandage for the navel, gloves, etc. (25). Their work was made difficult by the low cultural level of the birth attendants, the difficult terrain, and the lack of communication. Regardless of these difficulties, which sometimes arose unexpectedly, and the great lack of education of women, the reception of visiting nurses during home visits was friendly. Mothers were interested in acquiring new knowledge, and were happy to listen to their advice, very happy to accept printed materials (25).

The goal of the work was to provide oral, written, and practical training based on modern socio-medical knowledge about the prevention and elimination of social factors of disease, as well as the constructive creation of favourable factors for health, that is, the gradual eradication of harmful practices in child health care that had their origins in folk medicine.

It was the beginning of organised health care for the most sensitive population in Bosnia and Herzegovina, in which modern attitudes of social medicine were incorporated with the aim of promoting disease prevention methods. The provision of health care to the population by official health institutions was broad, aimed at preventing the onset of disease by taking social-medical preventive measures in a timely manner. Health workers, especially doctors, were asked to keep the social condition of the patient and his environment when in mind working with patients.

The Children's Dispensary in Sarajevo was not only an institution that took care of the health of mothers and children, but it was also a place for the education of paediatricians from the interior of Bosnia and Herzegovina. They came to learn how a modern children's social-medical institution works, specifically the institution where Dr. Maša Živanović (4) and Dr. Mara Kurtović[4] (26) worked. One of the

Picture 10. Dr. Maša Živanović with colleagues from Sarajevo's Institute for Maternal and Child Health Care. Maša third from the right. Archives of Maša Živanović, with permission of the family.

Picture 11. The Children's Dispensary building on Skerlićeva Street (today's Vancaševa Street) in Sarajevo, c. 1972. With the permission of Cantonal Institute for the Protection of Cultural, Historical, and Natural Heritage–Sarajevo.

paediatricians who was trained for six months at the Children's Dispensary was the respected doctor Kornelija Rakić from Mostar (9, 27).

Constant professional development and expansion of the Children's Dispensary's activities from year to year, under the leadership of Dr. Maša Živanović, resulted in its growth to become the Institute for Maternal and Child Health Care at the end of 1931. The Institute had five departments and well-organized outpatient services. Dr. Maša Živanović, managed its work for many years later (Pictures 9 and 10).

After the capitulation of the Kingdom of Yugoslavia in World War II and the creation of the Independent State of Croatia, which included Bosnia and Herzegovina, Dr. Maša Živanović found herself in a difficult situation. She was attacked by the new authorities because of her national and political orientation. Together with her husband, Todor, and daughters, and with the help of one of his patients, she managed to leave Sarajevo and escape to Serbia.[5] They were registered by the Commissariat for Refugees in Belgrade on July 17, 1941. In occupied Belgrade, like most citizens, the Živanović family lived in great material poverty. Maša's private medical practice that was approved by the Chamber of Physicians in August 1942 (28) barely completed the domestic budget. In 1942, Teodor went to Soko Banja[6] due to his poor health, where he was then engaged in the treatment of refugees. As a former Yugoslav civil servant, Maša was accepted into the civil service only in August 1943. At first, she worked at the Department for Health Care of Mothers, Infants, and Young Children of the Central Institute of Hygiene and then, from January 1944, at the Children's Department of the General State Hospital. During the Allied bombing of Belgrade in April 1944, she travelled to Soko Banja with her daughters. After joining the Yugoslav Army in August 1944, she performed the duties of the director of the mobile hospital of the 19th Brigade of the XXV Serbian Division of the XIV Corps and the doctor of the Command of the city of Soko Banja (4).

After the liberation of the country, Maša and Todor returned to Sarajevo. Maša was demobilised on May 30, and already on June 4, by decision of the Minister of Health of the Federal People's Republic of Yugoslavia, she was placed at the disposal of the Federal Ministry of Public Health of Bosnia and Herzegovina (29). By the decision of the Minister of Health of the People's Republic of BH, Dr. Nedo Zec[7] (30), she was immediately appointed acting head of the Institute for Maternal and Child Health Care in Sarajevo, of which she was one of the founders and a long-time manager before the Second World War. At the end of the same month, in addition to her regular duties, she was assigned to the Ministry of Public Health of the People's Republic of BH to lead the report for Women's Counselling Centres (31). Dr. Maša Živanović first retired on July 29, 1950, but due to the needs of the service, she remained in the same position until her final retirement on April 14, 1958 (32).

Due to her attitudes and principles, to which she adhered firmly, and which often did not meet

with the understanding of those around her, she came into conflict with her superiors. The first time this happened was at the beginning of 1922, when she refused to participate in activities she did not agree with. This was also discussed in the press (33, 34, 35). In the autobiographical note, it is also stated that "in September 1948, she was punished by the Ministry of Public Health for disciplinary negligence" (36).

Živanović's Social and Cultural Work

In addition to the fact that the First World War brought about major changes in the social position of women, and the fact that numerous new female associations (the National Women's Association, the Alliance of Women's Movements in the Kingdom of Serbs, Croats, and Slovenes/ Yugoslavia etc.) (37) were established throughout the newly formed state, the roots of Maša's social engagement in the struggle for the equality of women, mothers' rights and child protection could be traced from an early age. Her schooling was crucial for the formation of her personality – both at the Lyceum and the Faculty of Medicine in Vienna.

Among Maša's teachers at the Lyceum for Girls in Zagreb, there were several prominent women responsible for the organization of the education of girls in Croatia at the end of the 19th and the beginning of the 20th century, such as the principal Marija Jambrišak, and teachers Štefa Iskra, Kamila Lucerna, Natalie Wickerhauser, and Milica Bogdanović (12). Among the older pupils, Mira Kočonda (Vodvarška) was one of the Maša's colleagues in the inter-war Women's movement[8]. While studying medicine in Vienna, Maša was a member of several associations which gathered students according to their profession (the association of students of medicine), or those of Southern Slavic origin (Zora, Zvonimir, Rad). Students went on trips together into the area around Vienna, listened together to various lectures (she mentioned Pero Slijepčević[9] and "his entrancing lectures on Maeterlinck, Ibsen, German art, music, lectures of Pero Mijatović and his associates etc.") (38). No doubt Dr Julius Tandler had a major impact on Maša's entire work in the field of medicine, but also on her engagement in the struggle for social justice, the care and protection of mothers and children, and finally her engagement in social medicine (39), and she received her academic degree from him.

After the First World War, and having settled in Sarajevo for work, as early as September 10 1919, Maša Živanović took part in establishing the *Society for Education of Women and Protection of their Rights,* which was later included in the *Alliance of Women's Movements in the Kingdom od SCS,* and the *Women's Movement*. Unlike other predominantly humanitarian or educational women's associations, the *Society* was feminist, aimed at struggling for the political, economic and legal equality of women and men, and dealing with the specific needs and problems of women. In the *Society,* Maša was engaged primarily in the field of health and social protection, as observed at the *Second Assembly of the Yugoslav Women*, held in Zagreb in July 1920. "That finally in our country the role of women doctors in the health of children and the entire nation, for their cultural influence on the environment, for the education of society in general, will be properly understood, Dr. Maša Živanović from Sarajevo gave us the greatest hope at the assembly in Zagreb. With her colleagues in the Society for Education of Women she developed the widest range of activities, the results of which will have to be seen in the shortest time" (40).

The first activities of the Society were courses in hygiene, and the very first course *About Infants* was run by Maša Živanović. Courses run by Dr. Staka Bokonjić, Dr. Pero Stjepanović and others followed, and were held in Sarajevo, as well as in the surrounding towns and villages. One of the results of the Society's work was the opening of the *Maternity Home for Pregnant Women, Mothers and Children* in Pofalići on December 1, 1919. The Society took care of this home until the end of 1920 (41), when the funding was taken over by the State Protection of Children and Youth. Supervision of the home's hygiene was carried out voluntarily by the doctors Mara Kurtović, Staka Bokonjić, Maša Živanović

and Katica Jakšić (42). In the spring of the following year, the Association took part in opening a kindergarten for the children of working mothers, organized lectures for members of the scout movement, etc. (43). Maša Živanović was the President of the Society for many years (and later when the *Society* was transformed into the *Women's Movement*), being elected unanimously several times.

Women's Movement was very active, reacting to many phenomena and changes that took place in the young state. When the Ministry of Education made a decision in 1927 to limit enrolment in secondary schools, introduce the *numerus clausus* and other restrictive measures, *Women's Movement* reacted, and "Dr. Maša Živanović condemned this kind of educational policy that 'limits the humanistic education of young people by closing schools when they do not open any equivalents', which "affected the interests of young women in particular" (44). She advocated the abolition of regulated prostitution and the introduction of an anti-venereal law. With this aim, being a representative of the *Alliance of Women's Movements* (not as a doctor!), at the Congress of Venereologists in Zagreb in 1927, she presented her views on the anti-venereal laws and the abolition of regulated prostitution (45). As the president of the *Women's Movement*, she also reacted when the Law on Child Protection was amended, proposing a change in the attitude towards illegitimate children, that is, seeking an equal attitude towards children born both in and out of wedlock (46). As the subject Hygiene was introduced into the school curriculum in 1928/1929, Maša Živanović was among the doctors who held lecturers at the Women's Teacher Training School for 3 hours a week (47).

As the president of the *Women's Movement* in Sarajevo, she participated in international feminist gatherings. *The Little Women's Entente* was founded as a transnational association at the International Woman Suffrage Alliance Conference in Rome in 1923. It brought together women's organizations from Yugoslavia, Romania, Greece, Czechoslovakia and Poland. It was inspired by the interstate political organization *Little Entente* (which consisted of Yugoslavia, Czechoslovakia and Romania). Maša Živanović took part in the conferences of the *Little Women's Entente* in Athens (1925), Prague (1927) and Warsaw (1929).

As the representative of the Yugoslav women's movement, Maša Živanović participated in the *10th Congress of the International Alliance of Women* in Paris in 1926. The cooperation of the *Alliance* with the League of Nations was established and the commission was chaired by Ruth Morgan. The president of the Alliance was Margery Corbett Ashby. Both of these prominent feminists were in the Alliance delegation that visited Yugoslavia in May 1931. After the main meeting held in Belgrade, they visited Sarajevo. The programme of their work presented in the journal *Women's Movement (*Ženski *pokret)* included the following topics: "The question of peace, disarmament, economic cooperation of countries in connection with the creation of a European Union (!)" (48). Their presentations were organized by the *Women's Movement* from Sarajevo, and were accompanied by articles in the press (12).

Maša Živanović (first from the left, standing) in Athens at the Congress of Little Women's Entente (1925). Archives of Maša Živanović, with permission of the family.

During the 1930'ies, anti-war themes and pacifism were increasingly present in the *Women's Movement*. When the Peace Academy was held in Sarajevo in February 1932, Maša Živanović spoke about the need for peaceful ways to resolve disputes as one of the goals of the women's movement (49). At that time, the Youth Section was formed under the auspices of the *Women's Movement* in Sarajevo. As one of the members remembered, Maša gave a speech entitled "For equal work - equal pay". Due to the increasing activity of young members, mostly communists, Smiljana Kršić and Maša Živanović were called to account. Threats by the police to ban the *Women's Movement* were realized in 1937 and the movement ceased to exist (6).

At a time, when a new world war was looming over Europe, the *Yugoslav Union for the Protection of Children* (founded in 1935 under the patronage of Queen Marija Karađorđević) dealt with plans for the care of children in a wartime environment. In 1939, when the *Society for the Care of Unprotected Children and Youth in the Drina banovina* was founded, Maša was on the steering committee of the *Society* (47). Having returned to Sarajevo after World War II, she joined the *Anti-Fascist Women's Front* (AFŽ) and devoted herself to the health and social protection of women and children, the fight against prostitution, and other social and medical issues which occupied her all her life.

Živanović's Publishing Activities

Almost all of Dr. Maša Živanović›s articles, whether she wrote as a doctor writing articles in medical journals or as a feminist activist writing articles in newspapers and social journals, were imbued, in a direct or indirect way, with a health-educational character, full of current medical knowledge, aiming to enlighten the entire readership public in Bosnia and Herzegovina. She knew very well that the realisation of various women's rights would have a significant impact on society in general and, thus, also on the health education of women, especially when they became pregnant and mothers, and that their acquisition of new knowledge would prevent many childhood diseases and enable children to grow up normally.

On the other hand, by writing articles for medical journals, she wanted to point out, on the basis of evidence, the real state of children's health care, to refresh or improve the knowledge of health workers about current children's pathologies, and thus help sick children. In all of this, she had visible success, thanks above all to her temperament, general education, her knowledge of foreign languages and education abroad, and the use of contemporary foreign paediatric literature.

It is not possible to write a complete overview of her journalistic activity for the time being, because no fundamental research into her writing work, which would enable the writing of an overall bibliography of her written work, has yet been undertaken. However, brief reviews of some of the available publications can best represent her writing work.

The article *The* Weaknesses *of Health Care of Preschool Children* by Dr. Maša Živanović, published in the journal *Socijalno-medicinski pregled* (Social Medical Review) in 1938, provides an overview of the work of pre-school institutions in Sarajevo at the time (50). The author states that the same text can reflect the work of similar institutions in other places in Bosnia and Herzegovina: "Because all these institutions have one evil in common: they do not have a system based on an anatomical, physiological, and psychological knowledge of the child with the aim of raising a healthy, valuable, and better person, but their child is a tool for propaganda and profit."

The research was done from the 1932–33 school year until June 1937. An examination of children, an inspection of the hygiene of playgrounds and schools, their environment, and surveys of the competent state and religious authorities, parents, and official staff were carried out by a doctor and a nurse. The survey is not presented in the paper, but in the presentation of the results, it is easy to see the questions whose answers should reflect the environment in which such a sensitive population resided every day. The results presented, although not very well systematized, indicate that the time spent by preschool children in these children's institutions had a negative impact on their physical and mental health.

Of particular interest are her health education articles: *Children's Infectious Diseases* (51), *On Children's Diarrhoea* (52), *Tuberculosis in Children* (53), *Gonorrhoea and Newborns* (54), *Syphilis, the Mother and her Child* (55), *Tuberculosis of Infants and Small Children* (56), and *Collection Points for Mother's Milk* (57), dedicated to the current health problems of children, and published in the journal *Život i Zdravlje* (Life and Health) after the Second World War, from 1946 to 1949. The articles are written in a simple style with an understandable vocabulary, and are clearly and precisely systematised in relation to the characteristics of the problems described.

Along with a series of articles on medical topics[10], Dr. Maša Živanović also wrote about the health care of mothers and children, hygiene and health education. She wrote about motherhood, marriage and the ethics of marriage, women's right to education and employment, and women's economic independence, in several popular magazines and journals. She published more than twenty articles in the *Women's Movement, the organ of the Society for the Enlightenment of Women and the Protection of Her Rights* alone. (58). One of the topics that she considered "the most feminist" was prostitution and the abolition of regulated prostitution. Dr. Živanović also wrote about the position of Muslim women in Bosnia (59).

In *Ženski pokret* (Women's Movement) in 1930 (60), a polemic on sexual ethics flared up between Živanović and the prominent writer and feminist Julka Chlapec Đorđević from Prague. However, this was no obstacle to Dr Živanović inviting Ms Chlapec to Sarajevo three years later, where the *Women's Movement* discussed birth control issues. The same journal published biographical articles by Dr. Živanović about two prominent women who worked in Bosnia and Herzegovina – Adeline Irby (61) and Teodora Krajewska (62). Dr. Živanović also wrote for *Žena danas* (Woman today) and took part in a special issue of this journal (no. 24) for 1939, dedicated to the women of Bosnia and Herzegovina.

In *Pregled* (Overview), a journal for political and cultural life in Sarajevo, she wrote about the history of feminism and current feminist events and gatherings. This magazine also published her report from the *Little Entente of Women* meeting in Prague in 1927, and also texts on the education of female youth, on the care of young people, as well as reviews of several books (63). Her many shorter articles written for the widest audience, with advice for caring for children, hygiene, and the treatment of certain individual diseases etc. should not be forgotten.

Concluding Remarks

Dr. Maša Živanović (1890–1960), one of the first paediatricians and a prominent social worker in Bosnia and Herzegovina, was equally dedicated to her medical profession as to her engagement in the Women's Rights Movement. Immediately after the end of the First World War, she was a follower of the then new concept of comprehensive paediatrics which, besides treating sick children, took care of their health from their very conception. This concept included prevention by eliminating social factors of disease, while at the same time creating favourable conditions for growth and development. For more than three decades, Maša Živanović was the head of the first social and paediatric institution in Sarajevo, founded in 1924 under the name the Dispensary for Mothers and Children. Under her leadership, this institution grew into the Institute for Maternal and Child Health Care in 1931, which was also a centre for the professional training of young paediatricians in BH. She managed the work of the Institute until 1958, excluding only the period of the Second World War. She successfully connected her medical mission with the mission as an activist for women's emancipation. She was one of the founders and long-time president of the *Women's Movement* in Sarajevo (1919–1937). In the newsletter of the Association, she published health and educational articles on child care, on the necessity of women's education for motherhood, and the legal protection of motherhood, on marriage and the family, and others. She was an advocate for birth control, the equality of women's and men's labour rights,

but also the abolition of regulated prostitution which she considered the "most feminist topic". As a delegate of the *Women's Movement*, she participated in international feminist gatherings. On the eve of the Second World War, she worked on the dissemination of pacifist ideas, and after the end of the war, through her work in the Women's Anti-Fascist Front (AFŽ), she was still engaged in solving the issues of the health and social protection of women and children, to which she had devoted her entire life.

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

Notes

[1] Teofil Skopczynski (? ?? ?? –Jan 12, 1855.– Zagreb, Aug 18, 1942). Construction engineer of Gospić and Brod na Savi districts; royal chief engineer of Požega County; „construction consultant" in the Royal Construction Office in Osijek. Lecturer at the School for Art Education, which was founded in Osijek in 1925.

[2] Maša Živanović's bequest, owned by the family, Belgrade. Hannah M. Stone to Maša Živanović, April 13, 1936, New York.

[3] Marion Shulevitz. Hannah Mayer Stone, 1893–1941. Jewish Women's Archive [cited 2023 Jan 12]; Available from: https://jwa.org/encyclopedia/article/stone-hannah-meyer

[4] Mara Kurtović (Belgrade, July 12, 1892–Sarajevo, Oct 17, 1979). She was the first expert in school hygiene in Bosnia and Herzegovina. She was the head of the School Polyclinic at the Institute for Maternal and Child Health Care on Skerlić Street in Sarajevo and befriended Dr. Maša Živanović.

[5] Her son, too, was living in Zagreb at the time. Despite being married to a Croatian woman, he had to live in secret and hide from the Ustaša authorities.

[6] Documents and writtings of Maša Živanović, family owned. Autobiographical notes by Maša Živanović.

[7] Nedo Zec (Mostar, July 12, 1899–Mostar, November 18, 1971). He 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.

[8] She was later the president of the *Women's Movement* in Zagreb.

[9] Pero Slijepčević (1888–1964), historian of literature, German scholar, university professor. One of the prominent members of the *Mlada Bosna* movement.

[10] Creating a bibliography of Dr. Maša Živanović›s papers, which are scattered throughout numerous newspapers and journals, would allow for a more comprehensive understanding of her contribution to social and medical issues.

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