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## The Prognostic Value of SUVmax of <sup>18</sup>F-FDG PET/CT in Patients with Metastatic Colorectal Cancer

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### Abstract

**Objective.** The aim of the study was to evaluate the prognostic value of the maximum standardized uptake value (SUVmax) of <sup>18</sup>F-Fluorodeoxyglucose (<sup>18</sup>F-FDG) PET/CT in patients with metastatic colorectal cancer, and to compare it with classical prognostic markers. **Materials and Methods.** The study included 70 patients with metastatic colorectal cancer who had not been treated for the metastatic disease. The patients underwent <sup>18</sup>F-FDG PET/CT as part of their routine diagnostic reevaluation. During the analysis, the value of the largest tumor diameter and SUVmax was determined for the lesion with the highest SUVmax observed. The values of CEA and CA 19-9 were recorded 7 days before the PET/CT analysis. **Results.** SUVmax and Carbohydrate antigen (CA)19-9 were found to be independent prognostic markers of disease progression within 12 months. Based on the Receiver Operating Characteristics (ROC) curve analysis, the patients could be divided into two groups: SUVmax≤4.1 vs. SUVmax>4.1. Patients with SUVmax values of 4.1 or less had significantly better progression-free survival within 12 months with an HR (95% CI) of 2.97 (1.4-6.3), relative to patients with SUVmax values above 4.1. **Conclusion.** SUVmax may be used as a novel prognostic marker of disease progression among patients with metastatic colorectal cancer. Values of SUVmax can be used to select patients with a more aggressive type of disease and higher risk for progression within 12 months of PET/CT analysis.

**Key Words:** PET/CT ▪ SUVmax ▪ Colorectal Cancer ▪ CA 19-9 ▪ Progression Free Survival.

### Introduction

Colorectal cancer is the third most commonly occurring cancer in men and the second most commonly occurring cancer in women. In 2018, 1.8 million new cases were reported and over the next 10 years, 50% more, newly diagnosed colorectal cancer cases are expected in the world (1). On the basis of the relevant literature, it may be concluded that PET/CT is present in almost all stages of management of this disease, and is only not indicated as part of the initial diagnosis of patients with colorectal cancer (2). The standardized uptake value (SUV) is the semi-quantitative

method most commonly used to determine <sup>18</sup>F-Fluorodeoxyglucose (<sup>18</sup>F-FDG) uptake in attenuation corrected PET images. With this technique, the tumor <sup>18</sup>F-FDG concentration is normalized to the amount of injected activity and the total volume of distribution (3). Although there are several studies that have evaluated the role of SUVmax as a predictive marker in such patients, only a few studies have examined the prognostic role of PET/CT parameters in relation to overall survival and progression-free survival in patients with colorectal cancer (4, 5). However, the results of studies investigating the role of PET/CT in the evaluation of treatments have revealed certain potential for

the formation of a prognostic model that would include SUVmax along with classical prognostic markers (6, 7).

Furthermore, pretreatment tumoral  $^{18}\text{F}$ -FDG uptake has been shown to represent an independent prognostic factor in patients with liver metastases undergoing any primary treatment modalities for colorectal cancer (8, 9).

The aim of our study was to evaluate the prognostic value of the maximum standardized uptake value (SUVmax) of  $^{18}\text{F}$ -FDG PET/CT in patients with metastatic colorectal cancer, and to compare it with classical prognostic markers. Considering that pre-existing prognostic models often include serological tumor markers, in our study we decided to investigate the relationship between SUVmax, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9, and to evaluate the role of SUVmax as an independent prognostic marker of progression-free survival.

## Materials and Methods

We performed an exploratory, descriptive-analytical study, with retrospective collection of data.

### Study Design

The study included 70 patients with metastatic colorectal cancer at the Clinic of Oncology, University Clinical Center of Sarajevo. The baseline was the date of the PET/CT analysis. After PET/CT analysis, the patients were followed up until the first radiologically verified disease progression. Patients had regular radiological examinations on a 3 month basis (as per guidelines), except for the patients who had earlier check-ups due to clinical indications of disease progression. In March 2019 we established a follow-up cut off when all the patients were examined for disease progression for the last time. The value of tumor markers (CEA and CA19-9) was recorded 7 days before PET/CT analysis. The values of the largest tumor diameter and SUVmax were recorded for the lesion with the highest SUVmax observed during the analysis.

### Inclusion Criteria

Patients were included when they met the following criteria: the diagnosis of colorectal cancer had been confirmed by histopathological examination; metastatic disease had been confirmed radiologically or by histopathological examination; patients had not received any systemic treatment for metastatic disease; patients underwent  $^{18}\text{F}$ -FDG PET/CT between January 2015 and December 2017 as part of their routine diagnostic reevaluation at the Clinic for Nuclear Medicine of the University Clinical Center of Sarajevo; patients had at least one-year period of adequate follow-up after the entry point; patients had regular evaluation on a 3 month basis, using an appropriate radiological diagnostic modality during the follow-up period.

### Exclusion Criteria

The exclusion criterion was not fulfilling one of inclusion criteria above.

### PET/CT Image Acquisition

The PET/CT was performed using a GE Discovery scanner (GE Healthcare, General Electric, Milwaukee, Wisconsin, USA). Multislice CT and PET emission data were acquired from the skull to the mid-thigh in all patients. Image acquisition started 60 min after intravenous injection of 370 MBq of  $^{18}\text{F}$ -FDG. We used the EARL reconstruction protocol with two iterations and 32 subsets.

### Statistical Analysis

IBM SPSS Statistics v. 23.0 was used for statistical analysis. The relationship between the largest diameter of the tumor, serological tumor markers (CA 19-9 and CEA), the number of months without disease progression, and SUVmax values was investigated using the Spearman correlation coefficient. The relationship of certain baseline characteristics, i.e. clinical and biochemical variables, and the parameters of PET/CT analysis, with the risk of disease progression within 12 months of PET/

CT analysis, was examined using multivariate Cox hazard analysis. In the survival analysis, progression of the disease within 12 months was the primary outcome measure. The study was performed on the sample of 70 patients since in the first year of follow up there were no deaths or patients lost to follow up. Progression-free survival was calculated from the date of the PET/CT analysis until the date of the disease progression for those patients who had progression of the disease within 12 months. The Kaplan-Meier method with the log-rank test was used to investigate the impact of SUVmax, defined as a dichotomous variable, on disease progression within 12 months.

A follow-up period of a minimum of 12 months was an inclusion criterion in this study. We had 6 patients who were lost to follow-up after 12 months. Those patients were censored at their last eligible and available radiology evaluation. The number of months without disease progression after 1 year of follow-up was used for descriptive statistical analysis only. The Receiver Operating Characteristic (ROC) curve was used to determine the optimal cut-off value for SUVmax. Data are presented as mean with standard deviation or as median with the 1st and 3rd quartiles.  $P < 0.05$  was an indicator of significance.

## Results

### *Patients' Characteristics*

The study included 70 patients with metastatic colorectal cancer, who underwent <sup>18</sup>F-FDG PET/CT at the University Clinical Center of Sarajevo (Table 1).

### *Correlation between SUVmax and Tumor Diameter*

The values of the largest tumor diameter and SUVmax were recorded for the lesion with the

highest SUVmax observed during the analysis. A statistically significant positive correlation between the largest tumor diameter and SUVmax was found. Higher tumor diameter values were associated with higher SUVmax values ( $\rho = 0.777$ ;  $P < 0.001$ ).

### *Correlations between SUVmax and Tumor Markers*

CEA and CA 19-9 values were recorded 7 days before PET/CT analysis. The median (1st -3rd quartile) CEA was 3.9 (1.6-21.7) ng / mL and CA 19-9 was 13.1 (4.4-46.6) U / mL. A statistically significant positive correlation was found between CEA and SUVmax. Higher CEA values were associated with higher SUVmax values ( $\rho = 0.494$ ;  $P = 0.0002$ ). The relationship between CA 19-9 and SUVmax was also examined, however, no statistically significant correlation was found between these variables ( $\rho = 0.036$ ;  $P = 0.797$ ).

### *Correlations between SUVmax and PFS (Progression Free Survival)*

The relationship between SUVmax values and PFS was investigated. A statistically significant negative correlation was found between the two variables. Higher SUVmax values were associated with a shorter PFS ( $\rho = -0.384$ ;  $P = 0.001$ ).

### *Prognosis of Disease Progression within 12 Months*

Among the 70 patients in our study, 36 (51.4%) experienced disease progression within 12 months of PET/CT.

Table 1. Summary of Patients' Characteristics

Variable	Age (years)*	Sex, N (%)	Tumor diameter (cm) <sup>†</sup>	SUVmax <sup>‡</sup>	Disease progression within 12 months, N (%)
All patients (n=70)	62.2±10.3 years (34-80)	M 29 (41.4)	1.5 (0.0– 3.6)	6.0 (1.5-10.5)	Yes 36 (51.4)
		F 41 (58.6)			No 34 (48.6)

\*Mean±standard deviation (minimum -maximum); SUVmax=Maximum standardized uptake value; <sup>†</sup>Median with 1st and 3rd quartile.

Table 2. Univariate Cox Regression Hazard Analysis of Prognostic Markers of Disease Progression within 12 Months

Variable	All patients (N=70)			
	B	P	HR	95% CI for HR
Age	-0.008	0.626	0.922	0.958-1.026
Sex	0.051	0.881	1.052	0.542-2.041
Tumor diameter	0.145	0.02	1.156	1.023-1.306
SUVmax	0.030	0.001	1.031	1.012-1.050
CEA	0.007	0.066	1.007	1.00-1.014
CA19-9	0.004	0.013	1.004	1.001-1.008

B=Unstandardized regression coefficient; P=p-value; HR=Hazard risk; 95% CI for HR=95% confidence interval for hazard risk; SUVmax=Maximum standardized uptake value; CEA=Carcinoembryonic antigen; CA19-9=Carbohydrate antigen 19-9.

Table 3. Multivariate Cox Regression Hazard Analysis of Prognostic Markers of Disease Progression within 12 Months

Variable	All patients (n=70)			
	B	p	HR	95% CI for HR
SUVmax	0.074	0.005	1.077	1.023-1.134
CA 19-9	0.005	0.01	1.005	1.001-1.009

B=Unstandardized regression coefficient; P=p-value; HR=Hazard risk; 95% CI for HR=95% confidence interval for hazard risk; SUVmax=Maximum standardized uptake value; CA19-9=Carbohydrate antigen 19-9.

Table 2 depicts the results of the univariate Cox hazard model of prognostic factors for disease progression within 12 months of PET/CT analysis. Tumor diameter, SUVmax value, and CA 19-9 value were found to be positively related with progression within 12 months (Table 2). Variables that were significant univariates were included in the multivariate Cox regression hazard analysis.

Table 3 shows the results of multivariate analysis. SUVmax and Ca19-9 were found to be independent prognostic markers of disease progression within 12 months (Table 3).

#### Measurement of SUVmax Cut-Off Value

ROC analysis identified a cut-off value of 4.1 as significant for SUVmax (area under the curve 0.65; P=0.031; 95% CI 0.52–0.78; Figure 1). The sensitivity and specificity at this value were 75% and 54.9%, respectively. On the basis of the ROC curve analysis, the patients could be divided into two groups: SUVmax $\leq$ 4.1 vs. SUVmax>4.1.

#### Survival Analysis

Survival without disease progression within 12 months was examined in relation to the cut-off

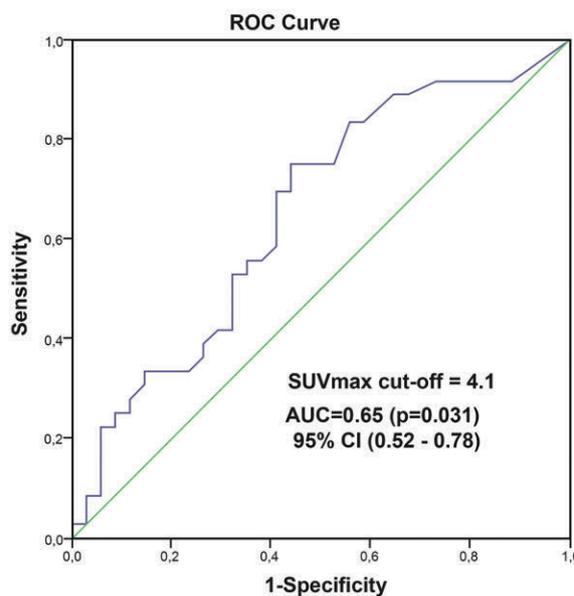


Figure 1. ROC curve SUVmax in prognosis of disease progression within 12 months.

ROC Curve=Receiver Operating Characteristics; AUC=Area Under the Curve; CI=confidence interval.

value of SUVmax determined by the ROC analysis (SUVmax=4.1) and the hazard risk was calculated (Figure 2).

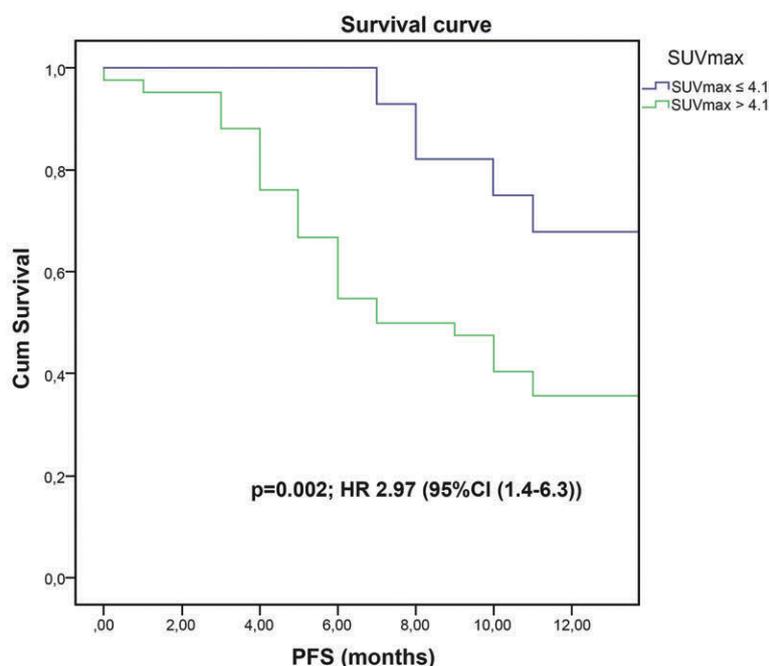


Figure 2. Kaplan-Meier curve of progression-free survival within 12 months for patients stratified based on a cut-off value of SUVmax of 4.1. PFS=Progression-Free Survival.

Average ( $\pm$  standard deviation) progression-free survival in patients with SUVmax above 4.1 was  $11.3 \pm 9.37$  months, and in patients with SUVmax below 4.1 was  $19.6 \pm 12.05$  months ( $P=0.001$ ).

## Discussion

The aim of our study was to evaluate the role of SUVmax in the prognosis of disease progression in patients with metastatic colorectal cancer. The relationship of SUVmax with serological tumor markers (CEA and CA19-9) was also evaluated. The aim of this testing is to try to find indicators that are routinely used in the evaluation of patients with colorectal cancer that might suggest that we could benefit from PET/CT analysis, which is an expensive diagnostic modality. Certain studies have shown a clear relationship between SUVmax and survival predictions in gastrointestinal cancers such as gastric cancer and esophageal cancer (10, 11).

However, only a few studies have been reported on the role of SUVmax in the prediction and prog-

nosis of outcomes in colorectal cancer. Such studies typically address the role of PET/CT parameters in evaluating the effectiveness of a particular therapeutic modality, rather than the possible impact on prognosis of overall survival or event-free survival (12, 13).

Due to the signs we had identified in the relevant literature that SUVmax could be a novel prognostic marker, we decided to investigate the clinical value of SUVmax as a prognostic marker through univariate and multivariate Cox regression analysis, and to compare it with classical colorectal cancer prognostic markers.

A significant relationship with disease progression within 12 months was calculated for the largest tumor diameter, SUVmax

value and CA 19-9. SUVmax and CA19-9 were the only independent markers of progression within 12 months. Statistical analysis showed that SUVmax was the best prognostic marker of progression of the included variables within 12 months. Although CA19-9 showed no significant correlation with SUVmax, multivariate analysis revealed a significant positive effect on disease progression.

Since SUVmax was the best prognostic marker of progression within 12 months, a ROC analysis was performed to determine the clinical value of this prognostic marker. The AUC score of our ROC analysis suggests that SUVmax is sufficiently reliable as a prognostic marker. It may be assumed that the AUC value would be even higher on a larger sample of patients. In order to prove the clinical value of the results we obtained, a Kaplan-Meier survival curve was performed. Patients with an SUVmax value of 4.1 or less (cut-off values obtained by ROC analysis) had significantly better progression-free survival within 12 months, with an HR of 2.97, relative to patients with SUVmax values above 4.1.

We compared our results with a study conducted by Shi et al. (5). In this study the authors included 107 newly diagnosed patients with various stages of colorectal cancer. In the multivariate Cox regression hazard analysis, it was demonstrated that TNM stage and SUVmax were the only independent prognostic markers of survival within 60 months in these patients. The SUVmax cut-off that was optimal for the prognosis of survival was 11.85, and it had a sensitivity of 73.3% and specificity of 75.3%. Patients with SUVmax below 11.85 had significantly longer survival (5). The SUVmax cut-off value in this study was higher than that observed in our analysis. A possible explanation may be that the patients in this study were newly diagnosed and had not been treated with any therapeutic modality so far.

Our results are compatible with the results of a study that examined the prognostic value of routine PET/CT in 70 patients with colorectal cancer, but who were found to have hepatic metastases. This analysis showed that patients with SUVmax below the cut-off value of 4.48 showed significantly longer progression-free survival (14). In our study, CEA showed a statistically significant positive correlation with SUVmax. Serological marker analysis is a cheaper diagnostic procedure than PET/CT analysis. The relationship between CEA and SUVmax demonstrated in this study may indicate the complementarity of the two methods. Increased CEA values may be an indicator for referring patients to PET/CT analysis. Lu et al. (15) conducted a meta-analysis of eleven studies, with a total of 510 patients, in order to investigate the diagnostic performance of  $^{18}\text{F}$ -FDG-PET or PET/CT in the detection of recurrent colorectal cancer occurring in patients with elevated CEA. The pooled estimates of sensitivity and specificity of  $^{18}\text{F}$ -FDG-PET/CT in the detection of tumor recurrence in CRC patients with elevated CEA were 94.1% and 77.2%, respectively. The results of this meta-analysis may contribute to the idea of using elevated CEA values as a sign for referring patients to PET/CT analysis. Certainly, future research with more patients may analyze this relationship more precisely and clarify it even further.

Although authors in some studies did not identify a significant relationship between CEA and SUV max, our results are certainly supported by the results of a study on 212 patients, where the authors identified a significant positive correlation between CEA and CA19-9 with SUVmax and other PET/CT parameters (16, 17). The authors of this study concluded that, although CEA is far from the ideal marker, its wide use and the fact that CEA reflects tumor metabolic features more than morphological ones, may certainly be of great benefit to clinicians (18). In the study conducted by Jones et al. (19), CEA was found to be an independent predictor of SUV-max values.

Our data could have an impact on the management of patients with metastatic colorectal cancer. Patients with a more aggressive disease (in our study patients with SUVmax values above 4.1) have worse prognosis and decisions on their further treatment should be made by a multidisciplinary team. Our recommendation for practicing surgeons is to postpone surgical treatment in this group of patients due to the possibility that surgery could shorten their survival. However, SUVmax with specific cut-off values has a limited role since the SUVmax variation is multifactorial and its use should be validated through further studies.

### ***Limitations of the Study***

Our study has several limitations. It is limited by its retrospective design and selection bias. We collected our data retrospectively while selecting patients who underwent PET/CT after confirmation of metastatic disease. A major problem with designing a randomized, prospective study with a control group on this topic is the ethical dilemma since it is well-known that PET/CT is superior to other radiological modalities when it comes to evaluation of malignant disease (20). The study was conducted at a single institution, with a relatively small sample of patients. The patient sample was heterogeneous since we did not divide patients up on the basis of the site of metastasis and type of therapy they had received. In this exploratory study we wanted to show proof of the concept

that SUVmax could be used as a prognostic tool in patients with metastatic colorectal cancer. The different sites of metastatic disease among our patients should not affect values of SUVmax, since Hofman et al. (21) concluded that the intensity of uptake of <sup>18</sup>F-FDG is parallels between primary tumors and metastatic tumors. Further, larger studies are needed to evaluate the relationship between SUVmax values, different types of therapy, different sites of metastatic disease and the joint effect of these variables on prognosis in patients with metastatic colorectal disease.

## Conclusion

SUVmax may be used as a novel prognostic marker of progression among patients with metastatic colorectal cancer. Values of SUVmax can be used to select patients with more aggressive types of disease and a higher risk for progression within 12 months of PET/CT analysis. The relationship between CEA and SUVmax demonstrated in this study may indicate the complementarity of the biochemical and radiological examination methods. Increased CEA values may be an indicator for referring patients to PET/CT analysis.

### What Is Already Known on this Topic:

*PET/CT is indicated in all stages of management and evaluation of colorectal cancer, except in the initial diagnosis. The parameters of the PET/CT analysis, such as SUVmax, have already been investigated as possible predictive markers in patients diagnosed with colorectal cancer. Previous studies have typically focused on the role of SUVmax as a marker of the effectiveness of a certain therapeutic modality.*

### What this Study Adds:

*Focusing on the role of SUVmax as a prognostic marker, this study could further clarify the possibility of identification patients with more aggressive types of colorectal cancer. Selection of these patients at an early stage of the disease would affect the treatment strategy. The complementarity of SUVmax and classical prognostic markers of colorectal cancer presented in this study could influence daily clinical practice.*

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## Systemic and Inhaled Corticosteroids, with or without Beta Agonists, as Adjuvant Therapy in Community Acquired Pneumonia

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### Abstract

The aim is to provide a narrative review of the role of corticosteroids, with and without inhaled beta agonist, in community-acquired pneumonia. Community and health-care associated pneumonia remain leading causes of morbidity and mortality despite appropriate antibiotic therapy. The pneumonia-associated adverse outcomes are not only related to the infectious organism, but also to a dysfunctional host-immune response resulting in overwhelming inflammation. Use of systemic corticosteroids as adjuvant therapy in pneumonia remains controversial. Multiple randomized clinical trials evaluating corticosteroids in patients with community acquired pneumonia have found discrepant results in terms of benefits and adverse effects. Inhaled delivery of corticosteroids offer the potential advantage of providing therapeutic benefits directly to the lung, with minimal to no adverse systemic effects. **Conclusion.** Although meta-analyses suggest potential benefits in a select group of patients with more severe pneumonia, the ideal timing, dose, route of delivery, duration, and patient selection remain to be established. A smaller body of literature suggests benefit of inhaled corticosteroids, with or without inhaled beta agonists, but future large scale clinical trials are needed to establish clinical benefit with inhaled delivery.

**Key Words:** Pneumonia ■ Corticosteroids ■ Beta-Agonists.

### Introduction

Community-acquired pneumonia (CAP) is the leading infectious cause of hospitalization and death in the United States costing more than 10 billion dollars in 2015 (1, 2). CAP and hospital associated pneumonia cause roughly 50% of the cases of acute respiratory distress syndrome (ARDS) resulting in a high mortality rate in patients requiring admission to the intensive care unit (ICU) (3-5). The mortality rate from pneumonia has not decreased substantially since initial improvement with widespread adoption of antibiotic therapy as standard of care in the late 1900's (6). The high mortality rate of pneumonia may be due to the broad range of infectious etiologies and clinically unidentified pathogens (7). This high mortality may also involve host-

specific factors including comorbidities and either an inadequate or overzealous host response (8).

Once a pathogen enters the host causing pneumonia, there is subsequent release of inflammatory cytokines that activate the vascular endothelium and recruit neutrophils to help contain the local infection (8). This host response also disrupts the normally preserved barrier between the alveolar (external environment) and vascular (internal systemic environment) compartments (9, 10). This barrier loss causes alveoli to flood with protein-rich edema fluid, leading to the loss of lung compliance, hypoxemia, acute lung injury, and ultimately acute respiratory failure (11). In addition, activation of vascular endothelium can promote a marked systemic inflammatory response syndrome (SIRS) leading to shock and multiorgan dysfunction, while increased vascular perme-

ability can promote dissemination of bacteremia. There has been ample research into adjuvant oral, intravenous, and inhaled anti-inflammatory and/or immunomodulating therapies to combat this dysfunctional host response in an effort to improve mortality in patients with pneumonia. Despite the current state of evidence, it remains unclear which patient subgroups are most likely to benefit from adjuvant therapies.

This review will summarize the data on corticosteroids, systemic and inhaled (with and without inhaled beta-agonists) to show their potential roles.

## Methods

For the literature search, we used PubMed MESH search headings “steroids” and “pneumonia” and included studies that have been published in the English language since 1990. We sequentially reviewed: Preclinical studies, human randomized controlled trials and meta-analyses of clinical trial data. We separately reported results of the studies using systemic versus inhaled steroids (with or without beta agonists), and included observational studies on role of systemic steroids in viral pneumonia.

## Results

### *Preclinical Studies Suggesting Benefits of Corticosteroid Use in Pneumonia*

Multiple preclinical studies have shown beneficial effects of systemic corticosteroids on the dysfunctional host response caused by pneumonia. One *in vitro* study showed suppressed bacterial replication in the presence of methylprednisolone when human monocytes were infected with different bacteria (12). Inflammatory markers including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were also reduced after treatment with methylprednisolone. In a mice model, Li et al. intratracheally introduced *Escherichia coli* and then either treated with hydrocortisone or placebo and found a decrease in serum levels of IL-6, INF- $\gamma$ , and nitric oxide in the mice that were treated with hydrocortisone (13). In a separate model of piglets bronchoscopically inoc-

ulated with *Pseudomonas aeruginosa*, Sibila et al. compared the effects of ciprofloxacin alone versus ciprofloxacin plus methylprednisolone (14). Pro-inflammatory cytokines and bacterial cell counts were both decreased in both the bronchoalveolar lavage (BAL) and serum in the group treated with corticosteroids.

## Clinical Studies of Systemic Corticosteroids in Pneumonia

### *Randomized Controlled Trials*

The following review is not intended to be a comprehensive one, but will highlight studies with important contributions to the current state of the evidence. A more comprehensive listing and brief descriptions of prospective trials in humans is provided in Table 1. For this review, a PubMed MESH search using headings “steroids” and “pneumonia” and including only randomized clinical trials in humans and in the English language was performed.

Two early preliminary studies found discordant results. The first by Marik et al. in 1993, randomized 30 patients with three or more British Thoracic Society criteria of severe pneumonia to receive 10mg/kg of hydrocortisone or placebo and found no difference in the intensive care unit (ICU) length of stay (LOS) in the treatment (4.3 $\pm$ 3.8 days) versus control group (4.6 $\pm$ 5.9 days) (15). Subsequently, Monton *et al.*, showed a decrease in systemic and lung inflammatory responses by measuring TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and c-reactive protein (CRP) levels in the serum and BAL fluid in mechanically ventilated patients receiving a large intravenous dose of 677 $\pm$ 508 mg methylprednisolone for 9 $\pm$ 7 days (16).

Confalonieri *et al.* showed improved clinical outcomes in 46 CAP patients randomized to hydrocortisone with a 200mg intravenous bolus followed by an infusion at a rate of 10mg/hour for 7 days versus placebo (17). The treatment group had a 60-day mortality of 0 vs. 8 (38%) (P=0.009) and a hospital LOS of 13 days (range, 10-53 days) vs. 21 days (range, 3-72 days) (P=0.03) despite hav-

Table 1. Summary of Prospective Trials on Systemic Corticosteroids in Pneumonia

Study	Target Population	Number of Patients (Control vs Intervention)	Intervention	Hospital Mortality (Control vs Intervention)	30-Day Mortality (Control vs Intervention)	Hospital LOS (Control vs Intervention)	Time to Clinical Stability (Control vs Intervention)
Marik <i>et al.</i> (15)	Adults hospitalized with CAP to the ICU	16 vs 14	10 mg/kg of hydrocortisone once	25% vs 7.1% (NS)	Not assessed	4.6 vs 4.3	Not assessed
Monton <i>et al.</i> (16)	Adults hospitalized with CAP requiring mechanical ventilation	9 vs 11	677 ± 508 mg IV methylprednisolone for 9 ± 7 days	67% vs 36% (P=0.37)	Not assessed	Not assessed	Not assessed
Confalonieri <i>et al.</i> (17)	Adults hospitalized with CAP	24 vs 24	Hydrocortisone 200 mg bolus followed by 10 mg/hour infusion for 7 days	30% vs 0% (P=0.009)	38% vs 0% (P=0.001)	21 vs 13 days (P=0.03)	Not assessed
Fernandez <i>et al.</i> (18)	Adults hospitalized with CAP	28 vs 28	200 mg bolus of methylprednisolone followed by infusion for 9 days	3.6% vs 3.6% (NS)	Not assessed	12 vs 10 days (NS)	7 vs 5 days (P=0.02)
Meijvis <i>et al.</i> (19)	Adults hospitalized with CAP	153 vs 151	5 mg IV dexamethasone daily for 4 days	5% vs 5% (P=0.98)	7% vs 6% (P=0.68)	7.5 vs 6.5 days (P=0.048)	Not assessed
Blum <i>et al.</i> (20)	Adults hospitalized with CAP	393 vs 392	50 mg oral prednisone for 7 days	Not assessed	2% vs 1%	7 vs 6 days (P=0.01)	4.4 vs 3 days (P=0.01)
Torres <i>et al.</i> (21)	Adults hospitalized with CAP and CRP > 150 mg/L	59 vs 61	0.5 mg/kg per 12 hours of IV methylprednisolone for 5 days	15% vs 10% (P=0.37)	Not assessed	10.5 vs 11 days (P=0.83)	5 vs 4 days (P=0.28)
Snijders <i>et al.</i> (22)	Adults hospitalized with CAP	109 vs 104	40 mg of oral or IV prednisolone daily for 7 days	Not assessed	5.5% vs 5.8% (P=0.93)	10.6 vs 10 days (P=0.16)	HR 1.14; 95% CI 0.82-1.59 (P=0.44)

LOS=Length of stay; ICU=Intensive care unit; CAP=Community acquired pneumonia; CRP=C-reactive protein; IV=Intravenous; HR=Hazard ratio; NS=Not significant.

ing higher CRP levels (mean, 55 [range, 14-349] vs. 29 [6-200], P=0.04) and a lower P/F ratios (the ratio of the partial pressure of arterial oxygen to fractional inspired oxygen) (mean±SD, 141±49 vs. 178±58, P=0.03) at study enrollment.

Larger trials, including patients with many different pneumonia severities, suggest that the anti-inflammatory properties of adjuvant corticosteroids may blunt the host inflammatory response and shorten time to clinical stability and hospital length of stay. In a single-center study, Meijvis *et al.* randomized 304 patients with a wide range of Pneumonia Severity Index (PSI) risk classes to receive 5mg of intravenous dexamethasone daily or placebo (19). LOS was reduced in the steroid group

by 1 day (6.5 vs. 7.5; P=0.048). Similarly, in the largest multicenter study to date, Blum *et al.* randomized nearly 800 patients in the moderate risk class (PSI II-IV) to 50mg of prednisone or placebo for 7 days (20). In the steroid treated patients, there was a reduction in time to clinical stability (3.0 vs. 4.4 days; HR 1.33; 95% CI, 1.15-1.50; P<0.001), and there was a 1 day reduction in hospital LOS (6 vs. 7 days, P=0.01). However, neither study found an improvement in mortality and rates of hyperglycemia were significantly increased in both trials (19, 20). Snijders *et al.* randomized 213 patients with mild to moderate CAP to receive either 40mg prednisolone daily or placebo for 7 days and found no difference in the primary endpoint of clinical cure

(2). Interestingly, more steroid-treated patients had worsening of symptoms after 7 days (OR 2.36; 95% CI, 1.05-5.31;  $P=0.04$ ), suggesting initial blunting of the SIRS response may have led to a delay in negative sequelae in this lower-risk population. However, CRP levels were higher in the treatment group (suggesting that these patients may have been more severely ill at baseline) and this appears to be an isolated finding, limiting confidence in this interpretation. Still, it is reasonable that the potential benefits of adjuvant corticosteroids are more limited in less severely-ill patients, thus increasing the risk for potential harms due to hyperglycemia and delayed immunosuppression.

In an attempt to study a more homogeneous patient population with more potential to benefit from systemic steroids, Torres et al. performed a multicenter trial of patients with severe CAP and a strong host inflammatory response signified by high baseline CRP level ( $>150\text{mg/L}$ ) (21). Severity of CAP was defined based on the modified American Thoracic Society criteria or a PSI V (3). Patients were randomized to receive  $0.5\text{mg/kg}$  of intravenous methylprednisolone every 12 hours or placebo for 5 days. Treatment failure—defined as development of shock, the need for mechanical ventilation, death, or radiographic progression ( $\geq 50\%$  increase in size of infiltrate)—was significantly reduced in the steroid group (OR 0.34; 95% CI, 0.14-0.87;  $P=0.020$ ). However, the overall improvement in the combined endpoint was driven mainly by the lack of late radiographic progression in the steroid group. In direct contrast to Sneijders et al., a post-hoc analysis found no difference in early ( $<72\text{h}$ ) treatment failures, but late treatment failure ( $>72\text{h}$ ) was reduced in the steroid group. Time to clinical stability, ICU and hospital LOS, and in-hospital mortality were not different between the groups, limiting definitive interpretation of trial results for these more clinically relevant endpoints. Finally, it took 8 years to recruit 120 patients at 3 hospitals, potentially limiting the generalizability of these more restrictive inclusion criteria.

In an attempt to make treatment more homogeneous, Lloyd et al. performed a blinded randomized trial of 816 patients with CAP who were

treated with a pneumonia bundle that included  $50\text{mg}$  per day of prednisolone acetate or equivalent (4). There was no difference in hospital length of stay (mean ratio of 0.95; 95% CI, 0.57-1.59), 90 day mortality (0.89; 95% CI, 0.48-1.63), and 90 day readmission rate (1.18; 95% CI, 0.69-2). This study included all patients with CAP and did not stratify by severity which about 50% of the patients in this study would have met criteria for severe CAP.

Combined, these trials suggest a possible decrease in hospital LOS in patients with severe CAP treated with corticosteroids, but with increased rates of hyperglycemia. It is possible that the associated hyperglycemia may partially explain the lack of mortality benefit as observational studies have found an increased mortality in CAP patients with hyperglycemia (25-27). However, the clinical relevance of increased hyperglycemia due to systemic steroids is uncertain. A large population based study of 327,452 patients that received a short course of steroids (median 6 days [interquartile range 6-12 days]) showed increased incidence of sepsis (incidence rate ratio (IRR) 5.3; 95% CI, 3.8-7.41), venous thromboembolism (IRR 3.33; 95% CI, 2.78-3.99), and for fractures (IRR 1.87; 95% CI, 1.69-2.07). This shows that even short courses of steroids may have potentially serious complications (28). More importantly, individual studies of heterogeneous populations lacking enrichment for patients most likely to benefit from adjuvant corticosteroid therapy have been likely underpowered to detect mortality benefit in low-risk patients (29). Based on these studies, however, it appears that the more severely ill patients with CAP may derive benefit with adjuvant corticosteroids.

### Meta-Analyses of Clinical Trial Data

A PubMed MESH search using headings “meta-analysis”, “steroids”, and “pneumonia”, found six meta-analyses all showing some benefit in clinical outcomes (30-36). A meta-analysis by Siemieniuk et al. of twelve trials and 1,974 patients provided a more nuanced interpretation of the discordant results seen in individual studies. While there was a nonsignificant reduction in all-cause mortality

(RR 0.67; 95% CI, 0.45-1.01), subgroup analysis of patients with “severe” CAP showed a significant improvement in mortality (RR 0.39; 95% CI, 0.2-0.77;  $P=0.01$ ) (30). There was also a significant reduction in the need for mechanical ventilation and development of ARDS among patients treated with steroids. However, the overall methodological strength of the included studies was considered to be low and one of the included trials, by Confalonieri et al. demonstrated remarkable benefit of corticosteroids towards mortality, which was considered to be an outlier to the other RCTs (17). Even after the exclusion of this trial’s data, there was a benefit of systemic corticosteroids towards all-cause mortality in patients with severe CAP.

A more recent meta-analysis addressed inherent heterogeneity by performing an individual data analysis including patients from 6 RCTs while excluding 5 smaller and 2 larger trials from 1970’s (1). Overall, there was no difference in mortality either with or without the Confalonieri trial. However, time to both clinical stability and hospital LOS were reduced by approximately 1 day in patients receiving corticosteroids, but so were the rates of hyperglycemia and CAP-related re-hospitalizations. No significant effect modifications were found, although trends existed for greater effects in severe pneumonia.

Stern et al. included 17 RCTs (including 4 RCT’s enrolling children) in their meta-analysis (2). Corticosteroid therapy reduced overall mortality in adults only with severe pneumonia (RR 0.58; 95% CI, 0.40-0.84). Corticosteroids also improved early clinical failure (death from any cause, radiographic progression, or clinical instability at days 5 to 8) in all patients regardless of pneumonia severity. Corticosteroid therapy was associated with hyperglycemia, but there were no differences for other adverse effects or secondary infections. This review included smaller and “lower quality” studies (11 of the 17 trials were single center, 9 were open-label, and 14 were assessed as high-risk for selective reporting bias due to inadequate blinding of participants and personnel) and it is unclear whether inclusion of these provided more precision for the true treatment effect.

## Corticosteroids in Viral Pneumonia

To date, there are no RCTs specifically investigating corticosteroids in viral pneumonia. A recent Cochrane review could only identify low quality observational studies addressing adjuvant corticosteroids in influenza pneumonia and suggested a three-times increased odds of mortality and an increase in hospital-acquired infections in the steroid treated patients. The differences in severity of illness could not be adequately addressed and the data was judged to be of very low methodological strength (37). Recently, Moreno et al. performed a secondary analysis of an earlier multicenter observational study to determine the effect of corticosteroids in severe influenza pneumonia (38). This study included 1846 patients with influenza pneumonia admitted to the ICU and treated with relatively higher doses of corticosteroids (median daily dose equivalent to 80mg of methylprednisolone) for a mean duration of 7 days. After a propensity-matched analysis, corticosteroid use was associated with an increase in ICU mortality (HR 1.32; 95% CI, 1.08-1.60;  $P<0.006$ ) (38). Another meta-analysis using ten trials with 6,548 patients showed that corticosteroids resulted in a higher mortality (RR 1.75; 95% CI, 1.30-2.36;  $P=0.0002$ ), longer ICU LOS (mean difference 2.14 days; 95% CI, 1.17-3.10;  $P<0.0001$ ), and a higher rate of secondary infection (RR 1.98; 95% CI, 1.04-3.78;  $P=0.04$ ) when compared to placebo (39).

There is a biologic explanation for these results for corticosteroids in viral pneumonia. Corticosteroids may provide benefit for bacterial infections by preventing negative systemic sequelae of a robust SIRS response while still allowing for effective bacterial clearance with potent antibiotics. On the other hand, lacking similarly potent antiviral therapy, a blunted host response may allow greater viral replication and dissemination resulting in greater systemic organ involvement and late-onset complications when treating viral pneumonia, such as influenza. The increased mortality could be related to an increase in secondary bacterial infections when these patients are given corticosteroids. These, plus other mechanisms could all result in

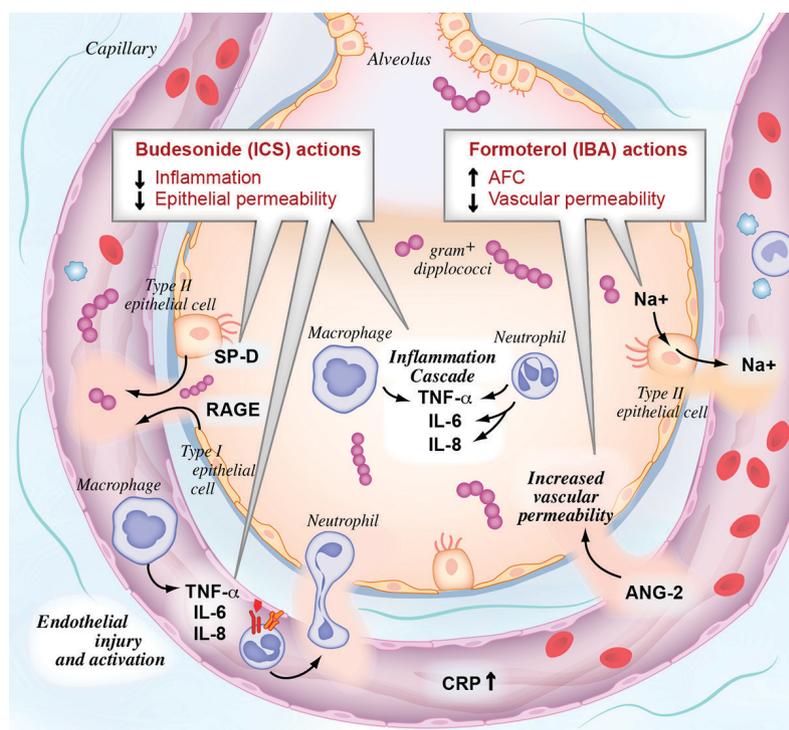
the increased mortality seen in adjuvant corticosteroid use in influenza pneumonia. The increased mortality observed in populations of patients with viral pneumonia could have implications in the previous studies of adjuvant corticosteroids as viral vs. bacterial pneumonia were not differentiated in the majority of the studies (although, the Torres trial (21) excluded patients with influenza). Moreover, the commonly used rapid influenza diagnostic test using an immunoassay has lower sensitivity when compared to PCR (40), allowing for misclassification of patients being treated for bacterial pneumonia and possibly increasing complications from adjuvant corticosteroids.

### Studies with Inhaled Delivery of Corticosteroids

There is a theoretical concern that systemic corticosteroids may have variable anti-inflammatory effects in the alveolar compartment, and that inhaled delivery of corticosteroids may provide more

effective anti-inflammatory benefits directly to the target organ while limiting negative systemic effects, such as, hyperglycemia and immunosuppression (Figure 1). A recent study showed that after instillation of endotoxin into the lungs of healthy patients, 40mg of intravenous dexamethasone markedly reduced anti-inflammatory markers in the peripheral blood, while only slightly affecting those in BAL fluid (41). As both systemic and inhaled corticosteroids affect the same inflammatory mediators, it is plausible that inhaled delivery would result in the same anti-inflammatory effects in the lungs without the adverse effects, compared to systemic delivery. This benefit needs to be contrasted with the long-term administration of inhaled steroids for chronic obstructive pulmonary diseases, where a higher incidence of pneumonia requiring hospitalization has been observed, but without an increase in mortality (42, 43).

Multiple studies involving experimental models of lung injury and/or pneumonia have demonstrated amelioration of inflammation, histo-



Legends: Key pathways in progression of lung injury. Infection causes neutrophils to release inflammatory cytokines IL-6, IL-8, and TNF- $\alpha$  which in turn causes epithelial injury (marked by increased levels of RAGE and SP-D) and vascular permeability (marked by increased ANG-2). There is potential for inhaled corticosteroids (ICS) to reduce this inflammatory response and limit epithelial permeability while inhaled beta agonists (IBA) could increase alveolar fluid clearance (AFC) and preserve vascular barrier function.

Figure 1. ICS=Inhaled corticosteroid; IBA=Inhaled beta-agonist; SP-D=Surfactant protein-D; RAGE=Receptor for advanced glycation endproducts; TNF=Tumor necrosis factor; IL=Interleukin; Na=Sodium; CRP=C-reactive protein; ANG-2=Angiotensin 2; AFC=Alveolar fluid clearance.

logic injury, and derangement in oxygenation, hemodynamics, and lung mechanics when treated with inhaled corticosteroids compared to placebo. Walther et al. studied effects of nebulized beclomethasone starting 30-minutes after infusion of live *Staphylococcus aureus* in mechanically ventilated pigs (44). Oxygenation, hemodynamics, and lung mechanics were better maintained in pigs receiving nebulized beclomethasone after onset of sepsis relative to placebo. Forsgreen et al. studied effects of prophylactic treatment with an aerosolized corticosteroid in a pig model of early ARDS induced by *Escherichia coli* (45). Pretreatment with aerosolized corticosteroids, either 15-minutes or 2-hours before the start of an endotoxin infusion, improved lung mechanics and hemodynamics relative to placebo. Jansson et al. also investigated the effect of pretreatment with aerosolized corticosteroids on lung injury and inflammation following varying doses of intratracheally instilled lipopolysaccharide (LPS) (46). Pretreatment with aerosolized budesonide decreased the production of inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and monocyte chemoattractive protein-1 after challenge with both low and high concentrations of LPS. Greater differences in pulmonary edema and development of ALI were seen at higher LPS concentrations suggesting that therapeutic effects of aerosolized budesonide were greater with more severe disease.

Timing of administration of therapy has varied across multiple studies. Wang et al. examined the effect of varying the time between injury and treatment with nebulized corticosteroids on lung injury in animal models (47). Nebulized budesonide was given immediately, 30, or 60-minutes following chlorine gas exposure. The mean arterial oxygen tension in animals treated immediately and 30-minutes post-delivery of budesonide was significantly higher than in controls or animals treated 60-minutes post-delivery. The recovery of lung compliance was enhanced and the pulmonary wet-to-dry weight ratio was lower in all three budesonide groups relative to controls, suggesting that while earlier is better, benefit may still occur with delayed treatment. Despite inherent hetero-

geneity, the above studies demonstrate relatively consistent beneficial effects of inhaled corticosteroids in animal models of lung injury caused by either inhaled or systemic insults.

Not all preclinical studies of inhaled corticosteroids in experimentally induced lung injury and pneumonia resulted in improvement relative to placebo. For example, Sjoblom *et al.* randomized 16 rabbits into groups receiving inhalation of ammonia, followed by 30-minute and 2-hour delayed treatments with 0.5mg inhaled budesonide or placebo (48). The ammonia inhalation resulted in severe ALI after only 15 minutes. In comparison with placebo, budesonide did not result in improved gas exchange or reduced airway pressure levels during the observation period. This result may exemplify the limitations of delayed treatment with a relatively low dose of inhaled corticosteroid in the setting of a potent inciting injury. In addition, most of these studies involved models of sepsis or direct lung injury using an injurious agent and not a live microorganism model of pneumonia. The use of inhaled corticosteroids appears to decrease the injurious host response in the lung, but results cannot be directly extrapolated to infectious pneumonia where the benefits of a decreased host immune response may be attenuated by increased bacterial or viral replication.

### Role of Adjuvant Beta Agonists

As previously described, pneumonia can increase vascular permeability resulting in loss of barrier function between the alveolar and vascular compartments. In experimental studies, beta agonists have demonstrated the ability to preserve pulmonary vascular stability and up-regulate alveolar fluid clearance. Spindler and Waschke compared effects of propranolol and epinephrine and concluded that activation of  $\beta$ -adrenergic receptor signaling contributes to the stability of endothelial barrier properties under baseline conditions (49). Therefore, beta agonists are likely to be most effective early in the course of developing lung injury when the alveolar epithelial and endothelial barriers are still relatively maintained. An extensive

physiological review by Matthay *et al.* provides a detailed discussion of the experiments that have enhanced our understanding of the complex regulation of lung fluid balance by active transport mechanisms across both the alveolar and distal airway epithelium (50). Pertinent to this review, the authors commented on the potential beneficial effects of both beta agonists and corticosteroids on the reabsorption of edema from the distal airspaces of the lung, during physiological and pathological conditions.

There are only four trials looking at beta agonists and their ability to prevent ALI. The BALTI trial was the first trial to demonstrate that beta agonist therapy reduced extravascular lung water in patients with ALI/ARDS (51). However, 5 years later, the BALTI-2 trial using the same treatment regimen of intravenous salbutamol did not decrease mortality in ARDS patients and was stopped early due to safety concerns related to arrhythmias (52). Concurrently, the ALTA trial, which randomized patients with ALI to either 5mg of aerosolized albuterol or placebo every 4 hours for up to 10 days, was also stopped early for futility after finding no difference in ventilator free days and hospital mortality between the treatment groups (53). The ALTA trial did show that systemic absorption of aerosolized albuterol in patients with ALI/ARDS was sufficient based on measurements of plasma albuterol levels. A common factor of these negative trials was that the enrolled patients were already mechanically ventilated with established ARDS, so the therapeutic window for potential effects of beta agonist therapy might have been missed. The only RCT to test whether pretreatment with inhaled beta agonists might prevent ARDS was performed in patients undergoing elective esophagectomy in 12 centers in the United Kingdom (54). Although the treatment with salmeterol 100 µg twice daily did not prevent the primary endpoint of early lung injury, the patients receiving salmeterol had lower pulmonary vascular permeability indices; lower serum levels of RAGE, TNF-alpha, and IL-1beta; and a lower incidence of postoperative pneumonia (7 vs. 17; OR 0.39; 95% CI, 0.16–0.96) (54). While not specifically limited to patients with pneumo-

nia, these studies are in patients with or at risk for developing ARDS with similar pathophysiology to patients with severe pneumonia suggesting that beta agonists may be beneficial as an adjuvant therapy in CAP, but future research is needed.

### Combined Use of Inhaled Corticosteroids and Beta Agonists

There is a synergistic benefit of combined inhaled corticosteroid and beta agonists in patients with obstructive pulmonary disease. The bronchodilating effect of inhaled beta agonists may improve distal delivery of inhaled corticosteroids to the alveoli, while also increasing the efficacy of inhaled corticosteroids by increasing the nuclear localization of corticosteroid receptors (55). Both inhaled corticosteroids and beta agonists are medications broadly available, inexpensive, and with established safety profiles when used in combination.

In a recent multicenter RCT, the authors studied the effect of combined inhaled corticosteroid and beta agonist therapy in patients at high risk of developing ARDS, defined by a lung injury prediction score  $\geq 4$  (56). Sixty patients were randomized to receive inhaled budesonide/formoterol versus placebo for up to 5 days. The primary outcome was change in oxygen saturation divided by the fraction of inspired oxygen (S/F) through day 5. The patients at risk of ARDS randomized to receive inhaled budesonide/formoterol, compared to those receiving placebo, had improved oxygenation based on more rapid improvement in the S/F ( $P=0.02$ ), with significant separation occurring at day 2. No patient in the treatment group had worsening S/F compared with eight patients (27%) in the placebo group. This benefit appeared to be restricted to patients with pneumonia ( $P=0.03$ ) versus those without pneumonia ( $P=0.51$ ) (56). Overall, patients in the treatment arm had lower rates of mechanical ventilation (53% vs. 21%,  $P=0.01$ ) and ARDS (23% vs. 0%,  $P=0.01$ ), as well as shorter hospital and ICU LOS. There was an imbalance in baseline severity of illness with more patients having baseline shock in the placebo group. However, shock was not associated with a change in S/F and

did not modify the effect of treatment on S/F. The median time from presentation to the first dose of study drug was <9 hours in this study. If proven effective in the upcoming NIH-funded, 600-patient ARREST Pneumonia trial (NCT04193878), it is likely that early treatment is crucial to provide protective effects prior to the development of more severe lung injury.

These studies highlight the need for future research with inhaled therapies in pneumonia. Some trials using systemic corticosteroids have shown an increase in rates of hyperglycemia and/or increase in delayed complications, such as secondary infections. The use of short-term, inhaled corticosteroid therapy would not be expected to yield these complications. Further studies are needed to determine whether the combination of inhaled corticosteroids and beta agonists may provide additional benefit. The potential synergy between the anti-inflammatory effects of corticosteroids and increased capillary stability and edema resorption from beta agonists combined with timely inhaled delivery directly to the target organ prior to the onset of respiratory failure has the potential to maximize clinical benefit while limiting negative systemic side-effects.

## Discussion

The consensus guidelines created by the Society of Critical Care Medicine and European Society of Intensive Care Medicine in 2017 recommended using corticosteroids for 5-7 days at a daily dose of <400 mg of intravenous hydrocortisone for all patients hospitalized with CAP. In this recommendation, they mention that patients with severe disease derived more benefit, but that all hospitalized CAP patients should be given corticosteroids (57). In contrast, the recent consensus guidelines for CAP by the Infectious Disease Society of America and American Thoracic Society (IDSA/ATS) published in 2019 did not recommend corticosteroids be used in patients with severe or non-severe CAP due to a lack of sufficient evidence (58). Clearly, additional clinical trials are needed to better define the patient populations that may benefit from the

adjuvant use of corticosteroids, as well as to elucidate the ideal timing, route, dose, and duration of therapy. In summary, there may be a benefit for adjuvant corticosteroids in patients with severe CAP, but their role still needs to be better defined. Given the remaining equipoise for this important question despite multiple multicenter RCTs, future trials need to assess timely (early) delivery and discern which patients would benefit the most relative to their host characteristics, severity, and etiology of pneumonia, along with the best route of delivery, dose, and duration of therapy.

## Conclusion

Despite conflicting guidelines, the strongest data currently supports using systemic corticosteroids for severe bacterial pneumonia. Using inhaled corticosteroids and beta-agonists has a theoretical benefit, but their exact role has yet to be determined.

### What Is Already Known on this Topic

*Adjuvant systemic corticosteroids may improve clinical outcomes in selected patients hospitalized with severe community-acquired bacterial pneumonia.*

### What this Study Adds

*Proposed use of adjuvant inhaled corticosteroid therapy, with or without inhaled beta agonists, would theoretically not predispose patients to systemic adverse effects, and as such may be an attractive alternative to adjuvant use of systemic corticosteroids in patients with community-acquired pneumonia.*

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

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## The Correlation between Enrolment Criteria and Students' Success in the Study of Medicine: a Five-Year Retrospective Study

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### Abstract

**Objective.** To investigate whether the students' study-success correlates with the achievements evaluated in the enrolment process based on the State Graduation Exam. **Methods.** The retrospective study included a total of 637 students enrolled in the study of Medicine at the Faculty of Medicine from the academic year 2010-11 to 2014-15. We analysed the structure of students enrolled on the basis of the State Graduation Exam, examined their success in two courses of the study programme and then correlated this study-success with individual admission parameters. In particular, we investigated whether the success in the study was influenced by the changes made in the enrolment requirements during this period. Pearson's correlation coefficient was used to measure strength of correlation between two variables. **Results.** The changes in the admission parameters, i.e., the inclusion of STEM subjects as obligatory enrolment requirements, made in 2012-13 enabled a better selection of students with high average secondary education grades and with significantly better results in Biology, Physics and particularly in Chemistry. This resulted in a higher success in the study, especially in the last two new-criteria generations. The Pearson correlation coefficient between students' success in Physiology and Pathophysiology II and the achievements evaluated in enrolment procedure as total cumulative score were statistically significant ( $r=0.27$ ,  $P<0.001$ ). Total points achieved in enrolment procedure was also correlated with students' success in Medical Physics and Biophysics and correlation was even stronger ( $r=0.52$ ,  $P<0.001$ ). **Conclusion.** Our results proved that a well-designed combination of the relevant admission parameters is a crucial prerequisite for a better success in the study.

**Key Words:** Enrolment ■ State Graduation Exam ■ Students' Study-Success.

### Introduction

The State Graduation Exam (SGE) was first introduced in Croatia in the school year 2009-10 and has since been carried out by the National Centre for External Evaluation of Education (NCEEE) pursuant to a government decision. The primary aim of the introduction of the SGE was to establish standardized instruments for evaluating the learning outcomes achieved during secondary education in order to obtain measurable results and comparable performance indicators, and thus establish a mechanism for monitoring the achievement of educational objectives and thus

assess the quality and/or identify the weaknesses of our educational system. Although the SGE was primarily conceived as a compulsory completion of grammar school ("certification" function), in the very first year of its introduction its results began to be used also for enrolment in study programs of higher education institutions (HEI) ("selectional" function), and thus became obligatory also for the students of four-year vocational and art schools who intended to continue their education at the tertiary level. This approach, however, disturbed the primary concept of state graduation, which was based on the students' obligation to take the level of subjects in the SGE that corresponded to the

curriculum of their respective secondary school. This situation has forced experts to continuously review whether the SGE must have both the output and input function, and whether it is an equitable system for enrolment in higher education (1-5).

Despite these constant doubts, it is indisputable that the application of the results of SGE as enrolment criteria for HEI has a number of advantages: centralized online application; a better overview of study programmes being offered; the possibility of applying for up to 10 study programmes; all applicants simultaneously write the same standardized tests; a better transparency of the final results.

However, the specific reservations expressed regarding the "selection function" of the SGE led to HEI being given the responsible task of constantly analyzing their enrolment criteria. Indeed, every academic year, HEI have the obligation to submit their current enrolment criteria and scoring system for verification. In this way, the students' results achieved in various compulsory and elective subjects in the SGE can be evaluated, together with their previous achievements during secondary education. Moreover, admission to some study programmes was also dependent on additional verification of knowledge, skills and competencies, and it was also possible to set a number of other preconditions which could narrow the screening framework. It is assumed that, if properly assessed, each enrolment parameter is one of the important preconditions for future success in the study and acquisition of learning outcomes of each study programme.

Every year, 1400 or more high school graduates apply for admittance to the University of Rijeka, Faculty of Medicine (MedRi), which enrolls 130 new students for the study of Medicine per year. The candidates are ranked according to a scoring system, with a maximum of 1 000 points. The original admission criteria, first determined in the year of inclusion of the SGE results, i.e., 2010-11, have been changed only once, in the 2012-13 academic year. An overview of these two scoring systems implemented in the selection process for the study of Medicine at the MedRi throughout all investigated academic years is shown in Table

1. For all investigated generations of medical students, the grade point average (GPA) of the four-year secondary education accounted for 25% (250 points out of 1000), while the grades obtained in the SGE amounted to 75% (750 points out of 1000). However, the manner of distribution of the points awarded for the results in the graduation test was changed in the academic year 2012-13. Namely, in the first two years of admission to the study on the basis of the SGE results (2010-11 and 2011-12), we evaluated the results achieved in the mandatory SGE subjects (Croatian language 10%, Mathematics 15%, foreign language 10%), all of which could have been taken at the basic level. An additional requirement for admission was just one elective subject (Biology, Chemistry or Physics), but if a candidate had passed all three elective subjects, their best result would be evaluated with 20% and the results of the other two subjects with 10% each. In the academic year 2012-13, we introduced a new scoring system, still in use, in which we evaluate only the grades obtained in three elective subjects in the SGE: Biology (25%), Chemistry (25%) and Physics (25%), all of which became mandatory for admission, with the remaining 25% still given for the secondary school GPA. We also included an additional precondition for admission, namely, compulsory previous attendance of Biology, Chemistry and Physics classes in at least two years of a secondary school program, and set the classification threshold at 400 points, below which no candidate can enrol. Our previous study (6) demonstrated some changes in the structure of students enrolled in the MedRi observed since the adoption of SGE results as enrolment criteria in relation to those previously enrolled on the basis of the entrance exam.

In the present study, we continued our previous research to determine how the introduction of the SGE, and particularly the changing of subjects taken into consideration for admission to the study of Medicine during this period, affected the quality of students enrolled, and whether there is a statistically significant correlation between the achievements valued in the admission process and the accomplishments of the learning outcomes

Table 1. The Enrolment Requirements for the Study of Medicine at the Faculty of Medicine University of Rijeka in the Period from the Academic Year 2010/2011 to 2014/2015

Academic year	Precondition	Evaluation of high school grades	Evaluation of mandatory State Graduation Exams	Evaluation of elective State Graduation Exams	Classification threshold
2010/2011 2011/2012	-	25%: high school average grade point	35%: Croatian language (10%), mathematics (15%), foreign language (10%)	40%: one elective exam (Biology or Chemistry or Physics) was obligatory, the best solved test was evaluated for 20%, the other two – each for 10%	-
2012/2013 2013/2014 2014/2015	Compulsory attendance of Biology, Chemistry and Physics at least two years of secondary school program	25%: High school average grade point	0%: Croatian language (0%), mathematics (0%), foreign language (0%); all exams have to be passed, but results are not evaluated	75%: Biology (25%), Physics (25%), Chemistry (25%); all exams are obligatory	400 points on the final ranking

of two courses: Medical Physics and Biophysics (MP&B), and Physiology and Pathophysiology II (P&PII), offered, respectively, in the first and second year of the study of Medicine.

## Methods

Retrospective data collection was performed at the archives of the MedRi and included a total of 637 full-time students enrolled in the study of Medicine between the academic years 2010-11 and 2014-15. The data collection was approved by The Committee for Ethical Issues of the Faculty of Medicine. We studied five successive generations of medical students. The following data were used: secondary school GPA, State Graduation Exam results in Biology, Chemistry and Physics and total points achieved at entrance to study, success in the end-of-course (EOC) exams of MP&B and P&PII. Croatian educational system uses grades 1–5 for appraising knowledge, where 1 indicates failure and 5 is the highest grade. The study-success was measured by two indicators. Firstly, we analysed the success in the EOC exams of, respectively, MP&B, which is taken in the first, and P&PII, taken in the second year of the study. According to the University of Rijeka Rules on Studies, the following grades are used for the first three years of the study: A 80-100% excellent (5); B 70-79.9%, very good (4); C 60-69.9%, good (3); D 50-59.9%, sufficient (2); E 40-49.9%, sufficient (2);

and FX and F, insufficient (1). For the purpose of correlation analysis, the SGE results and students' results of the two EOC exams were expressed in percentages. Students' performance was assessed with respect to the first two years of their studies. The five study groups were compared using the average values of variables for each group. The average grades were calculated on the basis of passed examinations only (grades 2-5).

Regarding the structure of enrolled students, we analysed how many of them had placed our study program high on the priority list in their online application (as their first or the second choice). According to the NCEEE rules, when applying for study programmes, the applicants can choose up to 10 programmes and the order of their choice in the final ranking list determines the study in which can enrol. If a candidate's total score on the final rankings is within the approved quotas for several study programmes, they have the right of entry only for the study which is placed highest on their priority list. We use this indicator as a measure of an applicants' interest for a specific study.

## Statistical Analysis

Statistical analysis was performed using STATISTICA (StatSoft, Inc., Tulusa, OK, USA), version 13.1. The variables were tested for normality using Kolmogorov-Smirnov test. Continuous variables with normal distribution were expressed as mean

± standard deviation (SD) and those with not normal distribution as median and interquartile range (IQR). Categorical variables were expressed as frequencies and corresponding percentages. Chi square-test was used for comparison of proportion of enrolled students who placed the study of Medicine as their first or the second choice on the priority list. The five study groups were compared in seven parameters (secondary GPA, SGE results in Biology, Chemistry and Physics, total points achieved at entrance to study and EOC exam results in MP&B and P&PII) using one-way analysis of variance (ANOVA) test, including post-hoc Scheffe test after testing for normal distribution. Pearson's correlation was used to investigate the association between the average grade obtained in the exams for the two relevant courses of the study of Medicine and the total skill score at admission to the study,

as well as the results in the tests for elective subjects in the SGE. The statistical significance level was set at  $\alpha=0.05$  ( $P<0.05$  being considered statistically significant).

## Results

### The Proportion of Enrolled Students Who Placed the Study of Medicine as Their First or the Second Choice on the Priority List

We analysed (Figure 1) the proportion of enrolled students in five generations since the introduction of the SGE in the selection process who had placed the study of Medicine at the MedRi high on the priority list, i.e., as their first or the second choice. The five study groups were compared in the proportion of enrolled students who had placed the study of

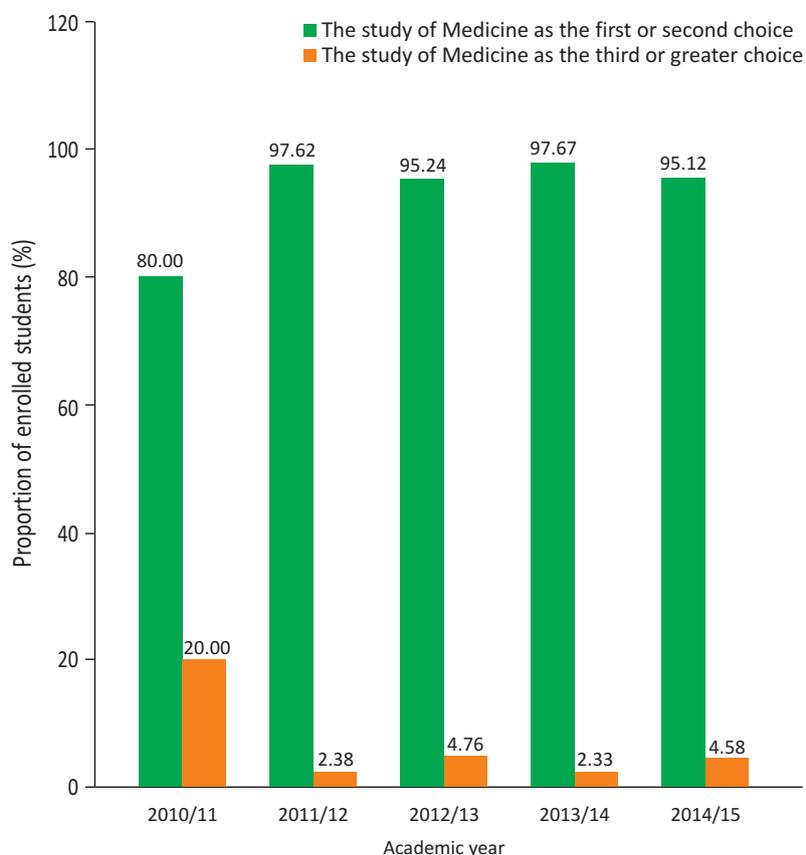


Figure 1. The place of the study of Medicine in Rijeka on the priority list of enrolled students. The proportion of enrolled students at the Faculty of Medicine University of Rijeka who set the study of Medicine as the first or the second choice (green bars) and the third or greater choice (orange bars) on the priority list in the period from the academic year 2010/2011 to 2014/2015. Values are expressed as percentages of enrolled students in particular academic year.

Medicine at the MedRi high on the priority list and the analysis showed that there was a statistically significant difference (Chi-squared=44.47,  $P<0.001$ ). Post hoc proportion test has shown that in the first generation the percentage of such students was significantly lower than in all four subsequent generations ( $P<0.001$ ), although the difference between the subsequent four generations were not statistically different ( $P>0.05$ ). Nevertheless, the change in the admission parameters made in the academic year 2012-13 did not significantly alter the percentage of students who had chosen our study of Medicine as their first or second choice when compared to the previous 2011-12 generation.

### The Structure of Students according to All Achievements Evaluated in the Enrolment Process

#### *The Distribution of Students according to Their Secondary School Grade Point Average*

Throughout all academic years involved in the analysis, we enrolled excellent students with very high secondary-school GPA (Figure 2), ranging

from 4.50 to 4.62. We have noticed a slight but continuous increase in the secondary school GPA in the last three new-criteria generations (4.50; 4.58; 4.62), but the differences were not statistically significant in comparison to the former-criteria SGE-enrolled generations.

#### *The Distribution of Students Based on their Grades in Biology, Physics and Chemistry in the SGE*

The average percentage of correctly solved questions in the SGE Biology test (Figure 3A) ranged from 68.98% to 79.64%, with the worst average grade in the first SGE-enrolled generation. A comparison of five generations with ANOVA test has shown that they differ significantly in their results on the SGE biology test ( $F=26.43$ ,  $P<0.001$ ). Post hoc analysis has shown that results in all the subsequent generations were statistically significantly better ( $P<0.001$ ) than in the first generation. Actually, we observed that already in the first new-criteria generation, the results of SGE Biology test were significantly better than in the very first former-criteria SGE generation (74.31% vs. 68.98%;  $P<0.001$ ), the last two new-criteria generations having significantly better

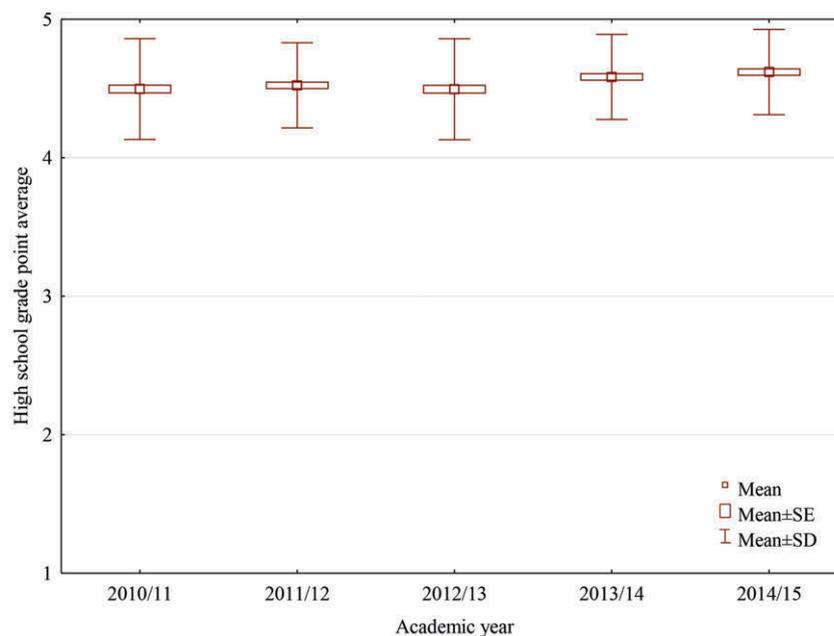


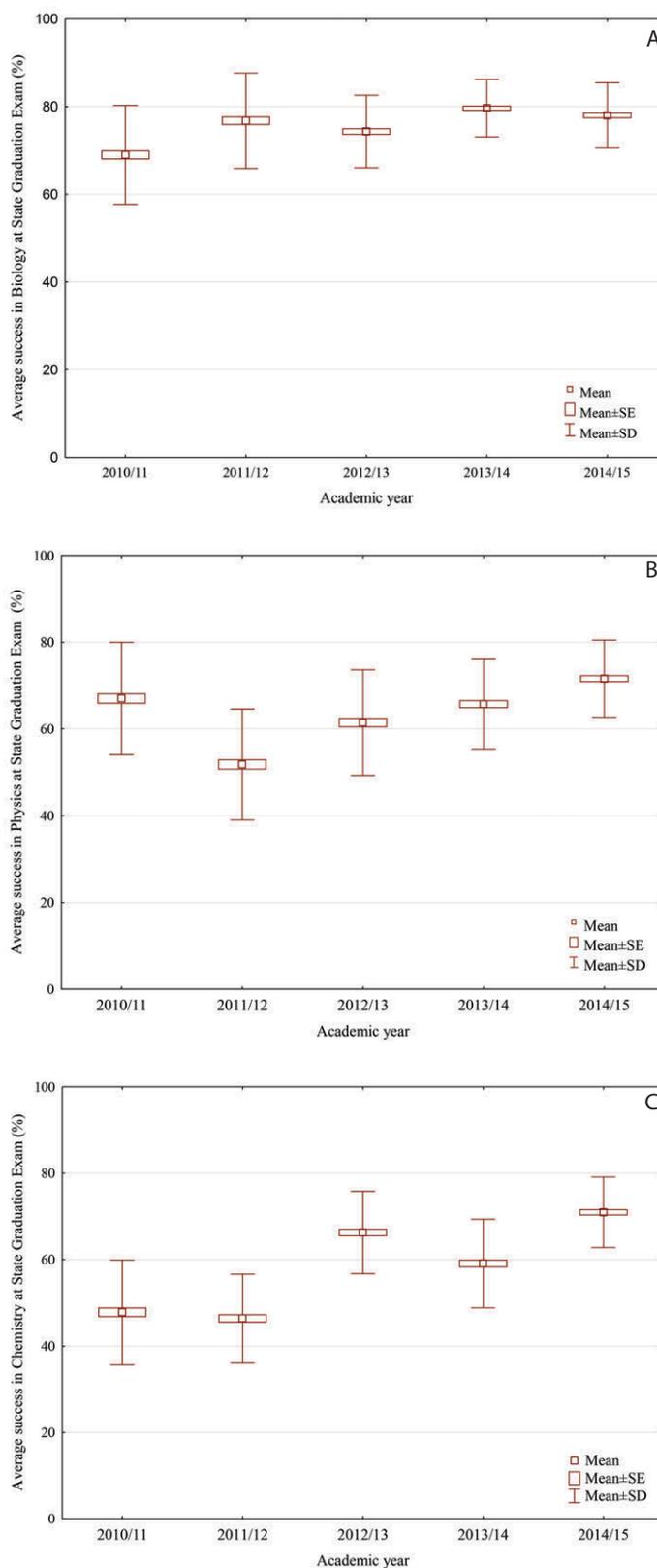
Figure 2. The high school grade point average of the enrolled students. The high school grade point average for the students enrolled in the study of Medicine at the Faculty of Medicine University of Rijeka in the period from the academic year 2010/2011 to 2014/2015. Grades are expressed as a mean ± standard error (SE)/ standard deviation (SD).

average grades even in comparison to the 2012-13 one (79.64%,  $P < 0.001$ , and 77.97%,  $P = 0.016$ ).

Figure 3B shows that the results of the SGE Physics test ranged from 51.78% to 71.6%. Comparison of all five generations has shown that the difference in success in Physics at SGE is statistically significant ( $F = 51.59$ ,  $P < 0.001$ ). The worst result in Physics was observed in the 2011-12 generation and was significantly lower ( $P < 0.001$ ) than the one achieved by the first SGE-enrolled generation (67%). However, we observed that all three new-criteria generations had significantly better average results also in Physics (61.45%; 65.7%; 71.6%;  $P < 0.001$ ) in comparison to the 2011-12 generation (51.78%).

The results of the SGE Chemistry tests (Figure 3C) ranged from 46.33% to 70.93% for the investigated period. A comparison of five generations with ANOVA test has shown that they differ significantly in their results ( $F = 144.08$ ,  $P < 0.001$ ). We observed that all three new-criteria generations had significantly better grades (66.25%; 59.06%; 70.93%;  $P < 0.001$ ) compared to either of the two former-criteria SGE-enrolled generations (47.75%; 46.33%). Unlike Physics, the improvement in the Chemistry test results was not continuous over the years, but it is worth noting that the last analysed generation had significantly better ( $P < 0.001$ ) average results (70.93%) in Chemistry compared to any previous generation.

Figure 3. The average success (percentages of correctly solved questions) in Biology, Physics and Chemistry at the State Graduation Exam. A=Biology; B=Physics; C=Chemistry at the State Graduation Exam for students enrolled in the study of Medicine at the Faculty of Medicine University of Rijeka in the period from the academic year 2010/2011 to 2014/2015. The results are expressed as a mean  $\pm$  standard error (SE)/ standard deviation (SD).



### ***The Comparison of the Total Score Achieved on the Basis of All Included Parameters of Evaluation***

The results of the total ranking score are shown on Figure 4, as median and interquartile range with the minimum and the maximum of all values on the ranking list. A continuous increase in the median value of total points throughout the analysed period was shown, starting from the first SGE generation. Comparison of all five generations has shown that the difference in success in total ranking score is statistically significant ( $F=39.69$ ,  $P<0.001$ ). Although we have noticed that the total points (716.1) were higher already in the first new-criteria generation, the increase became statistically significant in the second new-criteria generation ( $P<0.001$ ) in comparison to the former-criteria generations ( $P=0.017$ ). An even more obvious increase in the total points was observed in the third new-criteria generation in comparison to either of the two former-criteria generations ( $P<0.001$ ).

### ***The Students' Success in the Study of Medicine for the SGE-Enrolled Generations***

After a detailed analysis of the structure of students enrolled in the study of Medicine in five subsequent

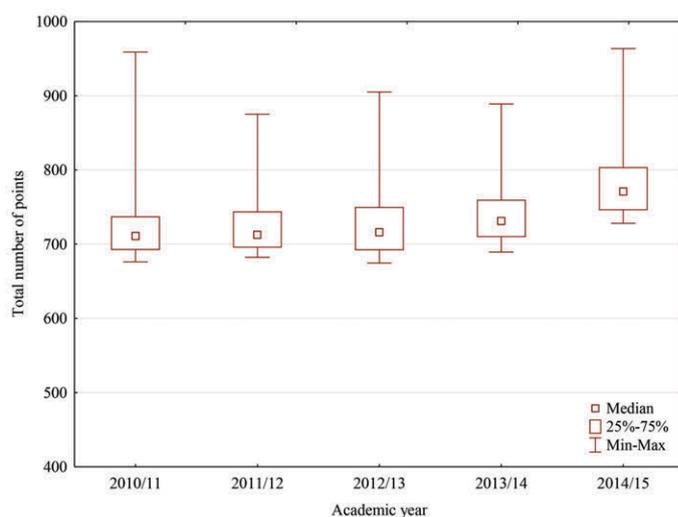


Figure 4. The total score on ranking list for students enrolled in the study of Medicine. The total number of points on final rankings for students enrolled in the study of Medicine at the Faculty of Medicine University of Rijeka in the period from the academic year 2010/2011 to 2014/2015. Values are expressed as a median, interquartile range (IQR) and minimal and maximal values.

generations starting from the introduction of the SGE in the selection process, we examined their success in studying and accomplishing the learning outcomes of two courses of the study programme: MP&B in the first, and P&PII in the second year of the study. The exam results for both courses are expressed as the average numerical grade calculated on the basis of passed examinations only (grades 2-5).

A comparison of the success in the EOC exam for MP&B is shown in Figure 5. To determine whether there is a statistically significant difference of mean grades between all academic years, one-way analysis of variance (ANOVA) test, including post-hoc Scheffe test was used. A comparison of all five generations in the success in the EOC exam for MP&B has shown that the difference is statistically significant ( $F=16.98$ ,  $P<0.001$ ). Although the data show that the average grade was higher already in the first new-criteria generation, post hoc analysis has shown that the increase became statistically significant only in the second new-criteria generation in comparison to the 2010-11 ( $P=0.034$ ) and the 2011-12 ones ( $P=0.002$ ). A more evident increase in the average grade was observed in the third new-criteria generation in

comparison to both former-criteria generations ( $P<0.001$ ). Moreover, the average score achieved in the EOC exam of MP&B in the last three analysed generations became significantly higher from year to year.

Figure 6 shows a comparison of the success in the EOC exam of P&PII in all analysed generations. Similar to the results in MP&B, comparison of all five generations has shown that the difference in success on the EOC exam of P&PII is statistically significant ( $F=7.15$ ,  $P<0.001$ ). A continuous increase in the average grade in the EOC exam of P&PII was also observed in all three new-criteria generations, but a statistically significant increase was observed only in the last two generations (2013-14,  $P=0.002$  and 2014-15,  $P<0.001$ ) in comparison to the last former-criteria SGE-enrolled generation.

The respective differences between the average grades obtained in the EOC exams of MP&B and of P&PII were not statistically significant within the same generation of students. Moreover, the average grades in both courses showed a similar trend of increase in all three new-criteria generations. This seems to suggest that the results achieved in the exams of two different courses from the study programme are comparable, as the same generation of students achieved similar success in two separate courses in two consecutive years of study.

#### ***The Correlation between the Results in the End-of-Course Exams of the Two Courses in the Study of Medicine and the Achievements Valued in the Selection Process***

In this study, we analysed whether there is a correlation between the results achieved in the EOC exams in two mandatory courses in the study programme of Medicine (MP&B from the first, and P&PII from the second year of the study) and any of the achievements valued in the selection process.

Figure 7 shows a statistically significant correlation between the results of this EOC exam in MP&B and all students' individual achievements valued in the classification procedure for almost all academic years. Thus, a significant correlation was observed between the secondary

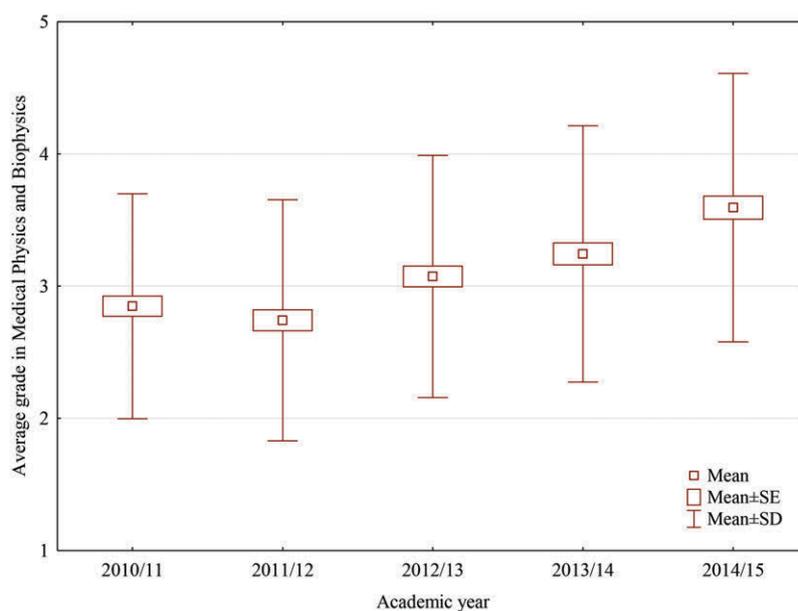


Figure 5. Students' success at the final exam of Medical Physics and Biophysics. The average grades at the final exam of Medical Physics and Biophysics in the first year of the study of Medicine at the Faculty of Medicine University of Rijeka in the period from the academic year 2010/2011 to 2014/2015. Passing grades (scale from 2=sufficient to 5=excellent) are expressed as a mean±standard error (SE)/ standard deviation (SD).

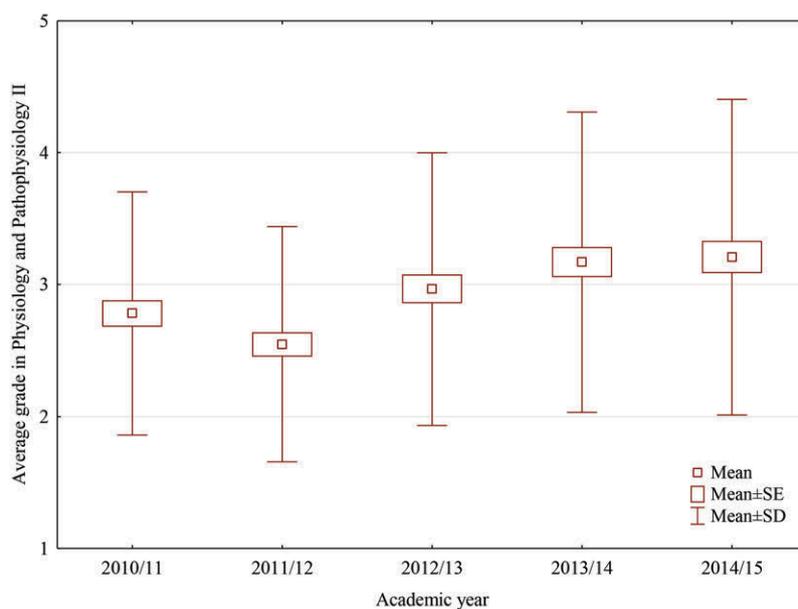


Figure 6. Students' success at the final exam of Physiology and Pathophysiology II. The average grades at the final exam of Physiology and Pathophysiology II in the second year of the study of Medicine at the Faculty of Medicine University of Rijeka in the period from the academic year 2010/2011 to 2014/2015. Passing grades (scale from 2=sufficient to 5=excellent) are expressed as a mean±standard error (SE)/ standard deviation (SD).

school GPA and this EOC exam results in all analysed generations, while a correlation the students' success in this EOC exam and their individual results in the SGE Physics, Biology and Chemistry was

found in some, but not all generations. Moreover, a significant correlation in all examined academic years was found between this EOC exam results and the overall score achieved in the SGE. In all exam-

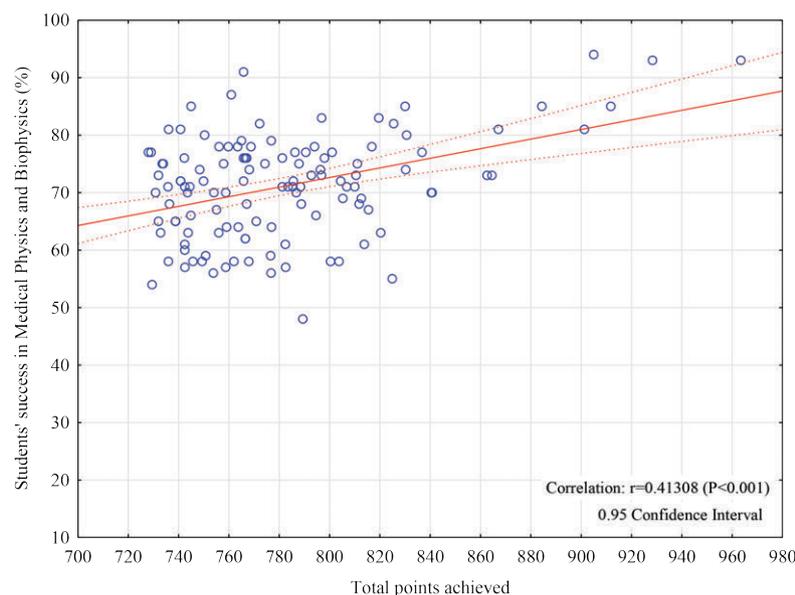


Figure 7. The correlation between State Graduation Exam results and students' success in Medical Physics and Biophysics. The correlation between the results of the final examination in Medical Physics and Biophysics and all individual achievements of students valued at classification to the study of Medicine at the Faculty of Medicine University of Rijeka in the academic year 2014/2015. Correlation was assessed using Pearson's test.

ined academic years the highest value of the correlation coefficient was obtained between this EOC exam success and the total score on the ranking list (Table 2). The Pearson correlation coefficient between students' success in MP&B and the achievements valued in enrolment procedure as total cumulative score for all students who passed EOC exam were statistically significant ( $r=0.52, P<0.001$ ).

As regards P&PII (Figure 8), a somewhat weaker correlation was obtained between the success in this EOC exam and all individual results included in the final ranking at enrolment. In contrast, in several generations (2010-11, 2012-13, 2014-15), a positive correlation between this EOC exam re-

Table 2. The Correlation between Students' Success in Medical Physics and Biophysics and the Achievements Valued in Enrolment Procedure for the Study of Medicine at the Faculty of Medicine University of Rijeka in the Period from the Academic Year 2010/2011 to 2014/2015

Performance parameters	Academic Year									
	2010/11		2011/12		2012/13		2013/14		2014/15	
	r	P	r	P	r	P	r	P	r	P
High school grade point average	0.351	<0.001	0.302	0.002	0.220	0.015	0.323	<0.001	0.347	<0.001
Biology at State Graduation Exam	0.263	0.008	0.071	0.475	0.199	0.028	-0.006	0.945	0.188	0.039
Physics at State Graduation Exam	0.258	0.009	0.443	<0.001	0.273	0.002	0.232	0.010	0.265	0.003
Chemistry at State Graduation Exam	0.312	0.002	0.330	<0.001	0.261	0.004	0.134	0.144	0.201	0.027
State Graduation Exam results	0.496	<0.001	0.479	<0.001	0.386	<0.001	0.217	0.017	0.329	<0.001
Total points achieved	0.594	<0.001	0.529	<0.001	0.453	<0.001	0.328	<0.001	0.413	<0.001

Correlation was assessed using Pearson's test. Statistically significant results ( $P<0.05$ ).

sults and the results in the SGE Biology was found. Moreover, in these generations there is also a statistically significant correlation between the success in the EOC exam and the overall grade achieved in the SGE. Finally, a statistically significant correlation between the success in this EOC exam and the total score on the ranking list was found in all gen-

erations (Table 3). The Pearson correlation coefficient between students' success in P&PII and the achievements valued in enrolment procedure as total cumulative score for all students who passed EOC exam were statistically significant ( $r=0.27$ ,  $P<0.001$ ).

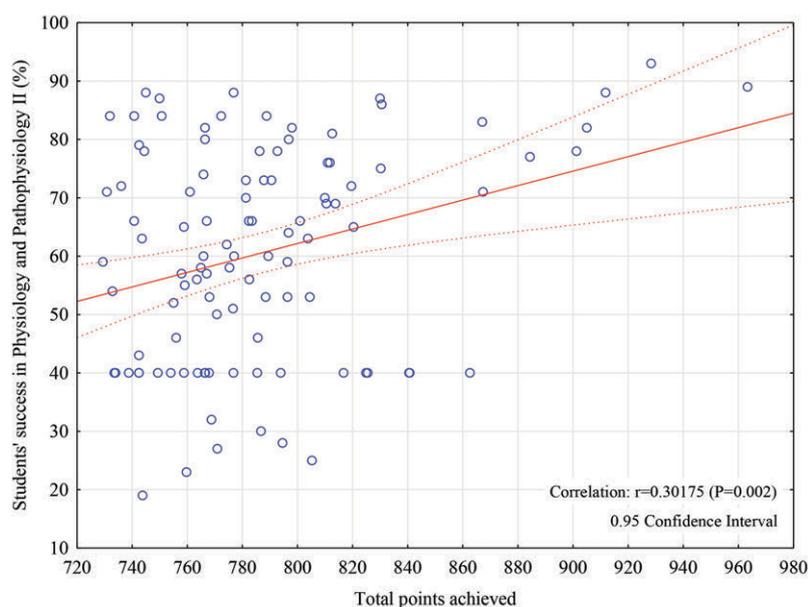


Figure 8. The correlation between State Graduation Exam results and students' success in Physiology and Pathophysiology II. The correlation between the results of the final examination in Physiology and Pathophysiology II and all individual achievements of students valued at classification to the study of Medicine at the Faculty of Medicine University of Rijeka in the academic year 2014/2015. Correlation was assessed using Pearson's test.

Table 3. The Correlation between Students' Success in Physiology and Pathophysiology II and the Achievements Valued in Enrolment Procedure for the Study of Medicine at the Faculty of Medicine University of Rijeka in the Period from the Academic Year 2010/2011 to 2014/2015

Performance parameters	Academic Year									
	2010/11		2011/12		2012/13		2013/14		2014/15	
	r	P	r	P	r	P	r	P	r	P
High school grade point average	0.185	0.103	0.215	0.051	0.103	0.293	0.183	0.057	0.241	0.014
Biology at State Graduation Exam	0.291	0.009	0.029	0.788	0.362	<0.001	0.106	0.271	0.249	0.011
Physics at State Graduation Exam	-0.005	0.966	0.221	0.045	0.051	0.606	0.035	0.714	0.127	0.200
Chemistry at State Graduation Exam	0.197	0.081	0.180	0.103	0.090	0.357	0.185	0.055	0.085	0.389
State Graduation Exam results	0.259	0.021	0.209	0.058	0.221	0.023	0.172	0.075	0.230	0.019
Total points achieved	0.307	0.006	0.260	0.018	0.251	0.010	0.230	0.016	0.302	0.002

Correlation was assessed using Pearson's test. Statistically significant results ( $P<0.05$ ).

## Discussion

In this study we found that one of the two enrolment regimes based on SGE, which is implemented in the academic 2012-13 year at the Faculty of Medicine in Rijeka, is proved to be a more successful screening method for our future students.

The introduction of the SGE in the school year 2009-10 has resulted in a series of changes in the education system of the Republic of Croatia. It was primarily designed as a tool for external evaluation of secondary education, but was at the same time adopted as an obligatory part in the classification procedure for enrolment in HEI. The selection role of the SGE has caused the most polemics in professional circles focused on the Croatian education system. Even before the implementation of the first SGE, the expert from the Centre for Educational Research and Development and the Institute for Social Research pointed to numerous inconsistencies in it (1). Some other important studies, published immediately after the implementation of the first SGE, analysed the results obtained in graduation exams in STEM subjects - Mathematics, Biology, Chemistry and Physics, and discussed in detail the doubts that emerged from the experience gained from the first generation (2-5). The objections to the introduction of the SGE were mostly related to the inaccuracy in defining the exact aims of the SGE, which led to numerous difficulties, regarding either the determination of the test contents and the level of difficulty of graduation exams, or the analysis of the results of graduation exams in order to prepare useful recommendations for improving future exams.

In order to contribute to the analysis of the results and of possible consequences of introducing the SGE into the enrolment procedure for HEI, we continued our research published in the paper (6), which shown that this new method of selection has enabled easier enrolment for students from other Croatian counties and has significantly reduced enrolment of students from vocational schools, but has had no significant impact on the female to male ratio (60:40) in comparison to former enrolment through the Entrance Exam. In the

present study we analysed the structure of enrolled students through some other indicators. The results showed that there was very high proportion of students in all analysed generations who placed our study programme on the top of their priority list, namely, as their first or second choice. In the first year of introduction of the SGE, 80% of students had placed our study of Medicine as the first or second choice, and in the following academic years this proportion was even significantly higher (95.24 to 97.67%). Placing the study of Medicine high on the priority list reflects genuine motivation for enrolment, which certainly represents one of the most important parameters that influence the success in the study. Although other authors (7) describe the existence of several different categories of motivation for selection of a particular study, the six-year study of Medicine certainly does not belong in the category of the "less demanding" ones; most of the other reasons, for example the desire for a well-paid and respected job, the possibility of working abroad later on, etc., can only be encouraging for finishing the study within the prescribed period. Furthermore, we aimed to find whether the changes in the enrolment parameters made in the academic year 2012-13 have had any impact on the structure of enrolled students and their success in studying.

Our results showed that, regardless of the parameters used during the selection process, the screening of excellent high school students was continuously successful throughout the examined period (GPA varied from 4.50 to 4.62). We found a statistically significant positive correlation between the success in the first-year course in MP&B and the secondary school GPA of enrolled students. However, although our results proved that the GPA achieved in secondary education is important and should be included among the parameters of classification because it evaluates the four-year secondary school success, we consider that GPA would not be good as the only parameter for selection because a more significant correlation was found between the success in the EOC exams of both examined subjects (MP&B and P&PII) and the overall score achieved on the basis of

all evaluated parameters. This points to a higher importance of the knowledge demonstrated in the standardized SGE, which all applicants write at the same time. Kim et al. (8) proved the importance of GPA in predicting the study success, while the others emphasised that secondary school GPA, as an indicator of the habits of continuous work, seem to be more important for study success than one-time results in state graduation exams (9, 10). Moreover, there are a number of studies that claim that the importance of grades from secondary school, and even the results achieved in graduation tests, are subordinated to other important and desirable personality characteristics which, according to them, should be assessed by various experts in the selection process and could be crucial in the future professional work as a medical doctor (possession of inter- and intra-personal skills, communication skills, motivation, stress tolerance, empathy, academic excellence, etc.) (11-20).

Analysis of the students enrolled in our faculty on the basis of their achievements in individual SGE tests in Biology, Physics and Chemistry showed that the last three new-criteria generations had significantly better results in Biology in comparison to the very first former-criteria SGE-enrolled generation, had significantly better results in Physics in relation to the second former-criteria SGE-enrolled generation, and finally, had significantly better results in Chemistry in relation to both former-criteria SGE-enrolled generations. However, the conclusions obtained by this analysis should be viewed in the context of all other changes that accompanied the classification process based on the state graduation. In detailed recommendations of Ristic Dedić et al. in 2011 (2-5), published after the first year of successful implementation of the SGE, the need to improve the contents and quality of test materials used in the selection to HEI was suggested. The authors have emphasized that for the selection purpose it is less important whether the test content is in compliance with the high school program, but greater attention should be paid if certain thematic areas and contents covered by the test are relevant for continuing education in a particular study programme.

Our analysis also showed a slight increase in the total number of points achieved already by the first new-criteria generation and, furthermore, a continuous and statistically significant increase in the subsequent generations. We believe that the new parameters of enrolment may serve as a powerful tool to select the best candidates. Our results also confirmed that the requirements for entry have been well chosen, and that simultaneous evaluation of the success in high school and the success in the state graduation tests in the ratio (25%:75%) ensures a good basis for successful accomplishment of learning outcomes later in the study.

In our previous analysis (6), we have already examined the students' study success for five generations enrolled on the basis of SGE by following up the average passing grade at EOC exam in MP&B, a course taken in the first year of the study, and compared the results with those achieved by the last generation enrolled on the basis of the Entrance Exam in 2009-10. In the present study, we continued to monitor the success of these students in P&PII, a course in the second year of the study. We have also compared the success in these two courses with each other and, additionally, examined if there is a correlation between the students' course success and their achievements in the SGE. Although in both courses we have noticed a continuous increase in the average passing grade in all three new-criteria enrolled generations, a statistically significant increase was found only in the last two generations (2013-14 and 2014-15) in comparison with any one or both former-criteria generations. The average passing grades for either course did not differ significantly within the same generations. Such findings have strengthened our assumption that better passing grades in the EOC exams for generations enrolled under the new parameters can be attributed to a better selection of high quality students that we have achieved by changing these parameters.

Regression analysis of the data from the enrolment and analysis of the passing grades at EOC exams showed a statistically significant correlation between the students' study success in the first and second year and the cumulative results they had

achieved in SGE tests, and in particular with the total score obtained in the admission selection process. We have shown that the success in MP&B in the first year of the study also correlated significantly with the results of each particular criteria parameter in almost all analysed generations, confirming our assumption that the selection process must evaluate exactly those secondary school achievements which will provide a good basis for easier accomplishment of the learning outcomes of the first-year subjects, mostly considered as a continuation of the secondary education with aim of creating a wider basis for all subsequent medical contents. As well, our regression analysis of results also showed that in all generations success in the exam of P&PII correlates significantly with the student's overall results in the SGE tests, as well as with the total score in the admission selection process. This supports our assumption that a properly selected combination of enrolment parameters is also very important for accomplishing the learning outcomes of the subjects in the second year of study but, nevertheless, much more important are the competencies and skills acquired at the end of all medical courses in the first year of the study.

Although our results clearly revealed some positive changes in the students' success after the introduction of the SGE in the enrolment procedure, the fact is that our study has some limitations and that the success in the study cannot be associated exclusively either with the average passing grade achieved in the EOC examination in only two courses or with the enrolment parameters. Indeed, numerous other factors, both individual and institutional, influence the study success (21-24). The impact of paying tuition on academic performance, for instance, has also been analysed, but has not been found to present a stimulus for better studying in state-owned schools (25, 26).

The importance of several other factors should also be emphasised, among them the manner of assessment of the learning outcomes, the level of achieved objectivity in the EOC exams and the high-set criteria that prevent "inflation of passing grades" (27). Further investigations should include

students' performance in other courses, especially in those that represent a significant barrier to studying and a common reason for interrupting the study of Medicine (Anatomy, Pathology, etc.) because of the complexity and scope of the contents, and we should correlate the outcomes achieved with the enrolment parameters. Moreover, other indicators of students' success should be found and put in correlation with the enrolment parameters of evaluation.

## Conclusion

Our analysis showed that the changes in the enrolment parameters, namely, the introduction of the three STEM subjects as compulsory components of the ranking criteria in the academic year 2012-13, continued to provide an even better selection of students with a high average grade achieved in secondary education, as well as a selection of students with significantly better fundamental knowledge in STEM subjects (Biology, Physics and in particular Chemistry). This resulted in improved success in the study, especially in the last two generations of students enrolled under the new conditions of admission. For MP&B, a course offered in the first year of the study, we have found a significant correlation between the success in the study and either individual or combined achievements which were evaluated at admission, while the success in the second-year course in P&PII correlated significantly only with combined results of all SGE tests, as well as with the total cumulative score of all evaluated achievements. Our results supported the assumptions that the changes of the conditions of entry in 2012-13 had been well considered and that they had a better selection role in admission to the study of Medicine in comparison to previous parameters of evaluation. Our results also point to the great responsibility that HEI have for students' study-success, not only for their role in maintaining and improving the quality of teaching, but also because for their role in determining the relevant parameters for selection of students.

**What Is Already Known on the Topic:**

Although the State Graduation Exam was primarily designed as a compulsory completion of grammar school and a tool for external evaluation of secondary education, it was adopted from the start as an obligatory part in the classification procedure for enrolment in higher education institutions. However, its dual role constantly provokes polemics in professional circles focused on the Croatian education system.

**What this Study Adds:**

In the present study, a statistically significant correlation between enrolment criteria and students' success in the study of Medicine was obtained, and thus we provided a scientific evidence that properly selected admission parameters could have a significant impact on future success in the study, which implies that higher education institutions have the great responsibility in determining the relevant parameters for selection of students.

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

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

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## The Experiences of Patients with Deep Brain Stimulation in Parkinson's Disease: Challenges, Expectations, and Accomplishments

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### Abstract

**Objectives.** Deep brain stimulation (DBS) is a safe and effective alternative treatment of some movement disorders such as Parkinson's disease. Although DBS is an effective treatment for Parkinson's disease, because of the necessity of surgical intervention, follow-up and the effects on symptoms, this study was carried out to determine the challenges, expectations and accomplishments of patients with DBS in Parkinson's disease. **Materials and Methods.** This qualitative study was carried out at the Neurosurgery Department of a research hospital in Turkey with seven patients who underwent DBS between 2008 and 2018. In the study, the challenges, expectations, and accomplishments of patients were investigated by using three focus group interviews in October 2018. **Results.** Among the participants, six patients were male, and one patient was female. The mean age of the patients was  $56.85 \pm 16.48$ . Three main themes were revealed in the study. These were (1) Reborn; decrease in dependence, sense of accomplishment, enjoyment of life, (2) Prejudice; perceived as severely ill by others and (3) Fear; not being accustomed to the device, loss of device function. **Conclusion.** The results obtained from this study can be used in the process of adaptation to this process by discussing and evaluating the challenges, expectations and accomplishments of the Parkinson's patient in DBS with healthcare professionals and other patients.

**Key Words:** Deep Brain Stimulation ■ Parkinson's Disease ■ Patient Expectation ■ Challenge ■ Accomplishments.

### Introduction

The main and early symptoms of Parkinson's disease (PD) include bradykinesia/akinesia, rigidity, and rest tremor, while later symptoms are postural instability and walking and balance disorders, and there is a progressive movement disorder (1, 2). In addition to the general symptoms, there may be symptoms such as cognitive and behavioural disorders, communication problems, urinary problems, sexual dysfunction, falls, dependence in daily life activities, walking and balance problems, weight loss, excessive sweating, sleep disorders and impulse control disorders particularly pathological gambling (2-5).

Deep brain stimulation (DBS) was first performed in 1987 by Benabid et al. (6). It is a safe and effective

alternative to treatment of some movement disorders (7) such as Parkinson's disease (8, 9), tremor (10-12), dystonia (13) and some neurological and psychiatric diseases (14) such as epilepsy (15) and obsessive-compulsive disorder (16, 17). It is well-known that PD is caused by loss of nigrostriatal dopaminergic neurons due to disruption of the dopamine and acetylcholine balance in the basal ganglia (18, 19).

The procedure provides electrical stimulation by burning the brain regions such as the subthalamic nucleus, the globus pallidus internus and the ventralis intermediate nucleus of the thalamus, using high frequency sound waves. It reduces the activity of abnormally increasing cells, or by increasing dopamine activity through deep electrodes placed in this region, for preventing

clinical signs and symptoms (9, 14, 18, 20). It does not cure the disease, but alleviates symptoms such as tremor, rigidity and bradykinesia, improves the quality of life of the patient, reduces or eliminates the amount of medication used, and provides independence in daily life activities (5, 14, 21).

There are different opinions on DBS in the literature (22-25). Cabrera et al. (22) stated that psychosocial factors, genetics, safety and efficacy of different targets play a role in the patient's response to DBS. Kim et al. (23) reported that DBS is important in patients with high response to levodopa treatment in vascular Parkinsonism. Additionally, when they compared elderly and young patients who underwent DBS, they found more surgical complications in the elderly group, yet psychosis was seen more in the younger group, but this difference was not very significant. Therefore, the procedure was as applicable in the elderly as in younger individuals.

Mueller et al. (25) studied changes in brain connectivity while comparing the effects of DBS to those of levodopa treatment in PD, and found that the treatment effect of DBS was associated with high connectivity inside the cerebello-thalamo-cortical network. They also found increased interconnectedness in the right and left motor cortices in patients treated by DBS. Little et al. (26), in their study on the effects of bilaterally adaptive DBS in Parkinson's patients, found a reduction in bradykinesia and axial symptoms. Mirzadeh et al. (27) determined that the mean off-medication motor scores and quality of life improved, and the levodopa equivalent daily dose was reduced in 6th months after DBS. They stated that there were no significant adverse events.

In the literature, it may be seen that studies have usually focused on clinical symptoms and procedures (18, 20, 22-26). However, since each patient's emotions are different, their direct statements as first persons and explanations of their experiences can guide other patients and healthcare professionals. In addition, the number of patients is low in the hospital where the study is being conducted because it is not a common procedure for Parkinson's patients. So, in this

study the views of Parkinson patients who had undergone DBS for ten years in the hospital were examined qualitatively.

The aim of this study was to determine the challenges, expectations, and accomplishments of patients with DBS in PD.

## **Methods**

### ***Design and Participants***

This qualitative study was conducted with three focus group interviews including seven patients with Parkinson's disease treated with DBS. There were rest tremors in 7 patients, bradykinesia/akinesia, urinary problems, sexual dysfunction, falls, dependence in daily life activities, and walking and balance problems in 5 patients, progressive movement disorder and weight loss in 2 patients, sleep disorders in 4 patients, and rigidity, cognitive and behavioural disorders and communication problems in 3 patients.

### ***Data Collection***

The data were collected in October 2018 through three focus group interviews using a semi-structured interview to determine the experiences of the patients. This study received the necessary ethics approval from the Ethics Board of Zeynep Kamil Education and Research Hospital in Istanbul, Turkey (01.08.18/123). A total of ten patients who underwent DBS between 2008 and 2018 at the Neurosurgery Department of a research hospital in Turkey were reached by the researchers, and given information on the purpose of the study. The study was performed in accordance with the Declaration of Helsinki. Verbal consent was obtained from the patients for the focus group interviews.

A quiet and comfortable room was arranged at the hospital for the interview. Seven patients volunteered for the study, and three patients did not. Before the interview, tags were prepared for the participants, the moderator and the rapporteur. The moderator and the rapporteur came to the meeting area before the focus group interview

and completed the preparations (table, chairs, papers, pencils, seating arrangement and food). The participants were also met by the moderator and the rapporteur. One recording device was prepared. Prior to the start of the interview, the moderator recorded the date, place, hour, who would manage the interview and the name of the rapporteur on the recording device. Thus, the device was checked. To prevent interruptions, a note was written on the outside of the door of the meeting room. While waiting for all the patients to arrive, nothing was discussed with the incoming participants. When the arrival of the patients was complete, the moderator stated that the interview would be recorded using a voice recorder. It was stated that these records would be examined by the researchers in the study, they would be used for scientific purposes only, and the names of the participants would not be included. The participants were asked to switch their mobile phones off before the interviews started. The patients were given a brief description of the purpose of the study and were provided information on the focus group interviews.

In the interviews, semi-structured questions were asked to clarify the experiences of the patients:

- What do you think about DBS? Would you recommend it to others?
- Did you experience any difficulties during and after DBS? What would you say?
- What do you think about the changes in your life after DBS?

Thus, the perspectives, experiences, thoughts, perceptions, feelings, attitudes and habits of the participants, and their psychological and social situations were evaluated in the three focus group interviews. The interviews lasted for 120 minutes.

### Data Analysis

In this study, the content analysis method was used to analyse the data obtained from the interviews. In the content analysis, the data were coded first. The researchers analysed the data they obtained and divided them into meaningful categories. So, the conceptual expressions in these sections were

examined. Then, each researcher created themes by coding the data. Finally, the researchers discussed the codes and themes together and agreed on the common themes.

### Results

Among the participants, six patients were male, and one patient was female. The mean age of the patients was  $56.85 \pm 16.48$  (30-78), and they had been diagnosed with Parkinson's disease  $11.85 \pm 4.48$  (7-20) years ago. DBS was performed  $6.14 \pm 2.34$  (3-9) years ago. The following themes emerged as a result of the interviews with the patients with Parkinson's disease: *Reborn: Decrease in dependence; Sense of accomplishment; Enjoyment of life; Prejudice: Perceived as severely ill by others; Fear: Not being accustomed to the device; Loss of device function.*

#### Reborn

In the study, the participants stated that DBS had given them a new life and identity, decreased their dependence on others, they had experienced a sense of recovery from the disease, and their enjoyment of life had increased.

**Decrease in Dependence.** The participants stated that DBS had significantly reduced their dependence on others, and they were able to continue their lives without need of others.

*"...I was born again by this process. It is like I am in another body. My hands were shaky before. I had difficulties in walking..."*. Male, age: 42.

*"...Now I ask for help from my loved ones less..."*. Male, age: 30.

*"...It is amazing how self-sufficient you are. First of all, thank God and then you (Doctor, nurse, etc.). I think the most important issue is its positive contribution to independence. Thank God..."*. Female, age: 64.

*"...The amount of medication I take decreased. It makes me happy, too..."*. Male, age: 54.

**Sense of Accomplishment.** In this study, the participants thought that their disease would always get worse before DBS, and they were

helpless regarding the disease. However, they stated that they now believed they would recover from PD, and they had the pleasure of being successful. Some expressions of the participants are included below:

“...I had been thinking that my life was going to get worse. But then I saw that this disease had a treatment. I am not scared anymore. Even if there are occasional problems, I can deal with these problems. I am confident...”. Male, age: 60.

“...Both in this disease and other diseases, I do not feel helpless. They (other people) should read about it. They should also trust their doctors and nurses...”. Male, age: 70.

“...A human being is such an entity that when you think you cannot get rid of this disease, the symptoms increase. You are worse. But when you see the survivors of this disease through this surgery, you can say “I can do it” with good reason...”. Female, age: 64.

**Enjoyment of Life.** In this study, two participants stated that life before DBS was painful, and feelings of pessimism were dominant.

“...Parkinson's is a very bad disease. It always progresses. What is going on? You are worried that dependence will increase. This surgery gave me my smile back. I wish I had never had this disease. ...but I still feel good when I focus on my progress...”. Male, age: 78.

“...I had a really bad time. Of course, in such a serious disease, when I was not able to carry a glass of water, it was very bad when others finished what they were doing in a few seconds. Now I can carry my cup more easily. I can drink water by myself. How nice. When I drink my water easily from the cup, there is no happier person than me...”. Male, age: 60.

## Prejudice

The participants stated that other people had prejudices about the surgery.

**Perceived as Severely Ill by Others.** In the study, three patients with PD stated that when the tool inserted into their brains, was felt by others, they perceived them as more severe patients than other people. Therefore, they reported that it

would be better if this surgery was not visible from the outside. Some of what the participants said is given below:

“...Sometimes it seems to me that when people see me, they think I am sicker. It seems like it would be better if I did not let them know that I have had brain surgery...”. Female, age: 64.

“...I wish it was not noticeable when somebody looks at me. It would be better...”. Male, age: 54.

“...When there is something in my head, even some of my relatives look at it strangely. ...but I also want them to care about me being well. It is more important for me to be well. Sometimes I think I can read their thoughts. Generally, when people have surgery, they reflect on the surgery as if they were more severe patients...”. Male, age: 60.

## Fear

In the study, some Parkinson's patients stated that they had some fears. They stated that they were afraid of losing their good and nice side with DBS. Additionally, two participants stated that they were concerned that the battery would not function properly after the surgery.

**Not Being Accustomed to the Device.** In this study, the participants stated that they were afraid of perceiving the device as a foreign body and not getting used to the device.

“...Actually, my doctor told me about the process. ...but, you are carrying a device that does not belong to your body. I wondered if I would have problems...”. Male, age: 42.

“...I tried to think I was going to be okay. So, I felt a bit of strangeness. Then I got used to it...”. Male, age: 60.

“...It is not just about this device. It is a foreign body inside you...”. Female, age: 64.

**Loss of Device Function.** Four participants stated that they feared that the device would not work, and they were troubled by problems. The patients expressed fears about the potential failure of the battery. Some statements by the participants are included below:

“...My doctor explained it to me very well. I can contact him immediately if there are any

*problems. However, in some moments, I am afraid of the battery stopping suddenly, so, I would have problems again, which would mean I cannot even drink water, I worry...*". Male, age: 60.

*"...I had this fear more at first. There were always questions. I suppose I was scared of going back to my old self. Thank goodness, I rarely think about it now..."*". Male, age: 30.

## Discussion

The results of our study focused on revealing the patients' challenges, expectations, and accomplishments rather than the effect of DBS on PD. In this study, the patients' feelings and opinions associated with their lives after DBS were included as their first-person experiences. As a result of this study, although the patients stated that they felt reborn, they said they had experienced prejudice and fear. We can say that DBS brings different challenges to patients, in addition to good outcomes. Gorman and Sultan (28) reported that evaluation is important not only of physical outcomes but also patients' experiences, difficulties and expectations.

According to the results of our study, the patients were satisfied with the DBS procedure, and they stated that they felt born again. As a result of this study, after DBS in Parkinson's patients, there were improvements in walking, they asked for help from others less, and they were more independent. Diseases such as PD, which cause physical movement disorders and psychological negativity, necessitate long-term treatment and the use of drugs in individuals. Additionally, the patient's dependence on others increases over the years despite treatment. This causes psychological problems, such as tiredness, exhaustion, depression and anxiety in patients and their relatives. These negative effects on the patient and their caregivers cause a decrease in the patient's progress in treatment, and lower their expectations and motivation in their struggle against difficulties (29, 30). Healthcare includes a holistic assessment of the patient and anticipating the problems that may or may not be present in the

patient. Therefore, evaluation of the physical and psychological status of Parkinson's patients and their dependency on others after DBS may improve the patient's adaptation capacity and develop their ability to cope with difficulties. Faivre et al. (29), in their animal model, stated that psychological disorders, such as anxiety and depression are seen in Parkinson's patients. Suzuki et al. (30) reported that the psychological problems of individuals with chronic diseases increased.

In the study, some participants stated that DBS stimulated their sense of accomplishment and their belief that they could cope with problems. The patients treated with DBS indicated that they felt happier because of its positive contribution to their well-being. Avanzino et al. (31) reported that the emotions of Parkinson's patients are important, and a lack of depression and anxiety in patients may increase walking speed, arm-swing, flexion of posture and step length, and provide initiation reaction time on patient.

Another result of this study is that patients stated that before DBS, life was painful, and they felt pessimistic. However, they stated that they were now very happy when they were able to perform their daily life activities independently and were less reliant on others. These results are supported by the literature. Lin et al. (32), in their study on 6-year follow up of patients' expectations and satisfaction with DBS for Parkinson's disease, determined that the quality of life of DBS patients increased due to the increase in mobility and daily activities, and additionally, all patients in the study felt they had made the right decision about DBS, and they would recommend DBS to others. The researchers also stated that the satisfaction levels of patients treated with DBS were always found to be high. Buhmann et al. (33) reported that patients treated with DBS were more successful in driving stimulation than those treated without DBS, and their cognitive abilities were better after DBS. Unlike the results of this study, Maier et al. (34) reported no improvement in the quality of life of patients after DBS.

In this study, some patients stated that DBS was perceived as serious surgery by others because it

was a surgical procedure on the brain. They thought it created prejudice against them. In particular, interventions made in the brain and central nervous system are more important because they are complex and directly affect the individual's vital functions. These procedures are important for the survival of patients. Therefore, surgical interventions on the brain are classified by the community as severe. Negative attitudes and prejudice against Parkinson's patients cause psychiatric problems. Therefore, these patients should benefit more from social support systems (35). Contrary to the belief expressed in this study that patients are perceived as severely ill by other individuals because of the surgical procedure, Vergani et al. (24), in their study evaluating 141 Parkinson's patients, stated that DBS was a safe procedure, had low mortality rates, and surgical complications were rare.

In the study, some Parkinson's patients stated that they were afraid that they would not get used to the DBS device and worried about the potential of the battery losing its function. Although they stated that they had received information and support from DBS-related healthcare professionals, they mentioned that these fears arose especially in the early stages. Although the applicability of the programmable pulse generator which was implanted in DBS was approved (22), patients' fear of the device and the battery losing its function may be associated with individual factors and healthcare. Unlike our study, Kim et al. (36) reported that patients with PD who underwent DBS had fears of complications and the economic burden. In order to understand the causes of fear, Gorman and Sultan recommended that the patients' personal reactions, maximum powers, factors affecting objectivity and psychological status should be examined (28).

### **Limitations of the Study**

The limitation of this study was that the study was conducted in a single centre with seven patients. The results are therefore valid only for the population of the study. In order to generalise the results, different centres and groups should be included.

### **Conclusion**

According to the results of this study, DBS contributes positively to the daily living activities of patients with PD, and increases their sense of reattachment and hope. However, as in any surgical procedure, patients experience fear. Therefore, in future studies, it may be recommended to establish protocols for adequate counselling for patients before the DBS surgery. The results of this study may be used for this kind of protocol. There was no specific questionnaire available to assess the quality of life of people with PD who underwent DBS. This focus group interview results could be used to structure a new questionnaire to assess the quality of life of people with PD who have undergone DBS. Also, support groups for patients with PD may help them to cope mentally with the burdens of the disease.

#### **What Is Already Known on this Topic:**

*Parkinson's disease (PD) is a common chronic neurodegenerative disease. In PD, motor symptoms are well known, and treatments (pharmacological / physiotherapy / surgical) are mostly focused on this area. As a surgical treatment, deep brain stimulation (DBS) is a safe and effective alternative to treatment of some movement disorders such as PD. DBS does not cure the disease, but alleviates the symptoms, improves the quality of life of the patient, reduces or terminates the amount of drugs used, and provides independence in daily living activities. In the literature, studies have usually focused on clinical symptoms and procedures.*

#### **What this Study Adds:**

*The success of treatment with DBS cannot be seen only in the initial recovery after surgery. Most patients require bio-psycho-social adaptation to their new experiences in life with implanted electronic devices. They should also be informed about their fears, and the possibilities and limitations of the treatment. As each patient's emotions are different, healthcare professionals must provide support to patients and caregivers in the pre- and post-operative period, by sharing their knowledge and experience, addressing the challenges, expectations, and accomplishments of the patients in a holistic manner, as stated in the results of this study.*

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## Is There a Role for Color Doppler Ultrasonography in Acute Cholecystitis?

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### Abstract

**Objective.** The aim of this study was to evaluate the utility of the Pulsatility Index (PI) of the right hepatic artery, measured by color Doppler sonography, in the diagnosis of acute cholecystitis. **Methods.** Seventy-five subjects were included in this study and divided into three groups, each consisting of 25 subjects: the cholecystitis group, the asymptomatic cholelithiasis group, and normal controls. Patients with acute cholecystitis fulfilled all the diagnostic criteria as stated in the latest Tokyo Guidelines. In all patients, the right hepatic artery was detected by color Doppler ultrasound and the PI was measured. **Results.** Patients with acute cholecystitis were found to have significantly higher PI values compared to both normal controls and cholelithiasis patients. Regression analysis revealed a significant positive correlation between the PI and the cholecystitis outcome. **Conclusion.** Measurement of PI by color Doppler ultrasound represents a useful aid in the diagnostic process of acute cholecystitis. More studies are needed before this method is incorporated in the relevant guidelines.

**Key Words:** Cholecystitis ■ Pulsatility Index ■ Color Doppler Sonography ■ Right Hepatic Artery.

### Introduction

The diagnosis of acute cholecystitis relies upon a combination of clinical, laboratory and imaging findings (1). Ultrasonography (US) represents the first line imaging tool for diagnosing acute cholecystitis. Sonographic findings suggestive of acute cholecystitis are wall thickening, pericholecystic fluid and gallbladder distention. Moreover, the presence of a sonographic Murphy's sign is highly indicative of acute cholecystitis. The accuracy of US in the diagnosis of acute cholecystitis has been extensively studied; sensitivity and specificity rates of 88% and 80% have been reported, respectively (2).

Nevertheless, a subset of patients present diagnostic difficulties, rendering the diagnosis of acute cholecystitis quite challenging (3). In approximately 10% of patients US fails to provide a definite imaging diagnosis of acute cholecystitis (4). The sonographic finding of gallbladder wall thickening is

common in both acute and chronic cholecystitis. In the former condition wall thickening represents an inflammatory edema of the wall on the grounds of hyperemia, while in the latter it is caused by the formation of fibrous scar tissue in the chronic setting. Furthermore, several chronic conditions, such as heart failure and cirrhosis, have been correlated with gallbladder wall thickening (5, 6). Last but not least, detection of a sonographic Murphy's sign, which is a key diagnostic finding, may be biased by analgesics administered to the patient (7).

Advances in sonographic imaging have provided the potential of overcoming these limitations of US. Color Doppler US and, more recently, power Doppler US have been used to measure several different parameters regarding blood flow in the affected area (7). The working hypothesis is that acute inflammation is accompanied by hyperemia in the affected organ. These parameters have been

evaluated in the gallbladder wall flow, hepatic artery flow, and right hepatic artery flow or, less commonly, cystic artery flow. Nevertheless, their use in the diagnosis of acute cholecystitis has not yet been widely adopted because relevant data are still scarce as well as conflicting.

The aim of this study was to evaluate the role of the right hepatic artery Pulsatility Index (PI), measured by color Doppler US, in the diagnosis of acute cholecystitis. The PI is a calculated flow parameter on ultrasound, derived from the maximum, minimum and mean Doppler frequency shifts during a defined cardiac cycle (8).

## Patients and Methods

This study was performed in line with the principles of the Declaration of Helsinki of 1975 and its amendments from 1983. Permission for the study was obtained from the Institutional Ethics Committee (approval number: 75/5-6-2019, General Hospital of Amfissa). Informed consent was obtained from all individual participants included in the study. Seventy-five subjects that underwent color Doppler US of the right hepatic artery over a 1-year period were included in this study. All participants were examined after a fast of at least 6 hours. In emergency cases, where the gallbladder was found to be contracted on US examination, the patient was admitted and re-examined at least 6 hours later. Data were collected prospectively. Subjects were assigned to one of three groups, each consisting of 25 patients: (1) the control group consisting of asymptomatic volunteers with normal gallbladders; (2) the cholelithiasis group including asymptomatic patients with gallbladder cholelithiasis without any other abnormal US find-

ings; (3) the acute cholecystitis group consisting of patients with a definite diagnosis of acute calculous cholecystitis, according to the latest Tokyo Guidelines (1) (Table 1).

Patients with equivocal findings were excluded from the analysis, in an attempt to counterbalance the non-availability of pathological confirmation of acute cholecystitis. Exclusion criteria also included the following comorbidities: choledocholithiasis (due to the fact that concurrent cholangitis may affect hepatic artery velocity, thus acting as a confounding factor); pancreatitis, congestive heart failure, liver cirrhosis, renal failure, hypoalbuminemia, and hepatitis (due to the fact that these conditions are known to cause gallbladder wall thickening thus being potential confounders) (5, 6, 9); arteriosclerosis, decreased intravascular volume due to severe dehydration, and hyperdynamic circulatory state due to high fever (because PI measurement is greatly affected by the condition of the arteries, the circulating blood volume and a hyperdynamic circulation state).

All patients were examined by a single consultant radiologist using a 2.5-5.5 MHz curved array or vector transducers, and a Voluson™ S8 BT16 (GE Healthcare, Waukesha, WI) US machine. Gray-scale upper abdominal US was followed by color Doppler US of the right hepatic artery. An attempt was made to evaluate blood flow in the cystic artery, but it was only feasible in a minority of cases. The Pulsatility Index (PI) was measured in all patients. Typical PI measurements in patients with uncomplicated cholelithiasis and acute cholecystitis are shown in Figures 1 and 2 respectively. The radiologist was intentionally not informed about the laboratory results of the patients.

Table 1. TG18/TG13 Diagnostic Criteria for Acute Cholecystitis

A. Local signs of inflammation: (1) Murphy's sign, (2) RUQ mass/pain/tenderness
B. Systemic signs of inflammation: (1) fever, (2) elevated CRP, (3) elevated WBC count
C. Imaging findings: Characteristic of acute cholecystitis
Suspected diagnosis: One item in A + one item in B
Definite diagnosis: One item in A + one item in B + C

CRP=C-reactive protein; RUQ=Right upper abdominal quadrant; WBC=White blood cell count.

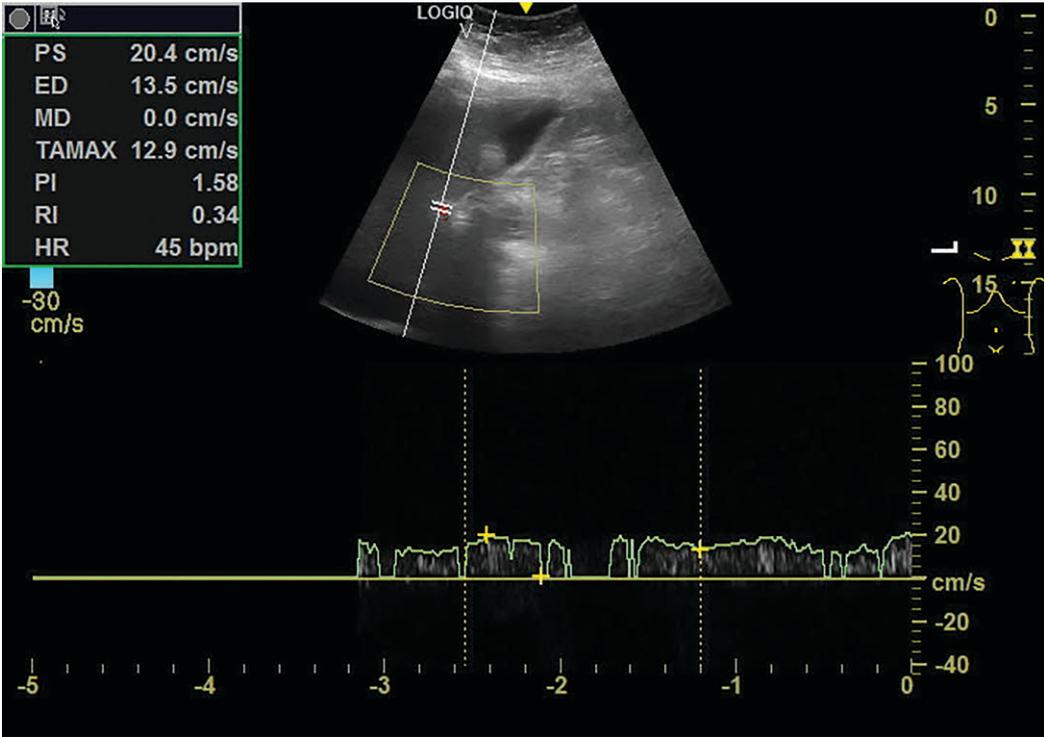


Figure 1. Typical Pulsatility Index in a Patient with Uncomplicated Cholelithiasis.

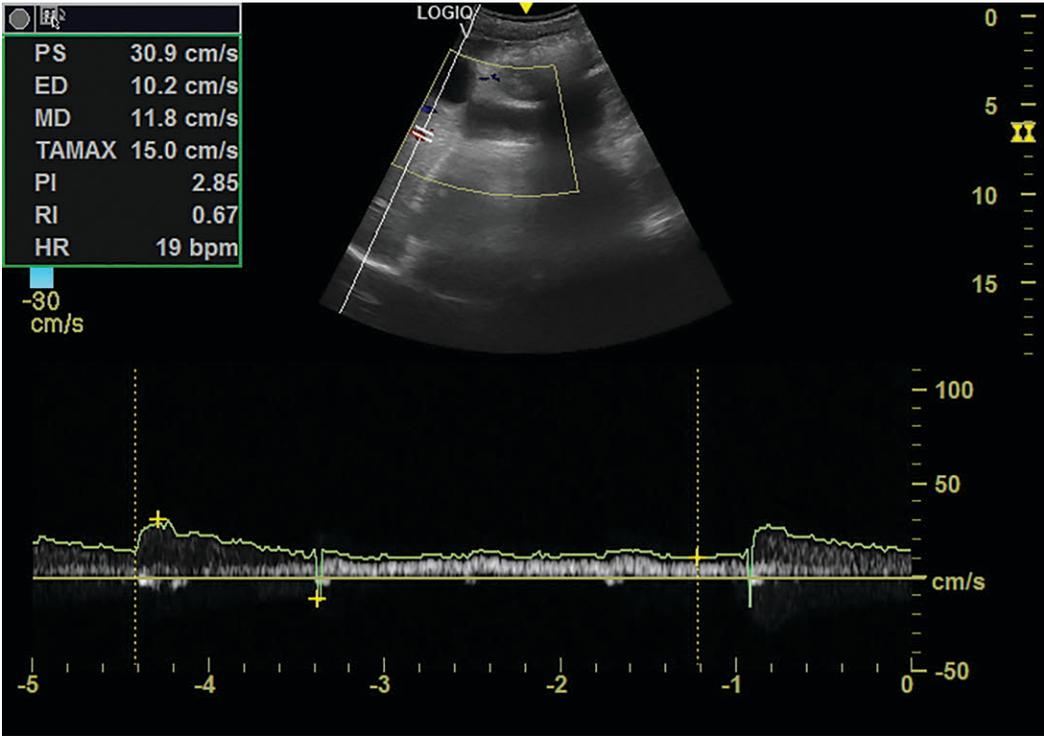


Figure 2. Typical Pulsatility Index in a Patient with Acute Cholecystitis.

### Statistical Analysis

Due to the lack of normality, non-parametric tests were performed. A multinomial logistic regression analysis was executed, comparing a nominal dependent variable and a continuous independent variable. All analyses were carried out using the SPSS statistical package, version 25 (Statistical Package for the Social Sciences, SPSS Inc., Chicago Ill, USA). All tests were two-sided, and statistical significance was set at  $P < 0.05$ .

### Results

The results of the descriptive statistics analysis are presented in Table 2.

Seventy-five subjects were enrolled in this study, with a median age of 69 years (range: 33-97 years). Twenty-five subjects were assigned to each group, as described before. All three groups were similar in terms of age and sex. Patient data are presented in Table 2. Patients in the third group fulfilled all the diagnostic criteria for acute cholecystitis. Specifically, all patients had acute right upper quadrant pain, with a positive Murphy's sign and fever ( $\geq 37.8^\circ\text{C}$ ); leukocyte count  $> 10,000/\mu\text{L}$ , C-reactive protein level  $> 0.5 \text{ mg/dl}$ ; sonographic

findings suggestive of cholecystitis (cholelithiasis, thickened gallbladder wall, pericholecystic fluid and gallbladder distention). The right hepatic artery was identified in all subjects.

The median Pulsatility Index was 1.8 in both the control and the cholelithiasis group, and 2.8 in the cholecystitis group. Due to the lack of normality, the Kruskal-Wallis test revealed that the PI was statistically significant different between groups [ $\chi^2(\text{df}2)=45.416 \text{ } P < 0.001$ ]. Post-hoc analysis using the Dunn-Bonferroni test on each pair of groups showed that there was strong evidence ( $P < 0.001$ , adjusted using the Bonferroni correction) of higher PI in the cholecystitis group (Mean Rank 61.68) compared to the cholelithiasis group (Mean Rank 27.46) and the control group (Mean Rank 24.86). Spearman's correlation coefficient revealed a strong correlation between PI and the group outcome ( $\rho=0.702, P < 0.001$ ).

We performed multinomial logistic regression analysis in order to determine the correlation between the independent variable PI (which was multiplied by a factor of 10) and the dependent variable outcome (which was coded as control group=0 – reference group, cholelithiasis group=1 and cholecystitis group=2). There were no missing values and all the values were included in the analysis, including the outliers. The regression coefficients are shown in Table 3. The overall model evaluation showed an improvement over the intercept-only model (Likelihood ratio test chi-squared ( $\text{df}2$ )=79.428,  $P < 0.001$ ). Goodness-of-fit test yielded a chi-squared ( $\text{df}20$ )=3.847 and was insignificant ( $P=1.0$ ), suggesting that the model fit the data well. According to the model, the log of the odds of a patient being diagnosed with cholecystitis was positively related to PI ( $P=0.006$ ; Table 3) compared to the control group. On the other hand, there was no statistically significant correlation between PI and the outcome of cholelithiasis compared to the control group ( $P=0.431$ , Table 3). Finally, as shown in the classification table (Table 4), the model correctly predicted 92% of the cholecystitis cases and 64% of all cases.

Table 2. Patients' Demographics and Data

Characteristics	Group		
	Control (n=25)	Cholelithiasis (n=25)	Cholecystitis (n=25)
Age (years)			
Mean	57.16	61.12	79.52
Median	59.00	62.00	79.00
Range	33-87	33-87	48-97
Gender (n)			
Male	9	7	15
Female	16	18	10
Pulsatility Index			
Mean ( $\pm$ SD)	1.79 ( $\pm 0.15$ )	1.82 ( $\pm 0.12$ )	2.70 ( $\pm 0.27$ )
Median	1.80	1.80	2.80
Range	1.50 - 2.00	1.50 - 2.00	1.80 - 3.00

Table 3. Regression Analysis Parameters Estimate with Parameter Coefficients

Group*		B	Standard error of B	Wald	Df	Sig.	Exp(B)	95% Confidence Interval for Exp(B)	
								Lower Bound	Upper Bound
Cholelithiasis	Intercept	-2.907	3.703	0.616	1	0.432	-	-	-
	PI	0.160	0.204	0.620	1	0.431	1.174	0.788	1.750
Cholecystitis	Intercept	-24.210	8.228	8.658	1	0.003	-	-	-
	PI	1.156	0.419	7.617	1	0.006	3.178	1.398	7.225

\*The reference category is: control; PI=Pulsatility Index; B=Parameter B (unstandardized logistic regression weight); Wald=test statistics  $\chi^2$ ; Df=Degrees of freedom; Sig=Significance;-Exp(B)=Exponentiation of B (odds ratio).

Table 4. Regression Analysis Classification Table

Observed	Predicted			Percent of correct
	Control (n)	Cholelithiasis (n)	Cholecystitis (n)	
Control	15	10	0	60.0
Cholelithiasis	15	10	0	40.0
Cholecystitis	1	1	23	92.0
Overall (%)	41.3	28.0	30.7	64.0

## Discussion

Data from the present study indicate a strong correlation between right hepatic artery Color Doppler findings (PI) and acute inflammation. Significant differences in right hepatic artery PI were found between patients with acute cholecystitis and both patients with asymptomatic cholelithiasis and normal subjects. Notably, PI measurements were similar between patients with asymptomatic cholelithiasis and normal subjects. Provided that a correlation between right hepatic artery and acute cholecystitis was established by the present study, further research should aim to assess this test in diagnostically challenging cases with equivocal findings. Such difficulties usually exist in patients with a thickened gallbladder wall and comorbidities that are known to cause gallbladder wall thickening themselves, such as heart failure and cirrhosis.

PI measurement in the right hepatic artery, instead of gallbladder wall flow, is theoretically advantageous because there are cases in which severe inflammation and the resulting ischemia diminish blood flow in the gallbladder wall. In such cases color Doppler sonography may give false negative results.

The idea of correlating inflammation with hyperemia of the affected organ, detected by Doppler sonography, is not a new one (10, 11). Several reports have tried to provide a link between increased blood flow in the gallbladder and acute cholecystitis. Nevertheless, this technique has yet not gained wide acceptance, and experts are hesitant to suggest its application (12). Parameters utilized to quantify hyperemia include PI, peak velocity and resistance index. These parameters are measured either in gallbladder wall flow or in supplying arteries, namely the hepatic artery, right hepatic artery and cystic artery. The majority of studies evaluate these parameters in the gallbladder wall flow for practical purposes. Sonographic visibility of the hepatic artery was not feasible using older generation US machines. Consequently, Doppler examination of the hepatic artery for diagnosis of acute cholecystitis was first reported by Loehfelm in 2017 (7). Visualization of cystic artery flow, however, still remains rather challenging, even with modern technology. Moreover, given that PI measurement is greatly affected by the condition of the arteries, the circulating blood volume and hyperdynamic circulation state, patients with

arteriosclerosis, severe dehydration and high fever were excluded from the study.

Regarding acute cholecystitis, the discouraging preliminary results reported by McGrath et al. in 1992, showed that cystic artery flow is diminished in acute cholecystitis below the level of detection (13). Similarly, Paulson et al. suggested in 1994 that color Doppler is of little value in diagnosing acute cholecystitis (14). Jeffrey et al. focused on the length of the cystic artery visualized and its pattern of distribution, as potential diagnostic criteria for acute cholecystitis. According to these authors, despite their high specificity, these findings have quite low sensitivity, thus limiting their role in patients with cholecystitis (15). The superior flow sensitivity of Power Doppler sonography did not meet the expectation of improving diagnostic accuracy in acute cholecystitis, due to its substantial false positive rate (4).

Hayakawa et al. studied gallbladder wall blood flow in several gallbladder diseases, and concluded that maximum velocity above 30 cm/sec is indicative of cancer, but not acute cholecystitis (16). Tochio et al. found a significant correlation between a maximum flow velocity in a thickened gallbladder wall of more than 40 cm/sec, or a resistive index of less than 0.75 with acute cholecystitis in patients with cirrhosis (6). Yamashita et al. suggested that gallbladder wall flow positively correlates with the degree of inflammation in the acute setting, proposing this method for monitoring treatment success (17). Cetinkunar et al. evaluated gallbladder wall vascularity using Power Doppler, and came to the conclusion that increased vascularity correlates with the presence of adhesions, but not with operation difficulty (5). More recently Loehfelm et al. proposed that a peak systolic hepatic artery velocity of more than 100 cm/sec represents a significant aid in diagnosing acute cholecystitis (7).

A possible limitation of our study was the high percentage of subjects in whom visualization of the cystic artery was not feasible, thus excluding this data from the analysis. Establishing a diagnostic link between acute cholecystitis and cystic artery flow would have otherwise been ideal. On-

going improvement in US modalities will probably render sonographic evaluation of cystic artery flow easier in the future. Another limitation of the study was the fact that US diagnosis of acute cholecystitis was not confirmed by intraoperative findings and surgical specimen histopathological examination.

## Conclusion

Data from the present study indicate a strong correlation between right hepatic artery color Doppler findings and acute inflammation. Information provided by color Doppler imaging is of paramount importance in patients with a thickened gallbladder wall and comorbidities that are known to cause gallbladder wall thickening themselves. In conclusion, it is the authors' opinion that large scale studies are needed to confirm these findings and establish the role of color Doppler US in the diagnosis of acute cholecystitis in the context of ambiguous clinical scenarios. Future research should also aim to answer the question whether color Doppler findings may predict the severity of acute cholecystitis and indicate the appropriate treatment.

### What Is Already Known on this Topic:

*Although gray-scale US is the gold standard imaging modality for the diagnosis of acute cholecystitis, the presence of a thickened gallbladder wall due to other causes poses significant diagnostic problems. Several studies have indicated color Doppler sonography as a potential diagnostic aid in acute gallbladder inflammation, the majority of which focus on gallbladder wall blood flow.*

### What this Study Adds:

*The results of the present study indicate a clear correlation between right hepatic artery PI elevation and acute cholecystitis. Consequently, measurement of PI by color Doppler ultrasound represents a useful aid in the diagnostic process of acute cholecystitis.*

**Authors' Contributions:** Conception and design: AK and VP; Acquisition, analysis and interpretation of data: VP, LK and VK; Drafting the article: IP, MN and MS; Revising it critically for important intellectual content: AK and IP; Approved final version of the manuscript: AK, IP and VP.

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

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## The Prevalence of Hypodontia and Hyperdontia in Orthodontic Patients

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### Abstract

**Objective.** The aim of this study was to assess the prevalence of hypodontia and hyperdontia among a group of orthodontic patients. **Materials and Methods.** This cross sectional study was conducted using radiographs of 4256 patients (2032 males and 2224 females) who attended the Department of Orthodontics, School of Dental Medicine, University of Sarajevo. Radiographs were examined for the prevalence of hypodontia and hyperdontia in permanent dentition. Hypodontia was recorded when a tooth was absent on the panoramic radiograph and hyperdontia was recorded as an increased number of teeth above that described by normal dental formula. All data were analyzed using descriptive statistics including frequency and percentage, and differences between groups were tested using the  $\chi^2$  test, or Fisher exact test. **Results.** In the sample of orthodontic patients, 4.08% subjects had a least one dental anomaly, hypodontia or hyperdontia. The observed prevalence of hypodontia was in 3.42% subjects, and it was more prevalent in females than in male subjects. The observed prevalence of hyperdontia was in 0.65% subjects and hyperdontia was more common in males than female subjects. **Conclusions** – By early diagnosis of a reduced or increased number of teeth, various modes of therapy with a multidisciplinary approach may be performed to correct the aesthetic and functional problems caused by hypodontia or hyperdontia.

**Key Words:** Prevalence ▪ Hypodontia ▪ Hyperdontia ▪ Orthodontic.

### Introduction

Dental anomalies are often encountered in orthodontic patients. They occur either in isolation or as a part of certain syndromes. Anomalies include irregularities in the number, size, shape, structure and position of the teeth. Factors leading to dental developmental disorders can be either congenital, such as inheritance, metabolic and gene mutations; or environmental factors that include physical, chemical and biological factors. In addition, some of these anomalies are caused by a combination of genetic and environmental factors (1). Disorders occurring in the initial stage of tooth development are manifested in a reduced or increased number of teeth (2).

There are several forms of reduced numbers of teeth: anodontia representing congenital absence of all teeth, oligodontia is the congenital absence

of six or more teeth and hypodontia represents the congenital absence of less than six teeth. The prevalence of hypodontia has been investigated in different communities and ethnic groups, and the results are presented in several studies (3-8). The reported hypodontia rates range from 3.48% in a Spanish population, to 9.4% in a Japanese population. The wide range of the prevalence of hypodontia is a result of racial differences, sample size and different diagnostic criteria.

The most commonly affected tooth agenesis is the second mandibular premolar, followed by the maxillary lateral incisor and the second maxillary premolar; while unilateral occurrence of tooth agenesis is more common than bilateral (2). Gender differences in the prevalence of hypodontia have also been observed, where females were more affected compared to males (9).

Hyperdontia, or supernumerary teeth, is defined as an increased number of teeth, meaning more than 20 deciduous teeth and more than 32 teeth in permanent dentition. Hyperdontia is more common in permanent dentition, and the prevalence of hyperdontia is between 0.3% and 3.8% in permanent dentition, while their occurrence in deciduous dentition ranges from 0.3% to 0.8% (4, 10-12). The etiology of hyperdontia still remains unclear. There are several theories that explain its occurrence, but heredity affects the appearance of hyperdontia (13).

The occurrence of supernumerary teeth may be single or multiple, unilateral or bilateral, or they may be impacted or erupted. Males are more often affected than females - approximately twice as often (14). Supernumerary teeth can be classified according to morphology as conical, tuberculate, supplemental and odontoma, and their localization as mesiodens, paramolar and distomolar (13). Several syndromes and disorders are associated with the occurrence of single or multiple supernumerary teeth, such as Gardner's syndrome, Cleidocranial dysplasia, and cleft lip and/or palate, and the occurrence of multiple supernumerary teeth without a syndrome is a rare phenomenon (13, 15).

The aim of this study was to assess the prevalence of hypodontia and hyperdontia among a group of orthodontic patients.

## Materials and Methods

The study was undertaken using the panoramic radiographs of 4256 orthodontic patients (2032 males and 2224 females), from the files of the Department of Orthodontics, School of Dental Medicine, University of Sarajevo, from January 2015 to December 2017. The study included all patients who needed orthodontic treatment, and whose diagnostic protocol included radiographs, which were analyzed for the purposes of this study. Patients aged from nine to sixteen years were included to take into account the onset of mineralization of all teeth, except the third molars. All patients included in this study were natives of Bosnia and Herzegovina. All

patients with syndromes or general development disorders and patients who were undergoing or had previously received orthodontic treatment were excluded from the study.

Hypodontia was recorded when a tooth was absent on the panoramic radiograph, excluding a history of loss due to trauma, caries, periodontal disease or orthodontic extraction. All permanent teeth were investigated, excluding third molars. On the basis of medical history the possibility of tooth agenesis due to extraction was excluded. Hyperdontia was recorded as an increased number of teeth above that described by normal dental formula. The overall prevalence was studied of hypodontia and hyperdontia, as well as the pattern of their occurrence regarding the affected side (left vs. right), jaw (maxilla vs. mandible), tooth type, tooth number and gender. All analysis of panoramic radiographs was carried out by one orthodontics specialist.

## Statistical Analysis

Data were analyzed using descriptive statistics, including frequency and percentage. The differences between groups were tested using  $X^2$  statistics, or Fisher exact tests. The level of significance was set at  $p < 0.05$ . The Statistical Package for Social Sciences (SPSS, version 20.0) was used for data analysis.

## Results

In the sample of 4256 digital panoramic radiographs of orthodontic patients, there were 2032 (47.75%) males and 2224 (52.25%) females. From the total of selected and analyzed radiographs, 174 (4.08%) had a least one dental anomaly: hypodontia or hyperdontia. Hypodontia was found in 146 (3.42%) subjects, out of which 64 (1.50%) were males and 82 (1.92%) female subjects. There was no significant difference between the genders ( $\chi^2=0.87$ ;  $P>0.05$ ) (Figure 1.). Hyperdontia was found in 28 (0.65%) subjects, of which 18 (0.42%) were male and 10 (0.23%) female subjects, and there was no statistically significant difference between them ( $\chi^2= 0.71$ ;  $P>0.05$ ) (Figure 1).

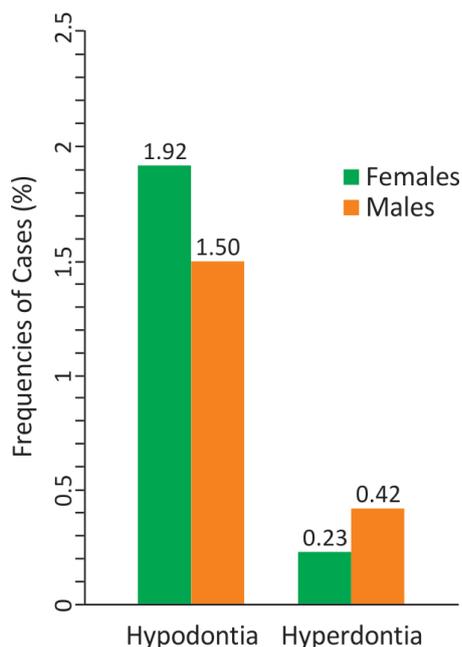


Figure 1. Frequencies of hypodontia and hyperdontia according to gender.

Figure 2 shows the data on the presence of tooth agenesis in relation to jaws. 61 (1.42%) subjects had agenesis in the upper jaw (0.72% males and 0.70% females), 67 (1.57%) in the lower jaw (0.68% males and 0.89% females) and 18 (0.39%) in both jaws (0.09% males and 0.30% females). The appearance of tooth agenesis in the upper or lower jaw had no statistically significant value ( $P > 0.05$ ). Unilateral agenesis of teeth was found in 37 (0.86%) male and 33 (0.77%) female subjects, and 27 (0.63%) male and 49 (1.15%) female subjects had bilateral agenesis; but the difference was not statistically significant ( $\chi^2=0.47$ ;  $P > 0.05$ ).

Figure 3 shows that the teeth most affected by agenesis were the lower second premolars, followed by the upper lateral incisors, upper second premolars and lower central incisors.

A total of seventeen (0.39%) subjects (0.28% males and 0.11% females) had supernumerary teeth in the upper jaw, 9 (0.20%) in the lower jaw (0.11% males and 0.09% females) and only two (0.04%) subjects (0.02% males and 0.02% females) had supernumerary teeth in both jaws. The appearance of supernumerary teeth in the upper

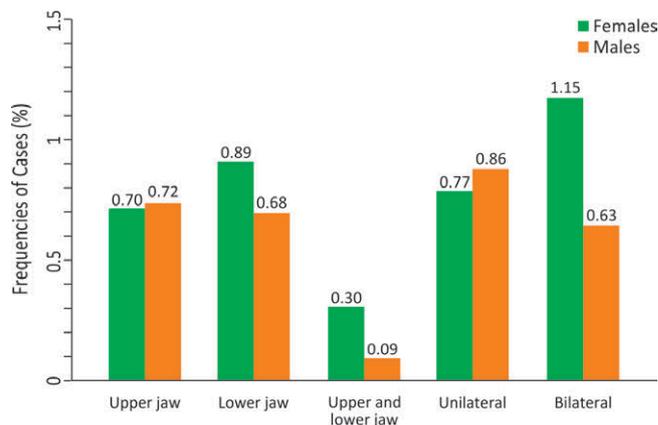


Figure 2. Frequency of hypodontia according to jaws, affected sides and gender.

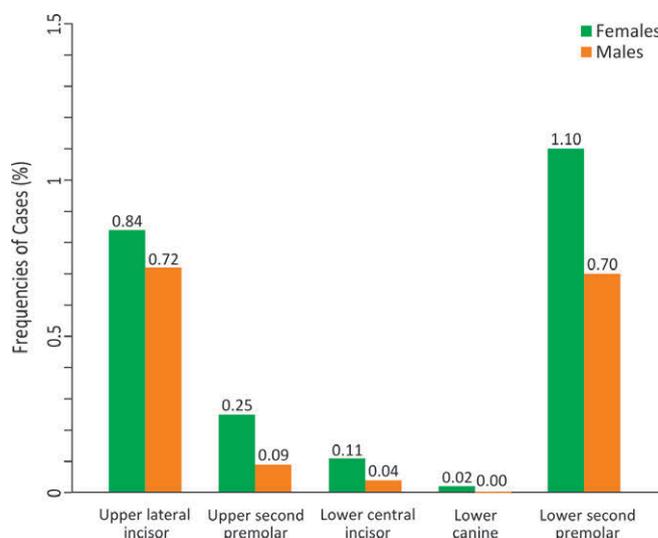


Figure 3. Frequency of hypodontia according to type of tooth agenesis and gender.

or lower jaw had no statistically significant value ( $P > 0.05$ ). These results are presented in Figure 4. Of the total number of subjects, 15 (0.35%) male and 7 (0.16%) female subjects had unilateral supernumerary teeth; and 3 (0.07%) male and 3 (0.07%) female had bilateral, but the difference was not statistically significant ( $\chi^2=0.21$ ;  $P > 0.05$ ) (Figure 4).

Figure 5 shows the number and type of supernumerary teeth occurring in male and female subjects. Among the male subjects, the most common type of supernumerary tooth was mesiodens (0.25%), followed by the lower premolars (0.07%),

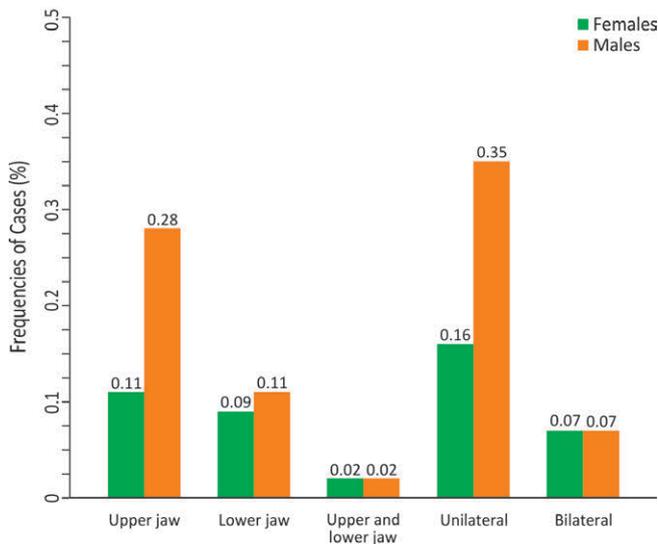


Figure 4. Frequency of hyperdontia according to jaws, affected sides and gender.

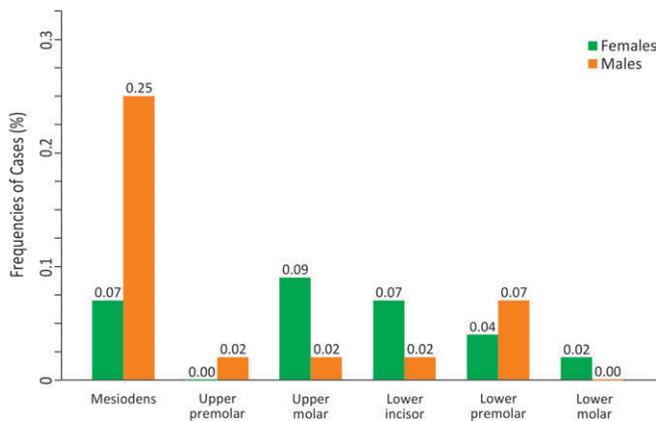


Figure 5. Frequency of hyperdontia according to type of supernumerary teeth and gender.

and the upper premolars and molars and the lower incisors were the least represented (0.02%). The most frequent supernumerary teeth in female subjects were upper molars (0.09%), mesiodens and lower incisors (0.07%), followed by lower premolars (0.04%) and molars (0.02%).

## Discussion

The reported prevalence of hypodontia, excluding the third molar, varies from 4.30% to 9.0% in the

population of orthodontic patients (5, 7, 11, 16, 17) and from 1.11% to 11.01% in the population of other dental patients (3, 4, 6, 8, 12, 18). The wide range of prevalence of hypodontia may be attributed to differences in sampling methods and sample size, as well as ethnic and racial differences. In this study, the presence of hypodontia was observed in 3.42% subjects among orthodontic patients, which is in accordance with previous, similar investigations. It was more prevalent in females (1.92%) than in male (1.50%) subjects, although this difference was not statistically significant, which is in accordance with reports from Japan, Norway, Portugal, Turkey, Iran, Croatia and Macedonia (3-7, 12, 16). Only in a sample of Spanish children was hypodontia more common in male subjects, but also without any statistically significant difference (8).

Similar to the results of other studies, our results showed a significantly lower prevalence of hyperdontia (0.65%) in relation to the prevalence of tooth agenesis. The prevalence of supernumerary teeth ranges from 0.3% to 3.8% (4, 10-12). Hyperdontia is more common in males, but with no statistically significant difference, which is in accordance with other research (10-12, 15).

Numerous studies have reported that tooth agenesis is more common in the mandible than in the maxilla (6, 7, 16, 18), and these results were in agreement with our results. However, other studies have shown that the tooth agenesis was dominantly in the maxilla (8, 15, 17). Bilateral tooth agenesis was more frequent than unilateral, where bilateral was more often present in female subjects, and unilateral in male subjects, but there were no statistically significant differences. However, the results of several studies have shown that the unilateral occurrence of tooth agenesis was more frequent than bilateral (8, 16, 18), but these differences were small and were not statistically significant.

In this research, agenesis of the second lower premolar was most common, followed by the upper lateral incisor, upper second premolars and lower central incisors. Most other studies confirmed the same (3, 5, 6, 8, 16-18), in contrast to some studies, where the upper lateral incisor was the most commonly affected tooth (11). Generally,

agenesis of certain teeth was more prevalent in female subjects (Figure 3), which is related to the overall prevalence of tooth agenesis in our sample. These results explain the common occurrence of hypodontia in the mandible in our research.

Regarding the location of supernumerary teeth, they can be found in any region of the teeth, and the results of our research have shown that the largest number is in the maxillary arch, but there was no statistically significant difference between the upper and lower jaw, or between the genders. These results are in accordance with the results of the studies by Haugland et al. (5), Kazanci et al. (11), Karadas et al. (15), Gurbuz et al. (19) and Schmuckli et al. (20). A greater number of male subjects had unilateral occurrence of supernumerary teeth compared to female subjects, but the difference was not statistically significant; while the bilateral occurrence of supernumerary teeth was equally represented in both male and female subjects.

The results of the present study showed that the supernumerary teeth most commonly found were mesiodens (0.32%) followed by premolars (0.13%), molars (0.13%), and lower incisors (0.09%). Similar results were presented in several earlier studies (5, 15, 19). By early diagnosis of a reduced or increased number of teeth, various modes of therapy, with a multidisciplinary approach, may be performed to correct the aesthetic and functional problems caused by hypodontia or hyperdontia.

## Conclusions

The prevalence of hypodontia was observed in 3.42% subjects among orthodontic patients, and was more prevalent in females (1.92%) than in male (1.50%) subjects. The prevalence of hyperdontia was observed in 0.65% subjects, and supernumerary teeth are more common in males than in female subjects.

### What Is Already Known on this Topic:

*Dental anomalies, especially tooth number anomalies, cause problems in dental arches and occlusion. By early diagnosis of a reduced or increased number of teeth, various modes of therapy, with a multidisciplinary*

*approach, may be performed to correct the aesthetic and functional problems.*

### What this Study Adds:

*This study provides important information about the prevalence of dental tooth number anomalies among orthodontic patients from Bosnia and Herzegovina, which should establish appropriate therapy modalities and minimize the complications caused by these anomalies.*

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

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

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## Evaluation of Solubility of Luting Cements in Different Solutions

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### Abstract

**Objective.** To evaluate and compare the solubility of three luting cements in three different solutions: distilled water and artificial saliva with different pH values (7.4 and 3.0). **Materials and Methods.** Resin-modified glass-ionomer cement (GC Fuji Plus) and two resin cements (Multilink Automix and Variolink II) were used. A total of 45 specimens, 15 specimens (15x1 mm) for each cement, were prepared according to ISO standard 4049:2009. The solubility of the cements was calculated by weighing the specimens before and after immersion and desiccation. Values of solubility in water (Wsl) in microgram/mm<sup>3</sup> for each of the five specimens were calculated using the following formula (ISO 4049:2009):  $Wsl=(m1-m3)/V$ .

The Mann-Whitney U nonparametric statistical method and Post hoc sample comparisons were applied. **Results.** GC Fuji Plus showed statistically significant higher solubility in comparison with Variolink II and Multilink Automix in all three solutions. In acidic artificial saliva (pH 3.0) Multilink Automix showed significantly higher values of solubility compared to Variolink II ( $P<0.016$ ). By studying the effect of pH value on the solubility of GC Fuji Plus cement, significantly higher values of solubility in pH 3.0 artificial saliva were confirmed ( $P<0.009$ ). The influence of the surrounding pH value on the solubility of the resin cements Multilink Automix and Variolink II was researched. No statistically significant difference was found. **Conclusion.** Solubility values were mainly influenced by the proportion of hydrophilic matrix, the type and composition of filler, and the pH value of the solutions.

**Key Words:** Solubility ■ Resin Modified Cements ■ Resin Cements ■ Prosthodontics.

## Introduction

Permanent cementing and the selection of cement are extremely important factors for the long-term success of fixed prosthodontics therapy. Success also depends on the type of restoration, the clinical circumstances and the characteristics of the cement material. Although achieving the optimal retentive and resistant form of the prepared tooth is of primary importance, the role of dental cement is not negligible (1, 2).

Cement fills the space between the teeth and the restoration, and protects them from the harmful effects of occlusal force, while at the same time

representing a barrier against microbial leakage. At the margin of the preparation, dental cement is in constant contact with saliva, a fluid containing a spectrum of proteins with different antimicrobial characteristics dissolved in water (3, 4). Good dental cement must be resistant to disintegration and dissolution where a thin layer of cement may possibly be completely dissolved, creating a space susceptible to plaque accumulation and secondary caries, which, if not observed in time, leads to tooth decay, infraction of the margin of restoration, and debonding of the restoration (5-8).

Numerous authors have pointed out that water may contribute to the failure of adhesive to bond with dentin (5, 9-12). Solubility is therefore an im-

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portant feature in the assessment of the clinical durability of dental cements, and has been the subject of numerous experimental and clinical studies (6, 10-14). Tests which examine these changes in dental cements should be performed in saliva. There is a wide range of pH values in the oral cavity under different conditions, however, the pH value of the oral cavity is considered to be slightly alkaline. Short-term changes in its pH value occur, in terms of increased acidity due to the bacterial metabolism of dental plaque. However, the unstable nature of natural saliva makes it unsuitable for standardized in vitro studies. Artificial saliva that reacts with the test materials in a way similar to natural saliva, is a basic requirement for an artificial oral environment. In vitro tests require the chemical conditions that exist in the oral cavity. ISO 4049 is the standard and most commonly used method for testing the water sorption and solubility of resin-based cements (15). In this study, the method described by ISO 4049 was performed completely, except that the samples, besides storage in distilled water, were stored in artificial saliva with two different pH values. The choice of pH value of the artificial saliva followed the logic: a slightly alkaline buffered solution mimics normal natural saliva; the acid solution (pH 3.0) mimics the conditions in the oral cavity after the action of a bacterial biofilm; and the third solution (distilled water) has a neutral pH. In this way, we tried to simulate the dynamics of change in the pH of saliva, whose pH is from 6.2 to 7.6, and changes under the influence of a number of factors (4, 16-19).

The multiple purposes of dental cements have led to different types of cements with various properties coming onto the market, as no material has yet been developed that can meet all the necessary requirements. With the expansion of aesthetic dentistry, resin-based cements are becoming increasingly important and are unavoidable in the adhesive cementing of ceramic crowns and bridges, inlays, onlays, ceramic veneers, composite post and orthodontic braces (20-22).

Resin-modified glass-ionomer cements may be used for cementing restorations made of ceramics and, in addition to this, they are widely used

for cementing metal-ceramic restorations and cast posts as well. They have good mechanical properties, limited solubility and radiocontrast similar to composite cements, and easy handling and fluoride release with a caries-protective effect, similar to conventional glass ionomer cements (21-24).

Knowing the characteristics of the cement material will aid the appropriate choice of luting cement and thus the durability of fixed prosthodontic restorations, and decrease the possibility of the occurrence of secondary caries, postoperative hypersensitivity, pulpal inflammation and periodontal diseases (11, 25-29). The solubility of dental cements has been the subject of numerous studies, but studies of solubility in artificial saliva of different pH values are rare. However, changes in the pH value of the environment might affect the solubility of different dental luting cements.

The objective of this research was to evaluate and compare the solubility of three luting cements for permanent cementation in three different solutions: distilled water and artificial saliva with different pH values (7.4 and 3.0), and to examine the influence of the pH value of the artificial saliva on solubility.

## Material and Methods

The dental cements used in this study are shown in Table 1.

Fifteen specimens of each cement were made according to ISO specification 4049:2009 (15). Specimens were made in Teflon molds with an inner diameter of  $15 \pm 0.1$  mm and thickness of  $1 \pm 0.1$  mm. Preparation of specimens of GC Fuji Plus self-curing cement (GC Corporation Tokyo, Japan), a resin modified cement, was carried out as follows: a 50  $\mu$ m thick polyester film was put on a metal plate and over it the mold in which the cement was slightly overfilled, to minimize air inclusion. Another polyester film was put on top of the material in the mold and covered with a second metal plate to remove the excess material. The metal plates were bound together by clamps, and the specimens were immediately stored in an incubator at  $37 \pm 1^\circ\text{C}$ . After 60 minutes the specimens were re-

Table 1. Name, Type and Main Component of Cement

Name of cement and manufacturer	Type of cement	Main components
GC Fuji PLUS CAPSULE (reinforced glass-ionomer cement) GC Corporation Tokyo, Japan	Resin-modified glass-ionomer cement.	Powder: aluminofluorosilicate glass Liquid: polyacrylic acid, HEMA, metadimethacrylate, water.
Multilink Automix IvoclarVivadent AG; FL-9494 Schaan, Liechtenstein.	Resin cement.	Monomer: dimethacrylate, HEMA; Inorganic filler (40%): barium glass, ytterbium trifluoride, spheroid mixed oxide; Additional content: catalysts, stabilizer, pigments. The mean particle size is 0.9 $\mu\text{m}$ .
Variolink II IvoclarVivadent AG; FL-9494 Schaan, Liechtenstein.	Resin cement.	Monomer: Bis-GMA, urethane dimethacrylate, triethyleneglycoldimethacrylate; Inorganic filler (40%): barium glass, Ba-Al-fluorsilicate glass, ytterbium trifluoride, spheroid mixed oxide; Additional content: catalysts, stabilizers, pigments. The mean particle size is 0.7 $\mu\text{m}$ .

moved from the mold. Specimens were trimmed and polished with 1000 grit silicon carbide grinding paper until a final diameter of  $14.9 \pm 0.1$  mm was attained. The diameter was measured with a TESA 0-25 mm micrometer for external measurements, with measurement accuracy of 0.001 mm (TESA, Renens, Switzerland).

For preparation of the specimens of the dual cure cements (Multilink Automix and Variolink II), the metal plate was replaced by a glass plate, over which polymerization of specimens was performed. The light was tested for light output by means of a digital radiometer (Bluephase Meter, Ivoclar Vivadent, Schaan/Liechtenstein). The light tip of the polymerization lamp (Bluephase 20, Ivoclar Vivadent Ag, and FL-9494 Schaan/Liechtenstein) was directed over the center of the specimens for the correct time of exposure, and then eight peripheral overlapping sectors were irradiated for 20 seconds each, until the whole area had been irradiated. After that, the lower side of the specimens was polymerized in the same way as the upper one. The specimens were immediately stored in an incubator at  $37 \pm 1^\circ\text{C}$  for 60 minutes, and then finished as the previous one. After preparation was complete, all specimens were stored in desiccators with silicate gel, and the entire set was stored in an incubator maintained at  $37 \pm 1^\circ\text{C}$ . After 22 hours, the specimens were moved into another desiccator maintained at  $23 \pm 1^\circ\text{C}$  for 2 hours, and, after that, weighed on an analytical balance, (Sartorius LE244S 0-240 g) with accuracy of measurement of 0.0001 g (Sartorius Göttingen, Germany) until a constant mass of  $m_1$  was

obtained or until the mass loss of each specimen was not less than 0.01 mg over 24h. The diameter  $r$  (mm) and thickness  $h$  (mm) of each specimen was measured by micrometer, with accuracy up to 0.001 mm according to the ISO specification, and the volume  $V$  ( $\text{mm}^3$ ) was calculated according to the formula:  $V = \pi \times r^2 \times h$ .

Five specimens of each cement were immersed in distilled water, five specimens in artificial saliva pH value 7.4, and five specimens were immersed in artificial saliva pH value 3.0. All specimens were stored in a Culture Incubator (Ivoclar Vivadent, Schaan / Liechtenstein) at  $37 \pm 1^\circ\text{C}$  for 7 days. Tomasi's solution of artificial saliva pH value 7.4 was prepared for this research at the Department of Chemistry, Faculty of Natural Science and Mathematics of Sarajevo University. (3) In order to obtain a pH-value of 3.0, Tomasi's solution was modified by the intentional acidification of the solution.

After the first day of storage, the specimens were taken out of the liquid, washed entirely with water, air dried for 15 seconds, and weighed one minute after they were taken out of the water to record their mass. The same procedures were repeated at 48h, 72h, 96h and 168 hours, when the final mass  $m_2$  from the second cycle was recorded. This second cycle showed the combination of water sorption and dissolution of the soluble components from the specimens.

After weighing in the second cycle the specimens were stored in the desiccator and incubator again in the same way as in the initial cycle, and afterwards the  $m_3$  mass was recorded. This third

cycle allowed measurement of the mass loss. The values of solubility in water (Wsl) in microgram/mm<sup>3</sup> for each of the five specimens were calculated using the following formula (ISO 4049:2009):  $Wsl = (m1 - m3) / V$ , where m1 = mass of specimens ( $\mu\text{g}$ ) before immersion, m3 = mass of refined specimens ( $\mu\text{g}$ ) and V=volume of specimens (mm<sup>3</sup>).

### Statistical Analysis

All data were analyzed using the IBM SPSS v.17 statistical software. Preliminary statistical analyses were carried out to determine the distribution of the dependent variables, and to decide on the application of parametric or nonparametric statistical methods. Since the dependent variables were asymmetric, the Mann-Whitney U nonparametric statistical method was applied. As the required

alpha level of significance for rejection of the null hypothesis, the level 0.05 (5%) was taken. To avoid first category statistical error, in subsequent (Post hoc) sample comparisons, matching with Bonferroni was used, where the required alpha level of significance of 5% was corrected, i.e. divided by the comparison number ( $P < 0.05/3 = P < 0.017$ ).

### Results

The arithmetical means and standard deviations of solubility for each dental cement in three different solutions are shown in Table 2. GC Fuji Plus showed statistically significantly higher solubility in comparison with Variolink II ( $P < 0.009$ ) in all three solutions (distilled water, artificial saliva of pH values 7.4 and 3.0). Post hoc comparisons are shown in Table 3.

Table 2. Mean and Standard Deviation of Solubility between Groups

Solubility in different solutions cement	Type of cement	N	Mean	SD
Solubility-distilled water	GC Fuji Plus	5	7.12	2.67
	Multilink Automix	5	-3.06	2.80
	Variolink II	5	-5.25	0.28
Solubility-artificial saliva pH 7.4	GC Fuji Plus	5	3.46	3.16
	Multilink Automix	5	-3.20	2.92
	Variolink II	5	-5.29	0.07
Solubility-artificial saliva pH 3.0	GC Fuji Plus	5	13.22	2.90
	Multilink Automix	5	-4.10	2.29
	Variolink II	5	-5.41	0.13

Table 3. Post Hoc Comparison between Groups

Type of cement	GC Fuji Plus	Multilink Automix	Variolink II
	Solubility - distilled water		
GC Fuji Plus	-	P=0.009(0.828)	P=0.009(0.826)
Multilink Automix	P=0.009(0.828)	-	NS
Variolink II	P=0.009(0.826)	NS	-
	Solubility-artificial saliva pH 7.4		
GC Fuji Plus	-	NS	P=0.009(0.828)
Multilink Automix	NS	-	NS
Variolink II	P=0.009(0.828)	NS	-
	Solubility-artificial saliva pH 3.0		
GC Fuji Plus	-	P=0.009(0.826)	P=0.009(0.828)
Multilink Automix	P=0.009(0.826)	-	NS
Variolink II	P=0.009(0.828)	NS	-

Mann Whitney U test; P (effect size =  $Z/\sqrt{n}$ ); NS=Not statistically significant.

A statistically significant difference was found between the solubility in the distilled water solution and the artificial saliva with pH value 3.0 between Multilink Automix and GC Fuji Plus ( $P < 0.009$ ). The samples of GC Fuji Plus cement showed a higher degree of solubility. In the solution of artificial saliva with pH value 7.4 ( $P < 0.05/3 = 0.017$ ), no statistically significant difference ( $P = 0.024$ ) in solubility between Multilink Automix and GC Fuji Plus was confirmed. However, at a level of significance of  $P < 0.05$ , it would show statistical significance. Post hoc comparisons are shown in Table 3.

No statistically significant difference was found in solubility levels between the cements Multilink Automix and Variolink II in distilled water ( $P = 0.173$ ), or in the artificial saliva solution with pH of value 7.4 ( $P = 0.600$ ). In artificial saliva with a pH of 3.0, a statistically significant difference was detected in the solubility of cements Multilink Automix and Variolink II, with a probability of  $P < 0.016$ . Multilink cement showed a higher degree of solubility. Post hoc comparisons are shown in Table 3.

Analysis of the effect of pH value on the solubility of dental cements confirmed a statistically significant difference ( $P < 0.009$ ) of solubility level in GC Fuji Plus cement between the solutions of artificial saliva with pH 7.4 and artificial saliva with pH 3.0. GC Fuji Plus cement showed a higher degree of solubility in artificial saliva with pH 3.0. Post hoc comparisons are shown in Table 4.

Table 4. Post hoc Comparison between Groups of Solubility (GC Fuji Plus)

Solubility	Distilled water	Artificial saliva pH 7.4	Artificial saliva pH 3.0
Distilled water	-	NS	NS
Artificial saliva pH 7.4	NS	-	$P = 0.009(0.828)$
Artificial saliva pH 3.0	NS	$P = 0.009(0.828)$	-

Mann Whitney U test; P (effect size  $= Z/\sqrt{n}$ ); NS=Not statistically significant.

The influence of the environmental pH value on the solubility of Multilink Automix cement was researched. No statistically significant influence of

the saliva's pH value on Multilink Automix cement was confirmed, with a probability of  $P = 0.583$ . Studying the effect of pH values on the solubility of Variolink II dental cement no statistically significant difference was found in levels of solubility of Variolink II cement in the different solutions, with a probability of  $P = 0.248$ .

## Discussion

The resin-modified glass ionomer cement Fuji Plus exhibited significantly higher solubility when compared with the resin cements (Variolink II and Multilink) in all solutions, except in the solution of artificial saliva with a pH value of 7.4, where no statistically significant difference in solubility was confirmed between Multilink Automix and GC Fuji Plus at the level of significance of  $P = 0.024$ . However, at the level of significance of  $P < 0.05$  it would show statistical significance. The hydrophilic nature of resin-modified glass-ionomer cements (that include HEMA in their composition) most probably contributed to the significantly higher solubility compared to the resin cements in our study (30-32). The values which Mortier obtained for Fuji II resin modified cement completely correspond to our results, as well as the fact that in their study resin modified glass ionomers showed significantly higher solubility values than composite cements (12).

The mean solubility values in our study for Fuji Plus are somewhat lower than the values obtained by Mese and Gerdole in their research (11, 33), which may be explained by our use of encapsulated cement and mechanical mixing, in comparison to the manually mixed cements used by these authors. The mixing process can lead to the formation of air bubbles, which may increase the surface exposed to water, which leads to the formation of inhibitory zones of unpolymerized material, and accelerates the water sorption and solubility of cement (10, 34, 35).

Knobloch, Al-Shekhli obtained similar results to ours with respect to the solubility of resin modified glass ionomer cements, which showed significantly higher solubility compared to composite ce-

ments (21, 36). Yohsida et al. also concluded that composite cements were significantly less soluble than conventional cements, but the results cannot be compared directly with our results because the authors expressed the solubility results in percentages (28). Although resin modified glass ionomer cement showed higher solubility values than composite cements, the presence of the resin matrix in resin-modified cements reduces the diffusion of solvent into the cement, and there is consequently less solubility compared to conventional glass ionomers (37). For significantly lower solubility values than conventional cements (zinc phosphate, polycarboxylate and ionomer cement), the choice of luting cement for metal-ceramic restorations, while ensuring adequate retentive and resistant tooth form and good marginal adaptation, would be resin-modified glass ionomer cement.

When composite cements, unlike resin modified glass ionomers, must be used for the bond, the *locus minoris* in the bonding chain must be kept in mind. When predicting prosthetic durability, the contact surface of the tooth and cement must be taken into account as well. Numerous studies have shown that adhesive systems have water sorption and solubility values 30 to 150 times greater than the corresponding resin based materials. The adhesive is exposed to oral fluids on a small surface, but has significant contact with dentin. Dentin tubules occupy about 20-40% of the median coronary dentin surface, and water accounts for about 22% of the volume of dentin (33). As for the water, 75% of its entire quantity is in the tubules, and 25% is bound in a mineralized matrix around the mineral crystals or collagen (33).

Over time, tubular fluids may damage the adhesive that connects the dentin to the restoration. This should be borne in mind when applying dental cements clinically and making a decision when selecting the appropriate cement. If this fact is taken into account, the importance of resin-modified cements in daily clinical practice is not diminished. With respect to the solubility in different pH values of saliva, with a demanding alpha level of Bonferroni adaptation ( $P < 0.05/3 = 0.017$ ), a statistically significant ( $P < 0.009$ ) difference in the solu-

bility level of GC Fuji Plus cement was confirmed between artificial saliva with pH 7.4 and artificial saliva with pH 3.0.

These results correspond with the results of Hajmiragh and Bharali, which confirmed the higher solubility of conventional cements, but also the resin modified glass ionomer and composite cements in saliva with a lower pH (38, 39). These authors report that, in addition to pH, the solubility of the cement is influenced by the storage time, the concentration of solvent in the solution, the shape and thickness of the sample, as well as the ratio of powder to liquid in the cement (39).

Yanikoglu et al. found significantly higher solubility of cements stored in an acidic solution in their study. They tested the solubility of zinc phosphate, polycarboxylate and conventional glass ionomer cement, whereby the glass ionomer cements showing the lowest solubility (40). The positive effect of an acidic over a neutral solution is the greater ability to release fluoride in resin modified glass ionomer cements (41).

As we have already pointed out, composite cements are less soluble than glass ionomer cements. Our solubility results are consistent with the data found in the literature; composite cements dissolve less than resin modified glass-ionomer cements (11, 21, 28, 34, 37). However, all the materials in the study showed some degree of solubility, i.e. they all interacted with water. The interaction between resin-based cements and water involves two opposing phenomena: the first is water sorption, which leads to the swelling of the material and an increase in weight, and the second is dissolution of materials (fillers or monomers) in the water. Release of unreacted monomers or fillers that will dissolve in water will result in weight loss, which we define as solubility (11, 42). It has also been shown that a portion of the absorbed water tightly bound to the resin matrix cannot be removed (31). The part of the water that is loosely bound to the resinous matrix after the drying period will disappear, but a certain amount of water remains inside the material. Therefore, when the amount of water which remains bonded to the resin matrix is greater than the amount of components lost

during the desiccation process, this will result in negative solubility values, suggesting an obvious gain in mass (11, 31, 32). Ortengren explains this by the possibility of the precipitation of particles within the solute material in which the samples were stored, or by the formation of metal hydroxide on the surface of the particles as a product of the hydrolysis reaction, which will also result in an increase in mass (32). In our study, negative solubility values were obtained for both composite cements. It should be noted that these values are in accordance with the values for solubility prescribed by the ISO standard. On the other hand, the weld volume of the polymer is subjected to a certain degree of dissolution, which is highly correlated with the degree of conversion of the material, i.e. the conversion of the monomer to a polymer. The conversion of monomers to a polymer network is never complete. It is also influenced by the type of filler and its treatment, as well as by the presence of air bubbles in the material (31). The Mann Whitney test found no statistically significant difference in solubility levels between Multilink Automix and Variolink II cements ( $P=0.173$ ) in distilled water and saliva pH 7.4 ( $P=0.600$ ). A statistically significant difference was detected in Multilink Automix cements (Mean rank = 7.80) and Variolink II (Mean rank = 3.2) in the solution of artificial saliva with a pH value of 3.0, with a probability of  $P<0.016$ , which can be explained by the chemical composition of these cements, different compositions and ratio matrix and fillers. Multilink Automix contains HEMA, which flows more readily in water than Bis-GMA because of its lower molecular weight and hydrophilic chemical structure (43). The data from our study correlate with the data of Vrochari, who obtained negative solubility values for Multilink Automix (31). The solubility results obtained by Mese et al. for Variolink II completely correspond with ours (11).

Our results are also in agreement with those of Berger et al., who examined the sorption and solubility of three restorative composites with different filler contents. These authors obtained a value of -4.0 (2.9) for the Filtek composite tested (44). These authors state that it is possible that not all

the water was drained during the desiccation process and that negative values were obtained as a result. Some authors recommend a longer sample desiccation period (45). Swizero et al. tested the solubility of the Z 250 micro hybrid composite, and their results were consistent with ours (46). When studying the effect of pH on the solubility of Variolink II dental cements ( $P=0.248$ ) and Multilink Automix ( $P=0.583$ ), no statistically significant difference in levels of solubility between different solutions was confirmed. Our results correlate with those of Toledano et al., who found that pH values had no effect on the solubility of composite cements (47).

Knobloch examined the solubility of cements in lactic acid, and found no significant differences in solubility between resin-based materials (21). Contrary to these studies, the study of Ortengren et al. concluded that solubility depends on the pH value of the solution, where solubility was reduced to pH4 and pH6, and increased to pH 8 (32). The influence of low pH on the wear of composite cements was also investigated by Buchalla, who concluded that an acidic environment had little effect on the resistance and wear of the composite cements (48).

Considering the above, higher solubility rates of composite cements are expected in the oral cavity. Tomas' solution of artificial saliva used in our study had no enzymes in its composition, and higher values for the tested parameters may be expected in the oral cavity. The presence of the enzyme may degrade the polymer through side-chain attacks, thereby producing potentially harmful by-products, as well as deteriorating the properties of the composite mesh (48) and reducing the material's wear resistance (49, 50).

The quality of the network formed during the polymerization process will also dictate how much molecular uptake and swelling occurs when the polymer is submerged in the solvent. It is important to ensure the optimum conversion of the monomer to a polymer, to ensure optimal mechanical properties, and to resist mechanical and chemical deterioration (46, 51). In complex oral conditions, we cannot expect maximum polym-

erization. Different clinical situations present a challenge for the use of the recommended polymerization technique, meaning the availability of the light source, its direction, and the preparation of the surrounding tissues (46, 52). Due to the low solubility of both composite cements used in our research, they are good choices in clinical practice. Both cements have a very wide spectrum of indication, where Multilink Automix is a self-cure, with optional light cure, and contains amines that may discolor the restoration and should be avoided when cementing ceramic veneers. Although the resin modified glass ionomer cement showed higher solubility values, this does not minimize the importance of its application in clinical practice in conventional cementation, since it has superior properties to conventional glass-ionomer, and a simpler use procedure than composite cement.

## Conclusion

The resin modified glass-ionomer cement Fuji Plus showed the significantly highest solubility values of all three examined solutions. Multilink Automix and Variolink II were found to comply with ISO requirements regarding solubility. The values of solubility were found to depend on the matrix hydrophilicity, the type and composition of the filler, and degree of polymerization. The pH value of the artificial saliva affected the solubility of the resin modified glass-ionomer cement.

### What Is Already Known on this Topic:

*There are numerous studies of the solubility of dental cements. Solubility of dental cements is an important factor in evaluation of their clinical durability and hence the durability of fixed prosthetic restoration. Knowing the properties of cement material enables the appropriate choice of cement for permanent cementation of dental restoration.*

### What this Study Adds:

*Studies of the solubility of dental cements in artificial saliva are rare. This research contributes to the knowledge about the solubility of dental cements in artificial saliva of various pH values, mimicking conditions in the oral cavity.*

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## Severe Leptospirosis in a Mexican Woman

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### Abstract

**Objective.** To describe a severe case of infection by *Leptospira* in a woman in the northwest of Mexico. **Case report.** A 55-year-old woman from Sonora, México arrived at the Intensive Care Unit due to severe multiple organ failure primarily affecting the respiratory, renal and hepatic systems. Diagnostic tests were performed, and they were positive for anti-*Leptospira* antibodies, IgM and IgG; and spirochetes were observed on dark field microscopy and confirmed by Polymerase Chain Reaction (PCR). Doxycycline and platelet apheresis transfusion were used as treatment, which led to a very slow recovery. **Conclusion.** The information presented in this study may help in the identification of pathology caused by spirochetes. This case report is the first to present a case of severe leptospirosis in Sonora, México.

**Key Words:** Vector-Borne Disease ■ Acute Febrile Illness ■ Leptospirosis ■ Renal Failure ■ Borrelia.

## Introduction

Spirochetes are Gram-negative aerobic bacteria with a double membrane and a helicoidal structure; these features allow the bacteria to survive in fresh water or wet soil (1, 2). In public health, one of the most pathogenic spirochetes with worldwide importance in humans is *Leptospira*. The infection may be transmitted by a chronic carrier, through contact with contaminated water, soil, and urine (3). These bacteria are endemic to unsanitary areas (4). A poor diagnosis may lead to inappropriate treatment and allow for the development of complications, and even death, due to the clinical mimicry of these infections of other acute febrile illnesses (AFI) (3). In Mexico, leptospirosis disease is not a very well-known pathology, with most cases being reported predominantly in animals (5).

The aim of this publication is to describe a case of severe leptospirosis infection in a Mexican woman.

## Case Presentation

On June 19, 2018, a 55-year old Mexican woman was evaluated at the emergency department of a public hospital, due to a 3-month history of arthralgia, asthenia, fever, diarrhea, and dyspnea. During the epidemiological survey, the patient referred to having 3 dogs as pets, and travelling to the city of Mazatlán, Sonora, a port city with access to the beach, where the patient participated in outdoor activities that put her in contact with free water during her stay, mainly swimming. Without clinical improvement and with compromised respiratory function and tachycardia, she was transferred to a private hospital. Clinical tests were later conducted which showed thrombocytopenia and leukocytosis, after which the patient was admitted to the Intensive Care Unit due to the development of multiple organ failure: predominantly affecting the respiratory, renal and hepatic systems. After be-



Figure 1. Diffuse ecchymosis in arms (A); Petechia (B).

ing admitted to the Intensive Care Unit, the same symptomatology was reported to persist for 3 more days. She was found to have no febrile antibodies and no antigen reaction to dengue when tested, but was reported positive for anti-*Leptospira* antibodies, IgM 3.510 Optical Density (OD) and IgG 1.574 OD (Range: Negative 0.0 to 0.3 and Positive >1.0; these antibodies were detected using commercial kits (Diagnostic automation, INC. Cat # 8204-3 and Cat # 8208-3 respectively). She was treated

with Doxycycline and underwent platelet apheresis transfusion.

The woman was then transferred to a public hospital where treatment was continued. A day later, she was admitted to the Internal Medicine ward in a very grave condition, with severe thrombocytopenia, generalized petechiae, a hyperemic rash that was hot to the touch, moderate hyperchloremia, mild elevation of LDH, grade III normochromic normocytic anemia, severe thrombocytopenia, and hepatomegaly (Figure 1). A skin-biopsy was conducted, and the results showed thrombocytopenic purpura. In addition, dark-field microscopy of a blood sample showed the presence of spirochetes characteristic of *Leptospira* and *Borrelia*; dark-field microscopy may be considered as a preliminary diagnostic tool associated with finding the morphological characteristics of spirochetes on samples, suggesting the presence of *Borrelia* and/or *Leptospira*. Enzyme immunoassay (EIA) IgM and IgG (Diagnostic Automation, INC.) was performed for *Borrelia burgdorferi* in order to exclude a coinfection by this bacteria, and leptospirosis was confirmed using conventional PCR, and Go Taq Flexible DNA polymerase wasevaluated (6); the tests performed were negative for borreliosis. After more than a month of being hospitalized, the patient was discharged with significant clinical and biochemical improvement.

## Discussion

Previous reports of leptospirosis conducted in Sonora, Mexico (2000-2010) have reported that the most affected demographic group is that of males between 25 and 44 years of age, predominantly those that originate from the southernmost regions of the country (5), the opposite of our current case. Due to the specific set of symptoms presented by this patient, it is highly probable that she was suffering from a severe case of Leptospirosis, since in other case reports where this diagnosis was made (7-9), similar symptomatology to that of our patient was reported. *Borrelia* spirochetes are not a common finding when performing dark-field microscopy, and their presence should not always

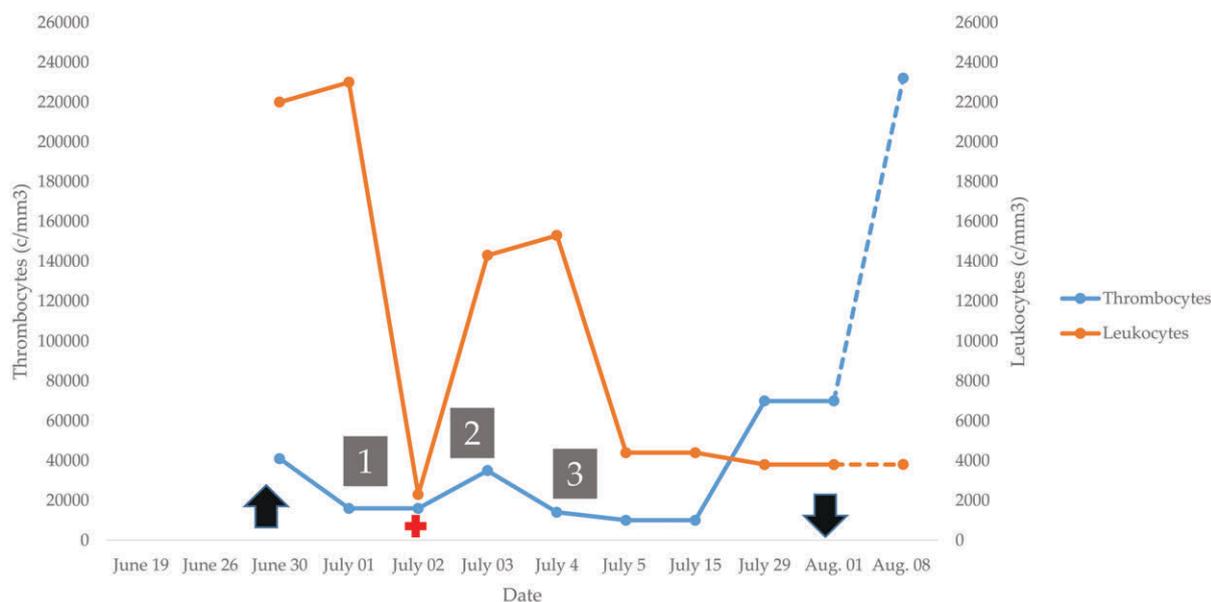


Figure 2. Clinical test and timeline of medical history. ↑=Indicates the onset of the symptomatology, 1=First visit to a public hospital; 2=Intensive care in a private hospital; +=*Leptospira* detection; 3=Transferred to a public Hospital to the internal medicine ward; ↓=Hospital Discharge.

be taken as an indication of a co-infection, because *Borrelia* serology may remain positive for months after the primary infection. It is however important to take into consideration that *Borrelia* and *Leptospira* may occur at different points in time and their sequelae may contribute to the clinical course of one another; during this study, the serological titers obtained for *Borrelia* were IgG 0.60 and IgM 0.30 OD (OD Range: Negative <0.90 and Positive >1.10), discarding coinfection. Upon questioning, the patient reported that she was in contact with pets, but not with rodents. In other leptospirosis case reports, the patients referred to going swimming in free non-sanitized water prior to developing symptoms (2), a risk factor also present in our patient, who said that she had traveled to the beach before becoming ill.

In our patient, the most difficult clinical problems to deal with were renal failure and thrombocytopenia. Figure 2 shows the increase in leukocytes next to the decrease in platelets, as reported in the medical history timeline, which strengthens our position that treatment based on doxycycline (1, 2, 7) and apheresis (10) was needed urgently. This proved to be a decisive factor in our

patient's improvement; plasma apheresis is helpful in eliminating the excess of microorganisms and inflammatory cells, and is a common treatment indicated for cases of multi-organ failure (11, 12). We consider that the severity of the case could have been reduced with preemptive antimicrobial therapy, even before the diagnosis of leptospirosis (13). In this case, even though the proper treatment was used, our patient had to be hospitalized for more than a month, unlike most cases of common leptospirosis where the patients are discharged after no more than two weeks, due to a case of severe leptospirosis (14).

## Conclusions

This report demonstrates the presence of *Leptospira*, and the unusual and severe clinical manifestations it causes in relation to the systemic nature of spirochetes. We report the case of a patient who presented with clinical deterioration that rapidly evolved into multi-organ failure involving the respiratory, renal and hepatic systems, together with severe thrombocytopenia and, which suggests immunocompromise. This study again confirms the

utility of plasmapheresis in severe leptospirosis. This report is the first to present a case of severe leptospirosis in the south of Sonora, México.

#### What is Already Known on this Topic:

*Leptospirosis is an under-diagnosed disease in Mexico. In this country, clinical cases in human patients have been historically scarce, and it has been more thoroughly studied in domestic animals. Its clinical diagnosis is difficult due to its polymorphism and because the clinical findings may be attributed to other more common diseases, such as dengue fever, paludism, brucellosis, flu-like syndromes, and others; therefore, complementary laboratory testing is key for its diagnosis and treatment.*

#### What this Case Adds:

*Confirmatory testing for leptospirosis, such as dark field microscopy and positive IgG and IgM, are a valuable tool for obtaining an early diagnosis, which, when coupled with the symptoms and suggestive clinical signs, allow for a speedier decision-making process regarding treatment, and rejecting other causative agents. The diagnosis is confirmed by a PCR test. In conclusion, with these measures, it is possible to help the patient, avoid multi-organ deterioration, and shorten hospital time.*

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## Anatomical Variation of a Communicating Branch between the Musculocutaneous and the Median Nerve: A Case Report

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### Abstract

**Objective.** The aim of our paper is to depict an anatomical variation of the brachial plexus, concerning a communicating branch between the median and the musculocutaneous nerve and its clinical significance. Anatomical variations of the brachial plexus and especially those of the musculocutaneous nerve are quite common. Awareness of these variations is of paramount importance in clinical practice, mainly in achieving best results in minimal invasive or surgical procedures. **Case Report.** After dissection in upper extremities in a 89-year-old male cadaver, a communicating branch between the median and the musculocutaneous nerve was found. This communicating branch was formed before the musculocutaneous nerve perforated the coracobrachialis muscle. It also derived from the level of the ansa medianis and its course was of an approximately 2cm length. **Conclusion.** The clinical significance of our study is the entrapment of the musculocutaneous nerve that may cause clinical findings similar to those of the carpal tunnel syndrome.

**Key Words:** Median Nerve ▪ Musculocutaneous Nerve ▪ Brachial Plexus Anatomy ▪ Communicating Branch.

### Introduction

Brachial plexus is formed by the contribution of all the anterior divisions of the C5 to C8 nerves, along with a sizeable part of the first thoracic nerve (T1) and a small communicating branch of the 4<sup>th</sup> cervical nerve (C4). These nerves are of similar size and the pattern of communication between them is subject to a non-negligible variation, so that there is no precise pattern that can be followed in all cases.

The Median Nerve (MN) is formed by a lateral and a medial root, the union of which forms the ansa medianis astride the lower third of the axillary artery. After its formation, the MN follows a course downwards along the midline of the arm and antebrachium, giving muscular branches. These branches are the anterior interosseous, the palmar cutaneous branch in the forearm and the three first common digital nerves as well as a motor branch that supplies the muscles of the thumb, in the palm.

The Musculocutaneous Nerve (MCN), or perforans Gasserii nerve, innervates the anterior brachial muscles as well as the outer part of cutem antebrachii. It derives from the lateral cord of the plexus brachialis and after perforating the Coracobrachialis muscle (CBM), it follows an oblique trajectory between the biceps and brachialis anticus muscles down to the elbow, where it finally turns to a cutaneous sensory nerve after perforating the deep fascia. As a cutaneous nerve, it divides into a posterior and an anterior nerval branch, after passing behind the cephalic vein (1).

The anatomical variations of the brachial plexus and especially the existence of anastomotic branches between its nerves, for instance between the MN and MCN or even the ulnar and radial nerve are not rare (2). More specifically the variations of the MCN are quite common as it may pass beneath or even through the biceps rather than

perforating the coracobrachialis, as well as it may send a communicating branch to the MN. Actually, as in our case study, some fibers of the MN are incorporated to the MCN and after covering some distance in it, they secede to unite with their originative nerve (1).

In this paper, we present a case of an interesting and clinically significant anatomical variation of the brachial plexus, concerning a communicating branch between the median and the musculocutaneous nerve.

## Case Report

The anatomical variation of our study was depicted in a 89 years old male cadaver of Greek origin, that was formalin-fixed. The cadaveric dissection took place in the Hall of Dissections of the Department of Anatomy, School of Medicine, National and Kapodistrian University of Athens, Greece. The axillary region of the cadaver was dissected and cleaned by the contributors. Finally, the anatomical specimen was properly photographed.

While proceeding a routine dissection of the left axillary region of a Greek male cadaver, we encountered a variation in the ipsilateral plexus brachialis. Between the median and the musculocu-



AA=Axillary Artery; AV=Axillary Vein; MN=Median Nerve; MCN=Musculocutaneous Nerve; UN=Ulnar Nerve; MACN=Medial Antebrachial Cutaneous Nerve; Mr=Medial root; Lr=Lateral Root; cb=Communicating Branch; CBM=Coracobrachialis Muscle.

Figure 1. The anatomical specimen of the cadaver's left axilla, depicting a communicating branch between MCN and MN. The major and minor pectoralis have been removed.

taneous nerve there existed an anastomotic neural branch that was derived before the musculocutaneous nerve perforated the CBM (Figure 1). This communicating branch stemmed from the level of the ansa medianis formation and its course was approximately 2 cm in length. The MN was formed by a normal median and lateral root, coalescing in the ansa medianis supra, in the outer third of the axillary artery, the course and supply of which were dissected and found normal (Figure 2). The anatomical variation was observed unilaterally.

## Discussion

There have been several case reports and studies about anastomotic branches between the median



AA=Axillary Artery; AV=Axillary Vein; MN=Median Nerve; MCN=Musculocutaneous Nerve; UN=Ulnar Nerve; MACN=Medial Antebrachial Cutaneous Nerve; Mr=Medial Root; Lr=Lateral Root (arrow); cb=Communicating Branch (double arrow); CB=Coracobrachialis Muscle.

Figure 2. The axillary cavity of a Greek male cadaver. A communicating branch between MCN and MN derived from the level of the ansa medianis. Its course is approximately 2 cm in length.

and the musculocutaneous nerve. There are also a number of attempts to classify this structural irregularity of the brachial plexus. According to Venieratos et al. (3), our case belongs to the Type I category, in which the communicating branch is given before the MCN pierces the CBM. Type II refers to those branches given after the CBM's perforation by the MCN and in Type III, which seems to be the rarest of the three, the MCN gives the anastomosing branch without entering the CBM. Additionally, in our case the MCN not only perforates but also innervates the CBM. However, there have been reported cases in which the MN is fused with the MCN and the CBM gets innervated from a small neural branch arising from the MN's lateral root (4).

It is not clear when to consider an anastomosis between the MN and the MCN as a communicating branch or a second lateral root of the MN. Buch-Hansen in 1955, has reported (5) that the segregation should be done comparing the thickness of the branch with that of the MN lateral root. In that terms, if the communicating branch is thicker of the lateral root or of equal thickness, then it is considered as a second MN lateral root, if it is not, then it constitutes a neural anastomosis. So, according to this study, our case is indeed a communicating branch.

Referring to the structural characteristics of the communicating branch that we found, it was of a length similar to those found in Elgseder's and Goldman's study (6), in which 108 upper limbs of American origin cadavers were dissected. According to this study, the most common length of the communicating branch was about 1.8 cm. In addition to these findings, Ballesteros et al. (2015) (7) found such branches with 5.78 cm length, significantly longer than our case's branch, as well as the case reported by Gelmi et al. (2018) (8) in which the communicating branch extended from the axilla down to the level of the elbow where it finally rejoined the MN beneath the cephalic vein. Finally, in our case the communicating branch that we encountered, gave off no other branch (motor or sensor), in contrast to other cases in the literature, as the one described by Patil et al. (9).

As already referred, the existence of a communicating branch arising from the MCN downwards to the MN is not a rare variation. On the contrary, a neural branch originating from the MN to the MCN, is not as common. In Ballesteros' study, MCN to MN branch was present in 17% of the upper limbs dissected, while the opposite was observed only in the 2.8% of the specimens. Also, according to Venieratos et al. 158 upper extremities were dissected and no MN to MCN branch was detected. Finally, Mat Taib et al. (2017) (10) found a MN to MCN branch in 13.6% of a total of 44 upper extremities, while Maeda et al. (2009) (11) dissected 453 axillae and found MCN to MN branch in 18.8% of them and MN to MCN branch in 12.8%.

In terms of the clinical significance that such an anatomical variation may have, according to El-Falougy et al. (2013) (12) and Wertsch et al. (1982) (13), entrapment of the MCN (usually while sleeping) may cause clinical findings similar to those of the carpal tunnel syndrome or MN lesion, in case of a communicating branch co-existence. Entrapment of the MCN may occur as a result of CBM spasm or more rarely by MCN's compression by the aponeurosis of biceps brachialis muscle, with its tendon against the brachialis muscle's fascia (14). In such a case, if an electromyography is not done, it may lead to an unnecessary operation in order to release the carpal tunnel. Also, this structural change of the axillary region's anatomy may lead to significant complications during an anterior or anteriolateral approach of the shoulder (deltopectoral incision) in terms of humeral fractures management and during an axillary block by anesthesiologists as well.

## Conclusion

The case described above is among the most common anatomical variations of the brachial plexus. However, being aware of such structural abnormalities, it is of a non-negligible practical significance, especially for surgeons operating in the axillary region and managing humerus fractures or anesthesiologists who perform axillary nerve blocks for operations in the upper limb.

**What Is Already Known on this Topic:**

*Anatomical variations of the brachial plexus are very common. Those concerning the median and the musculocutaneous nerve have the most significant clinical impact. Many attempts to classify structural irregularities of the brachial plexus, have contributed to perform safe operations in the axillary cavity, by avoiding disastrous neural complications.*

**What this Case Adds:**

*As a complementary in the existing literature, the anatomical variation of a communicating branch between the median and the musculocutaneous nerve, contributes a rare case. The clinical analogue of this finding might be the entrapment of the musculocutaneous nerve that may cause clinical findings similar to those of the carpal tunnel syndrome. It is of paramount importance the awareness of such structural abnormalities in order to further evaluate the anatomy of the axillary cavity, before performing minimally invasive or surgical procedures.*

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## Gisela Januszewska (née Rosenfeld), an Austro-Hungarian ‘Woman Doctor for Women’ in Banjaluka, 1899–1912

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The focus of this article is on the biography and medical activity of Gisela Januszewska (née Rosenfeld) in Austro-Hungarian (AH) occupied Bosnia and Herzegovina (BH) between 1899 and 1912. Rosenfeld, later Januszewska and then Kuhn(ová) by marriage, was the fifth of a total of nine official female physicians who were employed by the AH administration to improve the health and hygienic conditions among Bosnian and Bosnian Muslim women. In 1893, Gisela Kuhn moved from Brno, Moravia to Switzerland to pursue her medical studies; she was awarded her Doctorate in Medicine (MD) from the University of Zurich in 1898. In the same year, she took up her first position as a local health insurance doctor for women and children in Remscheid but was prohibited from practising in the German Empire. In 1899, she successfully applied to the AH authorities for the newly established position of a female health officer in Banjaluka and began working there in July 1899. She lost her civil service status upon marrying her colleague, Dr Wladislaw Januszewski, in 1900 but carried out her previously officially assigned tasks as a private physician. In 1903, she was employed as a ‘woman doctor for women’ at the newly established municipal outpatient clinic in Banjaluka. Upon her husband’s retirement in 1912, the couple left BH and settled in Graz, Styria. After, World War I Januszewska ran a general medical practice in Graz until 1935 and worked as a health insurance-gynaecologist until 1933. She received several AH and Austrian awards and medals for her merits as a physician and a volunteer for humanitarian organisations. Upon Austria’s annexation to Nazi Germany 1938, however, she was classified a Jew and was deported to Theresienstadt concentration camp (Terezín, Bohemia), where she died in 1943. **Conclusion.** Gisela Januszewska, née Rosenfeld (1867–1943) viewed her medical practice as a social medicine mission which she put into practice as a ‘woman doctor for woman’ in Banjaluka, BH (1899–1912) and Graz, Austria (1919–1935).

**Key Words:** Gisela Januszewska ■ Female Health Officer ■ Bosnia and Herzegovina 1878–1918 ■ Banjaluka ■ Social Medicine.

### Introduction

In 1878/79, the Austro-Hungarian (AH) Empire implemented a modernisation policy in occupied (formerly Ottoman) Bosnia and Herzegovina (BH) with the aim to transform the country—administratively and institutionally—into one of its provinces as quickly as possible. In order to achieve the public health and hygiene aspects of this goal, the AH administration established a public health infrastructure of hospitals at all administrative levels within just 20 years (1). In addition, the AH public health administration implemented a series of social

medical measures in rural BH, the introduction of which had been demanded by social reformers and Czech feminists for the industrial core regions in vain. In line with this reform concept, publicly employed female doctors were conceptualised to replace the then-popular medical consultation with midwives in order to create low-threshold access to modern public health utilisation for poor (i.e., working-class) women and children. Therefore, Czech and social-democratic feminists had demanded the admission of female doctors to practice in Austria since the late 1880s. While social medical institutions in the Austrian crownlands did not employ female

doctors,<sup>1</sup> the AH military administration of BH introduced the office of female doctors, although under the premise that (rural) Muslim women were their primary target population.

The first three female health officers—Anna Bayerová (Tuzla, 1892), Bohuslava Kecková (Mostar, 1893–1911), and Teodora Krajewska (Tuzla, 1893–1899)—had no other guidelines than to offer free treatment to local women and to visit Muslim women at home in order to guide them to modern standards of personal and public hygiene. According to an official public health report published in 1903, AH doctors had found offering their private practice to rural locals to be unrewarding. Arguing that doctors did not dispose of adequate facilities and that rural patients were reluctant to visit physicians at home, an ordinance dated January 22, 1897, stipulated that ‘community outpatient clinics’ had to be set up in all district towns to look after the ‘poor urban and rural population’ on a ‘neutral ground’ (2). The quickly expanding system of outpatient clinics ensured a genuine breakthrough in public health utilisation in BH. Nearly 50,000 patients were treated in the newly established public wards in 1901, not including the urban hospital outpatient clinics (2).

The outpatient clinic system was also established as the framework for the office of female doctors, which was expanded by two posts in Banjaluka and Sarajevo in 1899. The newly recruited official female physicians were expected to work predominantly as ‘woman doctors for women’ in public outpatient clinics.

Against this background of the reorganisation of the office of an AH female physician in BH, the biography and professional activities of Gisela Januszewska, recruited in 1899, are discussed.

### Gisela Januszewska’s Biography

Gisela Januszewska (Picture 1) is usually considered to be Austrian because she was born near Brno in

<sup>1</sup>The responsible Ministry of the Interior repeatedly stated with regard to the Austrian crownlands that the popular health care provided by state-certified midwives was sufficient and that a public health system based on the health care provision by academically trained physicians was not needed.

Moravia into a German Jewish family and assumed the Austrian nationality in 1918. In Austria, her characterisation as the older sister ‘Gisel’ (‘Gisl’) of the humorous Austrian writer, Alexander Roda Roda<sup>2</sup> (3) (Picture 2) likely exceeds her reputation as an Austrian female pioneer of medicine and ‘woman doctor for women’ in Banjaluka and, after World War I, in Graz.



Picture 1. Gisela Januszewska (née Rosenfeld). Her youthful portrait is used on a card dated July 28, 1906 containing a note written by Sándor Roda Roda to “[d]earest Gisl”. Published with kind permission of Vienna Library, Vienna, Austria (Fotografien von Gisela Rosenfeld, Wienbibliothek im Rathaus, Handschriftensammlung, Teilnachlass Roda Roda, ZPH 670, Archivbox 8).

<sup>2</sup>*Nom de plume* of Sándor (Alexander) Friedrich (Ladislaus) Rosenfeld-Roda, 1872–1945; cf. Vlado Obad. Roda Roda und die deutschsprachige Literatur aus Slawonien. Mit einer Anthologie unbekannter Texte. Vienna, Cologne, Weimar, 1996; Rotraut Hackermüller: Einen Handkuss der Gnädigsten. Roda Roda – Bildbiographie. Vienna, Munich, 1986.



Picture 2. Sándor (Alexander) Friedrich (Ladislaus) Rosenfeld-Roda, 1872–1945 brother of Gisela Januszewska (née Rosenfeld).

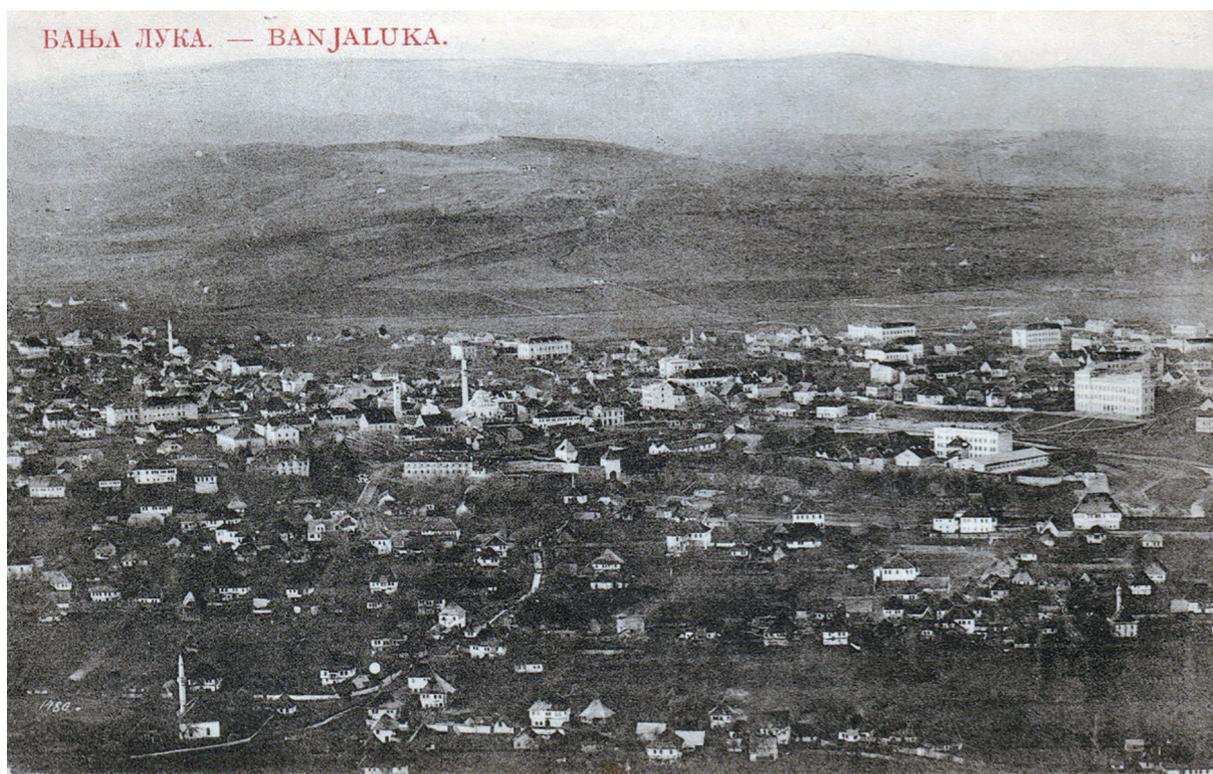
The most authoritative account of Januszewska's life and achievements is provided by Reinhold Aigner's (1979) collective biography of the female pioneers of medicine at the University of Graz (4). Aigner's biography of Januszewska is the basis of her frequently-quoted entry into an Austrian women's website of the Austrian National Library (5) and the 'Encyclopaedia of Austrian Women' (6, 7). Aigner is also cited as an authoritative source on Januszewska in Stibor Nečas' collective biography of the AH female health officers in BH (8). Nečas restricted his remarks on Januszewska to her activities as an official and semi-official female physician in Banjaluka, which were neglected by Aigner. Due to Januszewska's interlude in German Remscheid, which provoked a conflict that eventually resulted in the accreditation of female physicians in the German Empire, she is mentioned under her first marital name, 'Kuhn', in a series

of publications on female doctors in the German Empire (9–12). A recent biography by the Bosnian historian Milena Karapetrović (2014) draws on all available sources and is devoted to Januszewska as the first female physician in Banjaluka (13, 14).

Gisela Januszewska was born on January 22, 1867, in Drnovice, a rural village not far from Brno in the Austrian crownland of Moravia, today part of the Czech Republic. She was the second of five children of the local tenant and former army officer Leopold Rosenfeld (1829–1901) and his wife Rosalie Stein (1842–1920; 15). The family moved to Slavonia in 1872, where Leopold Rosenfeld assumed the position of a steward of the Puszta Zdenci estate near Orahovica and Našice from Count Ladislav Pejačević (1824–1901), who was the Ban of Croatia from 1880 to 1883. Gisela, therefore, spent her early childhood in Slavonia and spoke Croatian besides Czech and German as a native tongue. In Zdenci, the thoroughly assimilated Jewish Rosenfelds adopted the local vulgongame of 'Roda'; thus, Gisela's maiden name is frequently given as 'Rosenfeld-Roda' (16).

After finishing Croatian elementary school, Gisela was sent to relatives in Moravia to attend a higher girls' school in Brno. At the age of 20, she married the much older Jindřich (Heinrich) Kuhn,<sup>3</sup> who was the affluent partner of an import-export cloth trade business based in the same city. The writer Marie Liebermann-Rosenfeld (1875–1935)—Gisela's younger sister, who moved in with her in 1889 in order to attend a private higher girls' school in Brno—remembered according to a contemporary lexicon of German authors, that she had desired to study medicine as a pupil because her sister hardly talked about anything else (17). Against a background of Czech feminists campaigning for 'women doctors for women', in 1892, Gisela finally moved to Zürich to pursue her medical studies. Whether she was accompanied by her husband after he had sold his share in his business to his partner, as indicated by Nečas (8; p. 102), or she had already separated from Kuhn, as

<sup>3</sup>Januszewska's first husband's first name is often given incorrectly as 'Joachim', as an error spread by her brother Roda Roda's memoirs (3).



Picture 3. Banjaluka at the time when Dr Gisela Kuhn started working there. Published with kind permission of Museum of the Republika Srpska, Banjaluka, Bosnia and Herzegovina.

indicated by her brother, Roda Roda (3, p. 209), is uncertain. It is certain, however, that 26-year-old Gisela Kuhn(ová) enrolled at the Medical Faculty of the University of Zurich in 1893. Like most female students of her time, she had to first acquire her qualification for university entrance ('matura'). She commenced her medical studies without further delay and finished a thesis about respiratory diseases in 1897/98 (18). After completing her medical training with an internship at the University Clinic of Obstetrics, she was awarded her medical doctorate by the University of Zurich on April 12, 1898 (16).

On June 1, 1898, Januszewska was employed by the local agency of the German general health insurance (AOK) in Remscheid in the Prussian Rhine Province. Together with two other physicians, she was expected to look after the 645 female health insurance members and their children (11). After a few weeks, the supervisory authority prohibited her from practising because she had no

accreditation for the German Empire, which, at that point, she could in no way have provided, due to her female gender. While she finally succeeded in having her Swiss diploma recognised by the German Empire, she could not begin to practice because of another persisting conflict between the AOK and the German medical association. The German physicians repudiated the AOK system of in-house outpatient clinics and eventually went on strike. The conflict was only resolved after Januszewska left Remscheid because she had meanwhile successfully applied for the position of an AH female health officer in BH (11).

In the summer of 1899, Januszewska started to practice in Banjaluka (Picture 3) as a provisory female health officer in the rank of a captain of the AH army, yet her status never became permanent, because, during her first year in Bosnia, she rearranged her private life by divorcing Jindřich Kuhn and calling herself Rosenfeld again. As noted by Nečas (8, p. 104), she had herself baptised 'Lud-

mila' (as a second Christian name) with a Czech godmother. After her conversion to Catholicism, she married her Polish colleague Wladyslaw Januszewski<sup>4</sup>, 24 years her senior, who held the office of a district physician of Banjaluka and was her superior. As a married woman, she had to resign from her office, although, as shall be seen, she was not replaced, but pursued her previously officially assigned duties as a private doctor on behalf of the AH public health authorities.

When Januszewska's husband retired in 1912, the couple left Bosnia and settled in the capital of the crownland of Styria, Graz, a city in which many retired AH officers who had served in Croatia settled. In 1913, Januszewska enrolled at the University of Graz to have her Swiss diploma recognised in Austria. In 1915, the Austrian daily press reported that Gisela Januszewska, a physician formerly active in BH, had been awarded her 'third' Austrian MD at Karl-Franzens-University in Graz on February 24 (19). She then signed up for the AH military medical service, which had been established at the start of World War I in 1914, and remained the only military female physician in Styria and Carinthia. After working as an internist at the Styrian Provincial Hospital and the 'second hospital of the reserve' in Graz-Eggenberg, she was dismissed in 1918. Having been widowed in 1916, she started to run a practice as a general practitioner in Graz on January 11, 1919 (4). She also pursued her personal mission to work as a 'woman doctor for women' and entered into a contract with the Union of Health Insurances in Styria and Carinthia ('Verband der Krankenkassen für Steiermark und Kärnten'), where she was active as a gynaecological consultant. In the daily labour press, she announced that her gynaecological practice was open to all health insurance members and their dependents every day, including Sundays and legal holidays (20, 21). She treated the needy for free, supported them personally, and made her professional work available to a relief organisation of 'the widows and orphans of civil servants' ('Verein der Witwen und Waisen nach

öffentlichen Beamten'). She continued her medical service for this organisation even after she had resigned from her medical practice in December 1935 (4). In 1928, Januszewska was awarded the title of 'Medizinalrat' as the second female physician in Austria<sup>5</sup> and received a high-ranking Austrian order of merit ('Ritterkreuz des Österreichischen Verdienstordens') in 1937 (4). Upon Austria's annexation into Nazi Germany in 1938, Januszewska's brother Alexander Roda Roda, who had lived in Graz since 1933, asked her to leave the country with him, but she decided to stay. She was forced to leave her home in Graz in 1940 to be 'resettled' in Vienna. From Vienna, she was deported to Theresienstadt concentration camp, where she died a 'natural death'<sup>6</sup> at the age of 76 on March 2, 1943 (4).

### Januszewska's Professional Activities in Banjaluka, 1899–1912

Januszewska received her appointment as a provisory AH official female doctor in BH on May 24, 1899. She arrived in Banjaluka, then a city of 25,000 inhabitants, on July 9. She was sworn into her office and started work on the same day (8). From July 10, 1899, until July 20, 1900, in her official function, she treated 1,431 patients, among whom were 936 women and 479 children (2). From its very start, her outpatient clinic was evidently well utilised by women of all religions, especially considering that she was frequently dispatched to combat epidemics elsewhere in the districts of Banjaluka and Bihać. In Autumn 1899, for example, Januszewska was detailed to Dubica to combat smallpox and carry out the necessary 'emergency vaccinations' (22). In 1900, she performed emergency vaccinations in the Cazin subdistrict of

<sup>5</sup>The first female "Medizinalrat" in Austria was Gabriele Possanner von Ehrental (1860–1940) who was awarded this title in 1928; she had achieved the "nostrification" of her Swiss diploma in 1897 as the first Austrian woman and practiced from that point on in Vienna.

<sup>6</sup>According to Januszewska's death certificate from the Nazi ghetto of Theresienstadt.

<sup>4</sup>Wladyslaw Januszewski (1841?–1916), cf. <https://pl.communications-unlimited.nl/bosnia-i-hercegowina-polska-imigracja/>



Picture 4. Gornji Šeher: The Muslim quarter of the Banjaluka at the time when Dr. Gisela Januszewska (née Rosenfeld) managed a newly established municipal outpatient clinic. Published with kind permission of Museum of the Republika Srpska, Banjaluka, Bosnia and Herzegovina.

the district of Bihać.<sup>7</sup> Her brother Roda Roda, who was visiting Gisela in 1902, remarked that during her first winter, his ‘brave sister’ had been busy riding from village to village over ‘half of Bosnia’ to vaccinate approximately 20,000 children who were lifted up to her saddle by gendarmes (3). During the interwar years in Austria, on occasions such as the awarding of orders her merits as an ‘epidemic doctor’ (Epidemiearzt) in combating smallpox, typhoid, and typhus, as well as endemic syphilis and osteomalacia in BH used to be underlined (4–7). Gisela Januszewska—as with Teodora Krajewska in Sarajevo and Bohuslava Kecková in Mostar—was also commissioned to give hygiene lessons at a higher girls’ school in Banjaluka (23).

<sup>7</sup>The AH health authorities had been particularly concerned about the frequent occurrence of local smallpox epidemics in the Bosanska Krajina, next to the Croatian border (see Austrian National Archives, AVA Ministerium für Inneres, Allgemein 36/5, K. 1097).

After she had resigned as the official female doctor of Banjaluka upon her marriage in 1900, she seamlessly continued her medical work as a local private doctor. She opened a practice for general medicine in Banjaluka and worked, when needed, in official vaccination campaigns or diagnosed syphilis on behalf of the AH public health authorities in the rural areas of the district.<sup>8</sup> From 1903, she also managed a newly established municipal outpatient clinic in Gornji Šeher (Picture 4), the Muslim quarter of the city. According to Nečas (8), she received a monthly salary of 600 AH ‘Kronen’ besides 200 ‘Kronen’ per month for her expenses from the city government, which was subsidised by the AH administration in Sarajevo. Although the AH public health authorities’ choice of the lo-

<sup>8</sup>An AH medical commission charged with surveying the spread of ‘syphilis and other skin diseases’ in BH (1890) found endemic syphilis to be particularly widespread in the subdistrict of Kotor Varoš, among other places (2); cf. also Roda Roda’s memoirs (3, p. 520c.)

cality demonstrates that they were attempting to ensure that the female Muslim clientele was given special consideration, Januszewska seems to have been remembered as the fairly popular first ‘woman doctor for women (and children)’ in Banjaluka (13). According to her own records, between 1900 and 1909, Januszewska treated a total of 20,232 (female) patients, most of whom had visited her in her private office or in Gornji Šeher (24). This figure also covers all those women who sent for her, those whom she visited in their homes, and those whom she treated while detailed as an ‘epidemic doctor’ elsewhere. She was consulted for all kinds of diseases and disorders and also performed minor surgical interventions. She treated 17.35% of all her patients for osteomalacia (adult rickets), a then widespread affliction, and acquired a reputation as a particularly capable physician by helping women suffering from ‘rheumatic’ (in fact osteomalacic) pains.

### Januszewska’s Publishing Activities

In addition to her thesis on *Tracheitis membranacea* (1898), in 1910, Januszewska published her observations on osteomalacia as a widespread disorder in Bosnia in a German language Austrian medical journal (25). At that point, progressive and occasionally serious adult rickets or osteomalacia was a controversially discussed ‘female disorder’, the cause of which the German scientific community prominently considered to be female reproductivity (25). Teodora Krajewka, an AH official female doctor in Tuzla and Sarajevo, advocated this theory after she discovered that osteomalacia was widespread in some parts of the districts of Tuzla and endemic in the mountainous quarters of Sarajevo. Krajewka explained the large number of osteomalacia cases, which occurred predominantly among Muslim women, as the result of frequent pregnancies and ‘other functions of the sexual sphere (sexual intercourse, menstruation, lactation)’ (26), besides previously established factors, such as malnutrition and a lack of sunlight (27, 28). However, Januszewska advocated a completely different view, which she based on no fewer than 3,510 cases of

osteomalacia that she had diagnosed between 1900 and 1909, mainly in Banjaluka (25). The exceedingly high number of patients suffering from an acknowledged ‘rare’ disease was due to the fact that Januszewska did not only classify cases with visual bone deformations as osteomalacic. Instead, she posited that the osteomalacic deformation of bones was only the ‘third degree’ of symptoms that started with ‘rheumatoid’ pains, especially in the asternal ribs (*costae spuriae*), with pressure points on the bone-cartilage connections and pains in the lower back that extended into the thighs (‘first degree’) and eventually resulted in a duck-like walk<sup>9</sup> (‘second degree’). Subsequently, the softened bones begin to bend in the upper part of the spinal column, with the result of a scoliotic curvature of the spine, the chin approaching the sternum. The pelvic area deforms through the inward indentation of the coccyx and a beak-shaped protrusion of the pubic branch, with the effect of the much-described osteomalacic cordate (cardiform) pelvis (‘third degree’).

Although Januszewska considered that osteomalacia might be exacerbated by (numerous) pregnancies and breastfeeding,<sup>10</sup> she was convinced that osteomalacia was predominantly caused by malnutrition and a lack of sunlight (25). As evidence, she noted the significant frequency of the disorder among poor urban Muslim women compared to non-afflicted rural Muslim women working outdoors with uncovered faces (25). She further observed the increased frequency of symptoms in the cold season and its significant co-occurrence with tetany. She also noted the benign character of ‘Bosnian’ osteomalacia, whereby patients usually recovered quickly when administered the common medication consisting of phosphorus in cod liver oil.<sup>11</sup>

<sup>9</sup>Today also called a ‘waddling gait’.

<sup>10</sup>She gives, among others, a case description of a patient whom she ‘persuaded’ to have a Porro operation at the provincial hospital in Sarajevo, thus also indicating that she did not perform operations of this kind herself.

<sup>11</sup>Against a background of German gynaecologists treating osteomalacia as a disease of the ‘female reproductive system’ with oophorectomy (castration), the Austrian internist Maximilian Sternberg had demonstrated, based on French

Although Januszewska's contribution did not refer directly to Krajewska's conceptualisation of osteomalacia as a disorder of Bosnian Muslim women, she contradicted Krajewska's interpretations point-by-point. Based on Januszewska's abundant empirical data, she considered the cause of the disease to be neither predominantly sexual (puerperal) nor, as she termed it, regarding Krajewska's suggestion, 'racial' (25). Without a theoretical discussion of osteomalacia, she demonstrated that osteomalacia did not prevail among 'Bosnian Muslim women' but among poor urban women who never saw daylight without covered faces; such women were predominantly—yet not exclusively—Muslims.

### Concluding Remarks

Gisela Januszewska, née Rosenfeld (1867–1943), was born as the second of five children of an aspiring, fully assimilated Jewish middle-class family in Brno and grew up partly in a village near to Osijek in Croatia. She is, therefore, considered to have had a typical late 19<sup>th</sup>-century Austrian multinational bourgeois background, yet while her younger brother Alexander Roda Roda claimed to be Croatian (3), Januszewska adhered more to Czech tradition. In 1899, she had herself baptised 'Ludmila' after the Czech saint, Ludmila of Bohemia, and insisted on feminising her marital names in line with the prevailing Czech custom. Her career choice was heavily influenced by Czech feminists' campaigning for 'women doctors for women' and she considered her medical practice to be a social medicine mission, be it in Remscheid, Banjaluka, or Graz. Her marriage after one year of practice in Banjaluka might be interpreted as taking the opportunity to pursue her mission without serving as an AH official with a military rank. There is no sign, however, that she regretted leaving Bosnia in

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doctrine (Armand Trousseau) in the early 1890s, that osteomalacia (as rickets) could be cured with phosphorus in cod liver oil (29), which was the common therapy for rickets at the time. At first, this therapy was applied under the incorrect premise that the phosphorus—not the cod liver oil—was the essential curative agent.

1912 to settle in Graz, where she put her original plan to work as a health insurance 'woman doctor for women' into action. The official recognition of her merits as a physician, humanitarian volunteer, and philanthropist in the AH Empire and Austria did not save her from deportation and death in a Nazi concentration camp in 1943.

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## John Irvine Hunter (1898-1924): Australian Anatomist and Medical Educator

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### Abstract

This paper focuses on the short, but brilliant career of the Australian anatomist and medical educator, John Irvine Hunter. Hunter's biography is presented within the context of the early twentieth century anatomy and medical education. John Irvine Hunter was not only the youngest ever Professor of Anatomy at the University of Sydney, but he was also undeniably brilliant with regard to teaching and researching anatomy, physiology and anthropology. While his short career answered many questions in these fields, it raised more questions regarding what Hunter may have accomplished if only he had been given the chance. These unanswered questions have spawned what we now affectionately refer to as the "Hunter Legend". His most ambitious work on the dual innervation of striated muscle, while eventually disproven, formed an important stepping-stone in the bridging of anatomy and physiology. His thought-provoking concepts were viewed with much intrigue, and at the time were very well received. **Conclusion.** Hunter remains one of the most prominent and inspiring figures in the history of Australian anatomy and medicine.

**Key Words:** John Irvine Hunter ▪ Anatomy ▪ Medical Education ▪ University of Sydney ▪ Australia.

### Introduction

It is uncommon that an individual is prolific to the level of referring to their life as a "Legend", but that is precisely what John Irvine Hunter achieved in his short 26 years. Undeniably brilliant, and loved universally by his students and colleagues, he amassed an impressive research portfolio and gained a reputation as an excellent teacher and a wonderful friend. His first name and surname, shared with the famous Scottish surgeon/anatomist John Hunter (1728-1793), seemed to have "predestined" him for a career in anatomy and medicine. Indeed, he will be remembered for bridging the gap between anatomy and physiology, as well as heading one of the most important departments at a pioneering Australian medical school.

What is less well known of Hunter, and perhaps a driving force behind the development of the "Hunter Legend", is the important role that he played in a complex social climate in

aftermath of World War I. His mentors obviously saw something in Hunter that was needed in a turbulent time not only for the medical profession, but also to generate optimism and restore faith in the public. His astronomical rise was met with mostly praise, but also with criticism. By the time of Hunter's death, he was a treasured teacher, researcher and human being who served his role with immense passion and pride. Hunter rightly forged his legend, and firmly established himself as one of the great anatomical minds of his time.

This paper explores the life, academic career and untimely death of Professor John Irvine Hunter, and will provide a snapshot of the world of medical anatomy and research in the early 20th century.

### Early Life

John Irvine Hunter (Figure 1) was born 24 January 1898 in Bendigo, a town in Australia's state of

Victoria, where his father originally worked as a gold digger and later a furniture salesman. John Hunter, or as his family lovingly called him “Jack”, had a tumultuous upbringing, at least in part due to a protracted course of treatment for his bilateral clubfoot deformity. It was through this chronic and ongoing condition that he likely developed the patience and persistence that later formed the cornerstone of his academic prowess (1). His family were not wealthy, and they were often afforded concessions for the expensive treatment of his clubfoot deformity that he received from Dr W. L. Long, a local surgeon in Bendigo. His treatment was ultimately successful and afforded him the ability to play numerous sports and resume most of the typical childhood activities. Hunter later reflected back on these experiences, and wrote to Dr Long, thanking him for his generosity (1, 2).

At around the age of eleven, he was again struck down by illness that was characterised by pleurisy and blood-stained sputum, which some believe may have been pulmonary tuberculosis. Around the same time, his parents’ marriage broke down, and he was sent to live with his mother’s sister and her husband in Albury, New South Wales, Australia.

Hunter attended Albury Superior Public School and excelled academically. He excelled so much so that his Latin teacher and headmaster pushed for his admission at the highly regarded Fort Street High School. He was accepted to Fort Street and again, he excelled. From his time at Fort Street he was known not only for his academic and intellectual excellence, but also for his charming disposition (1). Even from an early age, Hunter demonstrated an innate ability to teach and educate his peers and was known for his ability to convey complex ideas in an understandable manner for his fellow students. This innate ability was again observed in medical school when he was noted to fill up entire halls to teach anatomy to his peers, many of which were senior to him. His headmaster at Fort Street later afforded that many people “never realised that he was such an incomparable genius”. This “genius” as many suggested, was probably masked or perhaps overlooked by the physical and economic hardship that he endured as a child (3, 4).

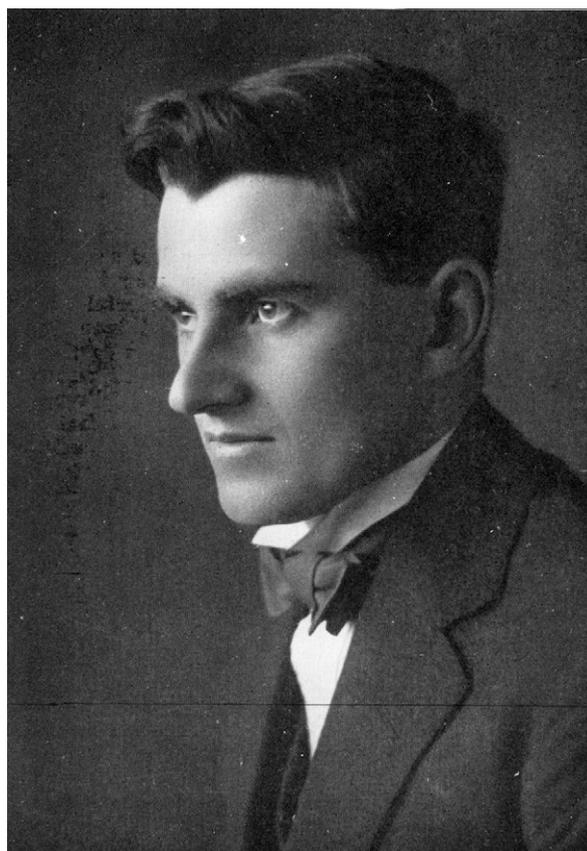


Figure 1. John Irvine Hunter in 1920 (courtesy of the University of Sydney Archives).

Upon completion of his time at Fort Street, he was able to gain entry to study medicine at the University of Sydney. After some period of uncertainty as to whether this would be financially feasible, he was ultimately able to secure a bursary that would cover his course fees and contribute a small amount towards his living expenses (3). Fortunately, his uncle was able to provide additional financial security to ensure that Hunter was able to study medicine, which he commenced in 1915.

### Medical School

Hunter commenced his studies during a turbulent time, not only for the medical profession but for Australia and many other parts of the world generally, as this was during the height of World War I. The founder of the medical school at the University of Sydney, Sir Thomas Anderson Stu-

art, had argued strongly against medical students enlisting in the defence forces prior to completion of their studies. Conversely, the Challis Professor (an eponymous professorship at the University of Sydney, honoring the nineteenth century philanthropist and University's benefactor John Henry Challis) of Anatomy at the time, and future great friend of Hunter's, James Thomas Wilson, had instead argued that the medical students should be enlisting (1).

Hunter, in 1916, enlisted in the forces, along with many of his colleagues, and after farewelling his loved ones and preparing to commence training, he actually received an official notice ordering him to return to his studies. It much later emerged that it was in fact Wilson who had contradicted his earlier encouragement towards his students to enlist and had instead ordered for Hunter to resume his studies. Wilson had observed, even in those early stages, something exceptional in Hunter, and had decided that this level of brilliance should be instead nurtured. The interest that Wilson had taken in the young Hunter is of particular importance, not only because he interrupted Hunter's plans of enlisting in the defence forces, but because it was Wilson who revitalised the department of anatomy at the University of Sydney. He did this in a number of ways, namely by reclaiming histology from the physiologists and incorporating histology into the teaching of anatomy, but also by laying the groundwork to facilitate Hunter's meteoric rise through the ranks of the university only a few years later. Perhaps the latter aspect of Wilson's plan for the university was based on the tumultuous social climate that was apparent in the aftermath of World War I and believed that by placing some faith in the youthful genius of Hunter, perhaps that could help to reinvigorate and promote optimism in society once again.

During his time at university, he rapidly became a source of anatomical knowledge for his colleagues, even those far more senior than him. He was required to run his anatomy tutorials off campus at the Darlington Town Hall, as the conventional lecture theatres at the University of Syd-

ney were not big enough to accommodate the students attending his lectures (1)! The notes that he wrote for his review of neuroanatomy were reproduced and given to all students as a core text. He obtained the highly prized role of prosector in just his second year of university, and he was highly regarded by his colleagues both as an excellent medical student, but also as an excellent human being.

Just as he succeeded in his secondary schooling, Hunter again demonstrated his academic excellence at university level, winning all major awards in his degree, aside from in his first year of study. In his final year of medical school, he won the Sandes prize for best surgical essay, a considerably large paper on visceral abdominal pain, that was later published in the Medical Journal of Australia (5). In addition to this, he also graduated with first class honours and claimed the university medal in medicine.

### **Professorship and Overseas Travel**

After graduating with a Bachelor of Medicine, Bachelor of Surgery degree in 1920, Hunter had the difficult choice of pursuing a career in academia or clinical medicine. It is likely that he would have succeeded regardless of what option he ultimately chose, having demonstrated an innate ability in clinical work as well as in academic roles. Ultimately though, he decided to pursue a career in anatomy, and immediately following his graduation he was appointed as an anatomy demonstrator at the University of Sydney (Figure 2).

The duties of demonstrators, at the time, were similar to those of the tutors/demonstrators in contemporary Australian Medical Schools and comprised assistance in practical instructions and dissections carried out in the anatomy laboratory. Hunter was renowned by his students and colleagues as being a brilliant teacher, and again demonstrated his innate ability to convey complex information in an approachable manner (1, 2).

His role as a demonstrator, however, was short lived. Challis Professor of Anatomy, James Thomas Wilson, resigned the very same year, and moved to Britain to take the Chair of Anatomy at the Uni-



Figure 2. The Anderson Stuart Building (formerly known as the "Old Medical School") at the University of Sydney, where the Department of Anatomy has been located (photograph by Sumple, licensed under CC BY-SA).

versity of Cambridge (6). As a result of Wilson's resignation and his desire to see Hunter promoted at the university, Hunter was appointed as Associate Professor of Anatomy. His astronomical rise through the ranks of the university was met with both positivity from his colleagues and negativity from those who did not know him. The Sydney Morning Herald was particularly critical, using Hunter's appointment as a catalyst to suggest that the university had lost touch with the citizens of New South Wales and claimed that the University of Sydney would "sink back into its peaceful obscurity" (1). As previously mentioned, Wilson's ambitious and optimistic promotion of Hunter was perhaps a reflection of the social climate and the need for change. Despite initially being met with criticism, Hunter's promotion was ultimately praised universally.

In 1921, Hunter was granted one year's leave on full pay to travel overseas and continue his studies,

primarily basing himself in London and travelling throughout Europe. Prior to leaving Australia, he became very interested in the work of orthopaedic surgeon Dr Norman Royle, who would later become both a close friend and research associate of Hunter's. In this one-year period overseas, Hunter was able to further his studies of anatomy, physiology and anthropology, as well as gain important life experience, that was later reflected in his correspondence with his aunt (1).

With his close friend and fellow anatomist and anthropologist, Grafton Elliot Smith, Hunter studied the famous Piltdown Man fossil in East Sussex, and while ultimately incorrect, they contributed to the growing body of research that this specimen generated (7). They believed that the skull of the Piltdown Man did indeed represent a "missing link" in human evolution, due to an abnormal mandible and the relatively small cranial fossa. However, it was later determined that the Piltdown

remains from which these hypotheses were generated was an artificially produced chimera, made up of both orangutan and human bones. It was not until much later that the Piltdown Man hoax was adequately exposed (8).

Hunter's association with Smith was no coincidence, with Smith being a student and somewhat of a protégé of Wilson's at the University of Sydney. Smith was another of those students that had been identified as exemplary at an early stage in their career and had been assisted by Wilson to reach their full potential. Smith was essentially the embodiment of British anatomy and revolutionised the teaching and study anatomy when he was appointed chair of anatomy at University College, London (9, 10). After the World War I Smith became one of the key architects of "the new British anatomy project", which enlivened the discipline of anatomy by reincorporating "physiology and other allied disciplines, whilst continuing to maintain gross anatomy at the centre" (10). None of this revolutionary work by Smith would have been possible without the very large amount of financial support bestowed upon him by the Rockefeller Foundation. Wilson's ability to identify future leaders (such as Smith) and nurture them was seemingly passed onto Smith, who dispatched his trainees around the world, and in 1938 around 20 of his former trainees held chairs of anatomy around the world. They introduced Smith's "holistic" philosophy to Medical School across the globe and established numerous centers of teaching and research excellence (11). Once again, the fact that both Wilson and Smith had taken a particularly close interest in Hunter begs the question of what Hunter would have become if he had not met his untimely demise.

When in London, Hunter had also been closely following the research of Professor Nikolai Konstantinovich Kulchitsky (who discovered enterochromaffin or Kulchitsky cells), particularly that of his work on innervation of striated muscle (12). As chance would have it, Kulchitsky, a Russian refugee, had recently commenced working at University College in London, alongside Grafton Elliot Smith, and as such, Hunter was able to form a close

personal and working relationship (1). Kulchitsky's initial research demonstrated two nerve fibre variants that were involved in the innervation of striated muscle, and this formed a major component of Hunter's most ambitious work that he later undertook, which was received with much curiosity and intrigue at the time.

Hunter also visited and worked in various centres of excellence in the continental Europe. While on the Continent Hunter worked with renowned Dutch neurologist and anatomist Ariëns Kappers. Under Kappers he carried out research on the Kiwi brain, which formed part of the work in neuroanatomy for which he would be awarded his Doctor of Medicine degree from the University of Sydney, in 1924 (13). Hunter returned to Australia in 1923 and assumed the role of Challis Professor of Anatomy at the University of Sydney. It was then that he resumed his close collaboration with the orthopaedic surgeon, Dr Norman Royle, and through countless experiments attempted to treat spastic paralysis by performing sympathetic ramisection procedures initially on goats and later on humans too (14). From his newfound knowledge overseas, Hunter and Royle postulated the dual innervation of striated muscle. Specifically, they argued that skeletal muscle fibres that received somatic innervation were involved in voluntary movement, but that the same muscles would also receive a second sympathetic innervation that would maintain a plastic tonus and would aid in supporting posture (15).

They further extended this hypothesis, postulating that this plastic tonus played an important role in poliomyelitis, myasthenia gravis, myotonia atrophica and spastic paralysis. Hunter believed that spastic paralysis was a pathological manifestation of the plastic tonus controlled by sympathetic innervation, and as such, he and Royle believed that a sympathetic ramisection could effectively treat this condition. Furthermore, due to this dual innervation, a sympathetic ramisection would have no adverse effect upon voluntary movement, as the somatic muscle innervation would remain unaffected (16-18). Royle actually performed this procedure on more than 80 pa-

tients, many of which are said to have had a good clinical response to the treatment.

At the time, Hunter and Royle's research garnered much interest from around the world, and in 1924 they travelled to New York where they delivered the John B. Murphy oration after being invited by the American College of Surgeons. The oration was very well received, and after demonstrating film of the outcomes from the sympathetic ramisection, some 2,500 surgeons in attendance broke into spontaneous applause (1). They were also invited to lecture at Harvard, Philadelphia, Chicago, Montreal and Toronto. While in Chicago, he received an invitation from Grafton Elliot Smith to lecture in London, which he promptly accepted (1). After delivery of his lectures in London, he travelled to Cambridge, where he spent some time with his close friend and mentor Wilson, however upon returning to London, he fell ill, and was admitted to hospital.

### **Death and Posthumous Events**

Hunter was admitted to University College Hospital and diagnosed with typhoid fever. It is believed that he became infected during his time in the United States. He had strongly positive serological investigations that confirmed the diagnosis, and he died a few days later on December 10, 1924, as a result of cardiac failure. During his final few days, Wilson remained by his bedside the entire time. News of his death reached Australia the following day (1).

There was controversy surrounding Hunter's death, with the exact cause being debated by many medical professionals back in Australia. There were a number of reasons for this. Firstly, the fact that Norman Royle returned from overseas also being unwell, and later developing Parkinson's disease lead many to consider that perhaps both he and Hunter had encephalitis, not typhoid fever. Secondly, typhoid was seen as a socially unacceptable condition at this point in history, and many did not want to accept that one as brilliant as John Hunter could have succumbed to such a deplorable condition. Ultimately however, the

medical team caring for Hunter when he died was unanimous in their diagnosis of typhoid fever, and along with his strongly positive serology, there can be little argument as to what his cause of death was. Hunter was survived by his wife, Hazel McPherson, a nurse from Royal Prince Alfred Hospital. The two had become engaged upon his return from Europe in 1923 and were married in 1924. Hazel gave birth to a posthumous son, Irvine John Hunter, in September of 1925 (1). It was Hunter's son who would establish and fund the John Irvine Hunter Memorial Lecture series, carried out under the auspices of the University of Newcastle, Australia. John Irvine Hunter Jr. noted, "I feel that series is a suitable means of remembering him because it epitomizes what he was striving for – excellence in education and research" (19).

While Hunter's work on dual innervation had been highly ambitious and well received during his tour of Europe and the United States, it was since disproven within around 5 years of his death. While the original hypothesis has been disproven, the potential that Hunter demonstrated is undeniable. It is important to remember Hunter also for the wonderful teacher that he was, who was loved by students and colleagues universally. As a researcher and anatomist, he was excellent. Wise beyond his years, and he proposed complex, thought-challenging ideas that ultimately benefited the field of anatomy.

Many have wondered just what Hunter may have been able to offer if he had not succumbed to illness. Perhaps his early demise is one of the reasons that the "Hunter Legend" lives on to this day. Grafton Elliot Smith, in his obituary for Hunter stated, "Had he lived, he might have become the foremost man of science of this age" (3). The world will never know what Hunter may have achieved, but even in his short life he has left a lasting footprint and will forever be known as one of the truly great anatomists and teachers of his time.

### **Conclusion**

In his short but rich life John Irvine Hunter managed to overcome numerous obstacles posed by

global politics, the local economy, personal health and complex family circumstances, rising to the unprecedented academic heights very early in his career. Although the results of some of his research projects are now falsified, at the time when they were presented, they constituted big steps towards understanding the phenomena studied. Hunter's teaching skills and ability to translate complex medical concepts to a language understandable to undergraduate students were extraordinary and he still stands as one of Australia's most remarkable medical teachers. Hunter, his life and work, remain an inspiration for generations of medical students, educators and anatomists.

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