



2021

52

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# SCRIPTA MEDICA

ISSN 2490-3329 (Print)

ISSN 2303-7954 (Online)

ASSOCIATION OF MEDICAL DOCTORS OF THE REPUBLIC OF SRPSKA,  
FACULTY OF MEDICINE, UNIVERSITY OF BANJA LUKA

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Major Adverse Cardiovascular Events After Implantation of Absorb Bioresorbable Scaffold: One-Year Clinical Outcomes

Risk Factors for Coronary Heart Disease and Family Medicine – What Can be Done?

Preparing a Rat Brain Tissue Samples for Acetylcholinesterase Activity Measurement - the MM method

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BANJA LUKA, December 2021





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## Design & Layout

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## Printed by

Grafix s.p., Banjaluka

## Publishers

Association of Medical Doctors of the Republic of Srpska,  
Faculty of Medicine, University of Banja Luka

## Indexed/Abstracted by

EBSCO, Google Scholar, Crossref, SCIndeks, DOAJ, KoBSON, Index Copernicus International (ICI) - Journals Master List, Dimensions, Sherpa/Romeo.

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# Comparative Analysis of Clinical and Laboratory Parameters of Autoimmune and Idiopathic Chronic Urticaria Patients

Đuka Ninković Baroš,<sup>1, 2</sup> Vesna Gajanin,<sup>2</sup> Bogdan Zrnić,<sup>2</sup> Živorad Gajanin,<sup>2, 3</sup> Gordana Katalina<sup>1, 2</sup>

## Abstract

**Background:** According to the cause, chronic urticaria is most frequently divided into autoimmune and idiopathic urticaria. Aim of the paper was to determine the frequency of autoimmune urticaria using autologous serum skin testing and a comparative analysis of chronic idiopathic and chronic autoimmune urticaria by disease course, severity and most common comorbidities.

**Methods:** Analysis covered 64 adult patients of both sexes with chronic urticaria, divided into two groups according to their positivity in autologous serum skin testing (group I with positive test and group II with negative test). General haematological and biochemical parameters, antithyroid antibodies, hepatitis serum markers, *Helicobacter pylori* and *Borrelia burgdorferi* antibodies were performed for patients in both groups. First group patients were treated by autologous blood therapy (autohaemotherapy). The analytical statistical tool SPSS (Statistical Product and Service Solutions) version 20 for descriptive statistics and statistical methods was used. The significance level used was  $p = 0.05$ .

**Results:** The frequency of positive autologous serum test in total population of patients with chronic urticaria was 43.8 %. The average duration of urticaria was 20 months in both groups. Statistically significant difference was found in weekly scores between the studied groups ( $p = 0.032$ ) in favour of chronic autoimmune urticaria with a positive autologous serum test. Subjects with chronic autoimmune urticaria had a significantly higher association with autoimmune thyroid diseases.

**Conclusions:** Direct relation was established between the use of autologous blood therapy in patients with autoimmune chronic urticaria and improvement of the clinical picture.

**Key words:** Autologous serum skin test; Autoimmune chronic urticaria; Idiopathic chronic urticaria.

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### ARTICLE INFO

Received: 16 August 2021  
Revision received: 26 November 2021  
Accepted: 29 November 2021

## Introduction

Urticaria is manifested by the appearance of erythematous, oedematous, usually pruritic urticarial papules and plaques, accompanied by a subjective itching sensation.<sup>1</sup> Urticaria affects persons of all ages. One in five persons will experience episodes of acute urticaria at least once in their lifetime, while the chronic form of the disease has an estimated annual prevalence of about 1 %. Fre-

quency of urticaria in the world ranges between 0.3 and 11.3 % depending on the studied population.<sup>2</sup> Zuberbier classified urticaria according to duration, frequency and cause into acute (AU) and chronic urticaria (CU).

AU implies changes lasting up to six weeks. CU is defined by the appearance of wheals on the skin

**Table 1:** Classification of urticaria according to duration, frequency and causes

Type of urticaria	Duration
<b>Spontaneous urticaria</b>	
Acute urticaria	Less than six weeks
Chronic urticaria - unknown aetiology	More than six weeks
<b>Induced urticaria</b>	
(i) Dermographism	(i) Application of mechanical forces to the skin (wheals appear in 1 to 5 minutes)
(ii) Delayed pressure urticaria	(ii) Vertical pressure (wheals appear after 3 to 8 hours of latency)
(iii) Urticaria secondary to cold	(iii) Cold air/water/wind
(iv) Urticaria secondary to heat	(iv) Localised heat
(v) Solar urticaria	(v) Ultraviolet (UV) and/or visible light
(vi) Urticaria/vibratory angio- oedema	(vi) Vibratory forces, usually pneumatic devices
(vii) Aquagenic urticaria	(vii) Contact with water, regardless of its temperature
(viii) Cholinergic urticaria	(viii) Stress, perception of body temperature elevation by the hypothalamus
(ix) Contact urticaria	(ix) Allergic or pseudo-allergic

and/or angioedema during a period longer than 6 weeks, most days of the week, for known or unknown reasons. CU comprises chronic spontaneous and chronic inducible urticaria (Table 1). The term physical urticaria has been revised into the term chronic inducible urticaria (CINDU) to indicate its physical trigger and inducible nature of urticaria and includes aquagenic, cholinergic and contact urticaria. Other forms of urticaria, which occur without an external trigger and via an endogenous mechanism are classified as chronic spontaneous urticaria (CSU).<sup>3, 4</sup> Possible causes of CU are medicines, food, infection, circulating immune complexes (CIC), autoantibodies in associated autoimmune disease, as in autoimmune thyroid disease and internal malignancy.<sup>5</sup> The concept of urticaria as a systemic disease is confirmed by its association with other autoimmune diseases, most commonly Hashimoto's thyroiditis, and this association is one of the evidences of the autoimmune aetiology of urticaria (ACU), as a variant of CU.<sup>6</sup> In most cases, it is an autoimmune disease caused by circulating functionally active IgG antibodies specific for the IgE receptors (FcεRI) present on mast cells or basophils (35-40 %) or antibodies to their own IgE (5-10 %).<sup>7</sup> Autoantibodies in ACU induce histamine release from mast cells and basophilic leukocytes directly via IgE receptors or IgE. Increased expression of Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) in patients with CU plays a role in the occurrence of inflammatory response. The presence of a late inflammatory phase explains why CU patients do not respond to the usual therapy used in acute urticaria.<sup>8-10</sup>

Urticaria management is based on the recommendations of the EAACI/GA2LEN/EDF/WAO guidelines (Guideline for the definition, classifi-

cation, diagnosis and management of urticaria: the 2013 revision and update, published in 2014.<sup>3, 11, 12</sup> Medical history can be considered the most valuable diagnostic 'tool' in identifying the cause of CU (Table 2).<sup>12, 13</sup> CU diagnosis and evaluation are supplemented by urticaria severity score (USS) and the laboratory findings: antinuclear antibody titre (ANA), complement components C3, C4, thyroid function, antibodies to thyroglobulin and tissue peroxidase, which indicate association of urticaria with other autoimmune diseases.<sup>14-17</sup> Diagnostic recommendations are presented in Table 3.<sup>3</sup> The simplest test in the differential ACU diagnosis is the autologous serum test in the form of an ID test with 0.05 mL of autologous serum (ASST). A positive ASST test indicates the presence of antibodies to high-affinity receptors on mast cells and basophils and/or IgE. The ASST test is useful in differentiating between ACU and ICU. It was first described by Grattan et al in 1986, with high sensitivity (70 %) and specificity (80 %), as well as by other authors.<sup>18-24</sup>

Lately, the basophil activation test is being used in the diagnosis of ACU.<sup>21</sup> The European Panel on Allergic Diseases suggests scoring to assess

**Table 2:** Anamnestic data in urticaria diagnosis

1. Signs and symptoms associated with the onset of changes: itching/burning
2. Duration of individual change, occurrence of angio-oedema, other symptoms including fever, weight loss, abdominal pain, joint pain
3. Trigger identification: new medication, such as antibiotic, NSAID, hormones
4. Aggravating factors, physical stimuli - heat, cold, alcohol, stress, food
5. Atopy
6. Medical history and changes in patient's health

\*Rule out other diseases by differential diagnosis

**Table 3: Recommended diagnostic tests in frequent urticaria subtypes (modified according to Zuberbier et al)**

Urticaria group/subtype	Routine diagnostic test	Extended diagnostic programme
<b>Spontaneous urticaria</b>		
Acute urticaria	No	No
Chronic urticaria	Differential blood count (DBC), C-reactive protein (CRP), sedimentation (ESR), suspected medicine (eg NSAID)	Potential infection (eg <i>Helicobacter pylori</i> ) and type I allergic reactions, antithyroid antibodies, thyroid hormones, antinuclear antibodies (ANA) physical tests, pseudoallergen, autologous serum skin test, serum tryptase, biopsy;
<b>Chronic inducible urticaria</b>		
Acquired cold urticaria	Cold provocation (ice cube test, cold water, wind)	DBC, ESR/CRP, cryoproteins, rule out other diseases, especially infections.
Pressure urticaria	Pressure (0.2-1.5 kg/cm <sup>2</sup> 10 and 20 min)	No
Heat urticaria	Heat provocation (warm water)	No
Solar urticaria	UV and visible light (different wavelengths)	Rule out other light-induced dermatoses
Dermographism /urticaria factitia	Elicit dermatographism	DBC, ESR/CRP
<b>Other urticarial diseases</b>		
Aquagenic urticaria	Wet compress on the skin 20 min	No
Cholinergic urticaria	Exercise or warm bath	No
Contact urticaria	Prick epicutaneous/test after 20 min	No
Exercise-induced urticaria/anaphylaxis	According to anamnesis exercise with or without food	No

the severity of urticaria, which is a weekly score summing the severity of itching and skin changes. Urticaria severity score (USS) is a valid score for monitoring the seriousness and severity of urticaria.<sup>25-27</sup> The method of calculating the score is shown in Table 4, modified according to Zuberbier.<sup>3</sup>

There are new guidelines in CSU therapy published by the American Academy of Allergy, Asthma and Immunology (AAAAI) and the European Academy of Allergy and Clinical Immunology (EAACI)/Global Allergy and Asthma European Network (GALEN)/European Dermatology Forum (EDF)/World Allergy Organization (WAO) and several other published guidelines with similar recommendations, including the Canadian. According to the new European guidelines, a stepwise approach is applied in CSU therapy:

**Table 4: Urticaria activity score (USS)**

Score	Wheal	
0	none and/or < 10 wheals	none
1	fewer (10-50 smaller wheals/24 h or < 10 large)	mild
2	moderate number (> 50 smaller wheals/24 h) or 10-50 larger	moderate
3	intense occurrence of wheals (> 50 wheals/24 h, generalised occurrence of wheals)	intense

Total score (0-6)

first-line treatment includes second-generation H1 antihistamines, second-line therapy includes increasing the dose of second-generation H1 antihistamines and third-line treatment includes a new medication such as omalizumab, which is preferred over cyclosporine.<sup>28</sup>

One of the significant differences in the guidelines is that the AAAAI algorithm uses first-generation antihistamines, unlike the European guidelines, due to their impact on the REM phase of sleep and impairment of cognitive functions. For similar reasons, they do not accept the use of tricyclic antidepressants. Both guidelines allow administration of systemic corticosteroids for 3 to 7 days, as well as the use of cyclosporine in treating refractory forms of chronic urticaria, and H2 antihistamines only in individual cases, but not as the first-, second- or third-line treatment. The AAAAI guidelines allow adding other second-generation H1 antihistamines and adding H2 antagonists, leukotriene receptor antagonists or first-generation H1 antihistamines at bedtime. In the third step, both guidelines include omalizumab, a recombinant humanised immunoglobulin G1 (IgG1) monoclonal antibody which binds to IgE. In binding IgE, omalizumab inhibits binding of IgE to the high-affinity IgE receptor.<sup>29</sup> TNF- $\alpha$  antagonists have been reported to be effective in 60 % of 20 patients with CU in a retrospective study, compared with healthy control cases.<sup>30</sup>

Autologous serum therapy and/or autologous whole blood therapy (autohaemotherapy) may be effective in patients with a positive ASST test at weekly intervals during six weeks, and even up to 12 weeks, leading to tolerance, desensitisation (hyposensitisation) of patients with chronic urticaria to pro-inflammatory cytokines.<sup>31-36</sup>

### Aim

1. Determine the frequency of positivity of the autologous serum skin test in patients with individual clinical forms of chronic urticaria.
2. Comparative analysis of clinical picture severity and laboratory parameters in patients with autoimmune and idiopathic chronic urticaria.
3. Determine the success rate of autologous blood treatment in patients with a positive ASST test (patients with autoimmune chronic urticaria).

### Methods

The study was conducted at the Skin and Venereal Diseases Clinic of the Republic of Srpska University Clinical Centre, by random selection, after obtaining permission of the Ethics Committee, as a prospective study of 64 patients with chronic spontaneous urticaria (Figure 1), who were made aware of the methodology and objective of the study. They verified their consent by their own signatures. Anamnestic data were collected and patients were subjected to clinical, dermatological examination and autologous serum testing. A sample of their serum was taken at the time of clinical worsening of the disease. Antihistamines and corticosteroids were stopped two days before the test and antidepressants 3 weeks before the test.<sup>37</sup> The original ASST test implies intradermal injecting (ID) of 0.05 mL of autologous serum and 0.05 mL of 0.9 % NaCl solution, and a histamine prick test (10 mg/mL), as a positive reference, at a distance of 3 cm on the skin of the volar aspect of the forearm. The test was read after 30 minutes. The test was positive if erythematodematos papule was 1.5 mm larger than the papule at the site of the injected saline solution (Figure 2). Based on the positivity of the autologous serum test, the subjects over 18 years of age were divided into two groups:

1. Group of CU patients with positive ASST test and positive immune parameters in

terms of presence of an associated autoimmune disease, ie autoimmune chronic urticaria ACU.

2. Group of CU patients with negative ASST test and negative immune parameters, which was at the same time both the control group and the studied group with idiopathic chronic urticaria (ICU).



Figure 1: Clinical picture of urticaria (papules, plaques, annular wheals appearance)



Figure 2: Autologous serum test (ASTT): positive test noted by presence of wheals

The study did not include pregnant women, children or patients with AU. At the beginning of the study, all patients were scheduled to perform the laboratory analyses required in the diagnosis of CU: complement components C3, C4 and ANA,

*Borrelia burgdorferi* and *Helicobacter pylori* serum antibodies, and hepatitis B and C markers. Stool was microscopically examined for parasites and candida. The following laboratory analyses were performed: erythrocyte sedimentation rate (ESR), complete blood count (CBC), routine urine examination, thyroid function test parameters, renal function test and liver enzymes. A prick test with inhalant and nutrient allergens was performed on all patients at the beginning of the study.

As clinical picture of urticaria is variable, self-assessment was performed over 24 hours, using USS.<sup>25-27</sup> The USS score is the sum of the scores of number of changes and itch intensity. The maximum value of daily USS score may be up to 6 and weekly up to 42 (Table 4). The patients were educated how to calculate the daily score required for calculating the weekly score that was recorded at follow-up examinations. Table 4 shows daily score calculation, modified according to Zuberbier.<sup>3</sup>

The first group of patients received autologous blood therapy, intramuscular (im) injections of 5 mL of whole blood at weekly intervals, during six weeks, with a non-sedating antihistamine and systemic corticosteroids in the exacerbation phase during 3 to 7 days. Depending on clinical picture severity, the second group of patients was prescribed an antihistamine with systemic corticosteroids therapy in the deteriorating phase during 3-7 days im, an antibiotic and an antifungal drugs according to the findings.

Results of the clinical part of the study based on USS score monitoring during the diagnostics and six weeks of treatment of skin changes in patients with chronic urticaria (CU) were obtained. Laboratory findings and other parameters, such as clinical picture severity measured by USS test, duration of urticaria and occurrence of angio-oedema, presence of autoimmune markers in patients with positive and negative ASST test, as well as effect of administered therapy were compared.

The analytical statistical tool SPSS (Statistical Product and Service Solutions) version 20 for descriptive statistics and statistical methods was used, while Microsoft Excel 2007 was utilised for graphical presentation. All results were presented in numbers, tables, charts and figures. Significance level used was  $p = 0.05$ .

## Results

The sample consisted of 64 patients with chronic urticaria, 20 (31.2 %) men and 44 (68.8 %) women. In the ACU group, there were a total of 28 (43.8 %) patients, including 4 (6.2 %) males and 24 (37.5 %) females. In the ICU group, there were a total of 36 (56.2 %) patients, of which 16 (25 %) were males and 20 (31.2 %) females. Use of the Fisher test in the total population of subjects yielded a highly statistically significant difference ( $p < 0.001$ ) in positive values of the ASST test in the group of CU subjects representing ACU patients (compared to the total number of subjects tested of 64).

The average age of patients with ACU was 46.5, and in patients with ICU it was 49.31. Use of the Mann-Whitney U test did not yield a statistically significant difference in the age of ACU subjects ( $N = 28$ ,  $Md = 48$ ) and ICU subjects ( $N = 36$ ,  $Md = 47$ ,  $U = 442.50$ ,  $z = -0.833$ ,  $p = 0.405$ ,  $r = 0.104$ ).

### Disease duration

The average duration of urticaria was 20 months (95 % CI: 13.42 - 26.03) in both groups of subjects (Figure 3). Use of the Mann-Whitney U test did not yield a statistically significant difference in urticaria duration between the subject groups with ACU ( $N = 28$ ,  $Md = 6.50$ ) and ICU ( $N = 36$ ,  $Md = 12.00$ );  $U = 502.00$ ,  $z = -0.027$ ,  $p = 0.978$ ,  $r = 0.03$ ).

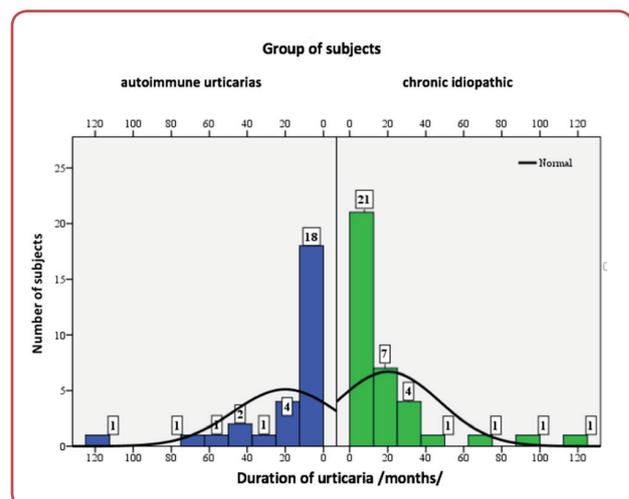


Figure 3: Duration of autoimmune and chronic idiopathic urticaria in weeks

### Haematological analysis

Anaemia was found in 4 (14.3 %) patients with ACU, and in 2 (5.6 %) patients in the ICU group. Use of the independent samples t-test yielded a statistically significant difference in the incidence

of anaemia ( $t = -2.603$ ,  $p = 0.012$ ) in the ACU group of patients compared to the ICU group of patients, while there was no statistically significant difference between the studied groups in the values of other haematological and biochemical findings.

### Urine findings

Urinary tract infection was found in 11 (17.5 %) subjects, as follows: in 3 (10.7 %) patients with ACU and 8 (22.9 %) patients with ICU. Use of the Fisher test yielded no statistically significant difference ( $p = 0.319$ ) in urinary tract infection presence between the groups of subjects.

### Thyroid function parameters

TSH reference values were found in 60 (83.8 %) subjects in the total subject population. Only 1 (3.6 %) patient in the ACU group had hypothyroidism, while 3 (4.7 %) patients in the total population of subjects had hyperthyroidism, ie 2 (7.1 %) in the ACU group and 1 (2.8 %) patient in the ICU group ( $p = 0.084$ ). FT4 reference values were found in 61 (95.3 %) subjects in the total population, with no statistical significance by group of subjects ( $p = 0.284$ ). Thyroglobulin antibodies were positive in 5 (17.9 %) patients with ACU and in the group of ICU patients, there were no patients with positive antithyroglobulin antibodies. Tissue peroxidase antibodies were positive in 5 (17.9 %) patients in the ACU group and in 1 (2.8 %) patient in the ICU group (Figure 4). Use of the Fisher test yielded a statistically significant difference ( $p = 0.013$ ) in the level of thyroglobulin antibody titre in patients with ACU, while use of the above test did not yield a statistically significant difference in the level of tissue peroxidase titre in the total population of subjects ( $p = 0.078$ ).

### Immunological parameters

ANA were positive only in 1 (3.6 %) patient in the total population of subjects, and use of the Fisher

test yielded no statistically significant difference ( $p = 0.438$ ) in ANA values between the groups of subjects. Consumption of complement component C3 was recorded in 5 (7.8) patients, ie in 2 (7.1 %) patients with ACU and 3 (8.3 %) patients with ICU. Elevated C3 values were found in 1 (2.8 %) patient with ICU, while the complement component was elevated in 2 (5.6 %) patients with ICU.

### Presence of fungi and parasites

No parasite eggs or intestinal protozoan cysts were found in stool of any subject from any group at the beginning of the study. Stool candida was isolated in 4 (14.3 %) patients with ACU and 11 (30.6 %) patients in the ICU group, but use of the Fisher test did not yield a statistically significant difference in the number of patients with the isolated candida in stool ( $p = 0.149$ ).

### Test for *Helicobacter pylori* and *Borrelia burgdorferi*

The subjects were tested for *Helicobacter pylori* at the beginning of the study. Positive values were found in 14 (50.0 %) patients in the ACU group and 11 (30.6 %) patients in the ICU group. Use of the  $\chi^2$  test with Yates correction yielded no statistically significant difference ( $\chi^2 = 1.751$ ,  $p = 0.186$ ) in the positivity of the *Helicobacter pylori* test by group of subjects, although a larger number of subjects was in the ACU group. Positive IgM antibodies to *Borrelia burgdorferi* were found in 4 (14.3 %) patients with ACU and 3 (8.3 %) patients with ICU, while IgG was positive in 3 (10.7 %) patients with ACU and 1 (2.8 %) patient with ICU (Figure 5).

Use of the Fisher test yielded no statistically significant difference in the values of IgM and IgG antibodies to *Borrelia burgdorferi* between the groups of subjects: IgM ( $p = 0.689$ ), IgG ( $p = 0.311$ ).

All patients in the ACU and ICU groups had negative hepatitis B and C markers.

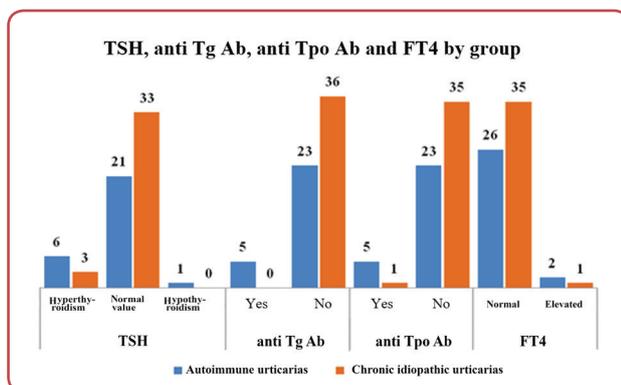


Figure 4: Thyroid gland hormones and anti Tg and anti TPO antibodies in patients with autoimmune and chronic idiopathic urticaria

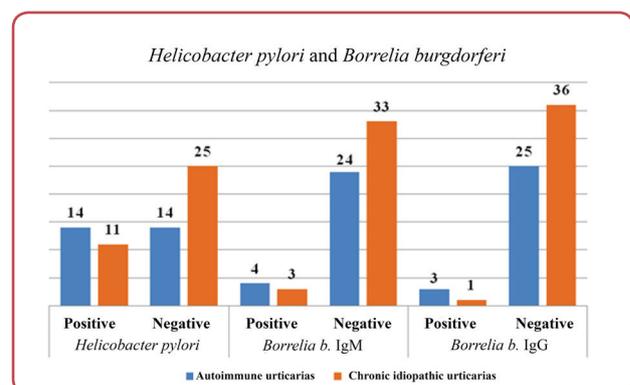


Figure 5: *Helicobacter pylori* and *Borrelia burgdorferi* tests in patients with autoimmune and chronic idiopathic urticaria

## Allergy testing

A prick test with inhalant and nutrient allergens was performed on all patients at the beginning of the study. The prick test with nutrient allergens was negative in all subjects, while the one with inhalant allergens was positive in 6 (21.4 %) patients with ACU and 3 (8.3 %) patients with ICU. The most common positive allergens were mixtures of grass, tree and weed pollen, as well as house dust mites. Use of the Fisher test yielded no statistically significant difference ( $p = 0.163$ ) in prick test positivity between the groups of subjects.

## USS score values

The mean USS value in the ACU patients receiving IM autologous blood therapy at weekly intervals was  $23.54 \pm 11.91$  in the first week, and  $1.39 \pm 2.47$  ( $F 1.13 \pm 2.401$ ,  $M 3.00 \pm 2.582$ ) in the sixth week. In ICU patients, USS was  $21.67 \pm 11.689$  in the first week and  $2.72 \pm 2.835$  ( $F 3.00 \pm 2.956$ ,  $M 2.38 \pm 2.729$ ) in the sixth week (Figure 6). Use of the Wilcoxon signed-rank test in monitoring ACU patients yielded a highly statistically significant difference ( $z = -4.375$ ,  $p = 0.000$ ) in weekly USS in the sixth week compared to the first week, due to the applied autologous blood therapy.

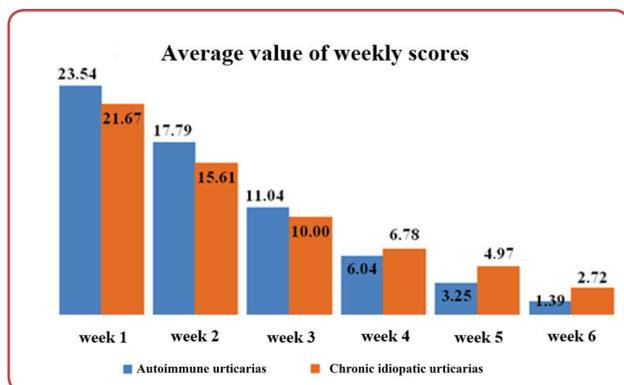


Figure 6: Median weekly values of urticaria severity score (USS)

## Applied therapy

Methylprednisolone in an im 40 mg dose once a day for seven days was prescribed during the exacerbation phase of urticaria in 27 (75 %) patients in the ICU group, and only in 9 patients in the ACU group (in 7 patients who received autologous blood). According to the  $\chi^2$  test with Yates correction ( $\chi^2 = 10.078$ ,  $p = 0.002$ ), this difference in the need for methylprednisolone therapy is highly statistically significant by group of subjects.

In patients with ICU, there was no statistically significant difference in weekly USS in the first ( $U = 126,000$ ,  $z = -0.612$ ,  $p = 0.558$ ) and in the sixth

week ( $U = 136,000$ ,  $z = -0.299$ ,  $p = 0.788$ ) by group of subjects. The use of methylprednisolone did not affect much the therapeutic response in patients with ACU and ICU, so remission was achieved mainly due to the use of autologous blood in the ACU group, and other conventional therapy in the ICU group.

Antibiotics were prescribed after obtaining of test results (to *Helicobacter pylori*, *Borrelia burgdorferi*), or in patients with inflammatory syndrome (accelerated sedimentation, leucocytosis, neutrophilia, thrombocytosis, high value of complement component C4). The antibiotic (azithromycin) was prescribed in 35 (54.7 %) patients, ie in 15 (53.6 %) patients with ACU and 20 (55.6 %) patients with ICU, in addition to other prescribed therapy.

Use of the  $\chi^2$  test with Yates correction yielded no statistically significant difference ( $\chi^2 = 0.000$ ,  $p = 1.000$ ) in the use of antibiotics in both groups of subjects.

Patients with candida isolated from the stool were prescribed fluconazole (Diflucan<sup>®</sup>, generic fluconazole) at 150 mg in weekly doses during four weeks in 15 (23.4 %) patients, ie 5 (7.8 %) patients in the ACU group and 10 (15.6 %) patients in the ICU group. No statistically significant difference ( $p = 0.149$ ) in the use of fluconazole between the groups of subjects was yielded by the Fisher test.

## Discussion

In the Te-Peng Tseng study, CU was more common in female population and there was no difference in age groups of the subjects with ACU (40 %) and ICU (22 %), while in the present study it was more common in females.<sup>36</sup> Urticaria occurred in the fifth decade of life in both sexes in this study, and Mozena et al note that ICU occurs more often in women in the third or fourth decade of life, at an average age of  $38 \pm 13$  years.<sup>38</sup> According to Kulthanan, CU mainly affects middle-aged women (70 %). Chaffari notes that urticaria duration varies from 2 months to 10 years (while in this study it lasted 20 to 26 months) and that urticaria lasts longer in patients with a positive ASST test. George et al compared clinical forms of ACU and ICU in 100 patients with CU, and noted that ACU patients had no special diagnostic, clinical or his-

topathological features compared to ICU patients, although they tend to have a more severe clinical picture of urticaria.<sup>39-41</sup>

Vohra et al specify the exact methodology and interpretation of ASST and Al-Hamamy et al note ASST as a diagnostic tool in ACU diagnosis. Twenty-two (40.7 %) patients had a positive ASST test and statistical analysis of clinical variables did not show a significant difference between the patients with positive and negative ASST, except for the distribution of wheals ( $p = 0.004$ ). Krupashankar et al deem that positive ASST is a way to select patients with ACU and to start immunomodulatory therapy faster.<sup>42-44</sup>

In the sixth week, the Mann-Whitney test established a statistically significant difference in weekly USS between the studied groups ( $U = 357.00$ ;  $z = -2.138$ ;  $p = 0.032$ ) in favour of ACU in our subjects and a lower USS in patients with ACU. Jariwala et al state that USS is a sensitive method, a valid and reliable instrument for monitoring the severity of urticaria and similar results were reported by Mathias and Jáuregui et al, as well.<sup>25, 26, 45</sup>

Positive values of the *Helicobacter pylori* test in subjects who participated in the present study were found in 14 (50.0 %) patients in the ACU group and 11 (30.6 %) patients in the ICU group. Yadav et al note a significant connection between HP infection and HP eradication response in patients with CIU. HP infection should be included in the diagnostic treatment of patients with CIU. Federman suggests conducting HP testing and applying appropriate antibiotic therapy. Moreira et al showed that the prevalence of HP infection in CIU patients was 71.4 % in the study of 21 CU patients.<sup>46- 48</sup> Research by some authors established association of *Borrelia burgdorferi* infection with several dermatoses. Warren et al note the occurrence of urticaria associated with *Borrelia burgdorferi* infection.<sup>49</sup>

Staubach et al state that further research should indicate the importance of fungal infection in CU, especially the importance of IgE antibodies to candida in the CU patients presenting intestinal colonisation with candida. In their study, Ghaffari noted the necessary laboratory findings in evaluating CU. Ghaffari states that aeroallergens can induce exacerbation of CU and the results showed that hypersensitivity to mites is more common in patients with CU.<sup>40, 50, 51</sup>

Patients with a positive ASST test received autologous whole blood injections (autohaemotherapy)

at weekly intervals in im 5 mL doses, during eight weeks. In line with other results, Tseng et al note the safety of whole blood therapy and long-term disease control, but also the need for further cohort (control) studies on a larger number of patients and a longer follow-up period.<sup>36</sup>

Staubach notes that autologous blood injections in patients with ACU lead to tolerance to histamine-releasing factor in these patients. The patients had satisfactory remission of urticaria, less need for antihistamines and better quality of life, although there was no statistical significance, most likely due to the limited number of patients treated. The results suggest that ASST-positive patients with CU may benefit from autologous whole blood therapy.<sup>52- 54</sup>

Federman et al report the effect of antibiotics on CU in patients with a positive HP test. Fukuda et al report complete remission of urticaria in patients with a positive HP test after antibiotic therapy. Kahn notes that the ultimate goal of therapy is to control urticaria, to reduce its impact on the quality of life of patients and negative effects of therapy and to use oral corticosteroids as rarely as possible.<sup>47, 55, 56</sup>

## Conclusion

1. Out of the total number of subjects in this study, 28 (43.8 %) patients with chronic spontaneous urticaria had a positive autologous serum test and 36 (56.2 %) patients have a negative test.
2. The average duration of urticaria in both groups of patients was approximately 20 months.
3. Subjects with ACU had a significantly higher association with autoimmune thyroid diseases. More frequent occurrence of anaemia was noted in patients with ACU compared to the group of patients with ICU.
4. The mean value of USS score as an indicator of disease severity in the first week was not statistically significant in subjects of any group, while in the sixth week a statistically significant difference of weekly USS was established among the studied groups in favour of ACU.
5. The use of autologous blood therapy in patients with ACU is directly related to the improvement of the clinical picture and lower USS score values.

## Acknowledgements

None.

## Conflict of interest

None.

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# Major Adverse Cardiovascular Events After Implantation of Absorb Bioresorbable Scaffold: One-Year Clinical Outcomes

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## Abstract

**Background/Aim:** Bioresorbable vascular scaffold (BVS) represents a novel generation of intracoronary devices designed to be fully resorbed after healing of the stented lesion, delivering antiproliferative drug to suppress restenosis, providing adequate diameter of the coronary vessel and preserving the vascular endothelial function. It was supposed that BVS will reduce neointimal proliferation and that their late bioresorption will reduce the negative effects of traditional drug-eluting stents, including the late stent thrombosis, local vessel wall inflammation, loss of coronary vasoreactivity and the need for the long-term dual antiplatelet therapy.

The purpose of this research was to investigate efficacy and safety of Absorb everolimus-eluting BVS implantation and the prevalence of major adverse cardiovascular events (MACE) at the mid-term follow-up.

**Methods:** The study encompassed 42 patients selected for BVS implantation and fulfilling inclusion criteria - 37 male and 5 female - admitted to the Dedinje Cardiovascular Institute, Belgrade, Serbia over the one-year period (from January 2015 to January 2016) for percutaneous coronary intervention (PCI). Coronary vessel patency before and after stenting was assessed by the Thrombolysis in Myocardial Infarction flow (TIMI) grades.

After the index PCI procedure with BVS all patients were clinically followed by regular (prescheduled or event-driven) visits during the next 12-month period.

**Results:** In the intention-to-treat analysis, all Absorb BVS procedures were successful, without the need for conversion to other treatment modalities. The complete reperfusion (TIMI flow grade 3) after the intervention was established in 97.6 % of patients and 100 % of them achieved the TIMI flow grade  $\geq 2$ .

The presence of angina pectoris was reduced significantly by the BVS procedure: stable angina 57.1 % to 11.9 %, ( $p < 0.001$ ) and unstable angina 31 % to 0 %, respectively ( $p < 0.001$ ). After the one-year follow-up, the MACE rate was 11.9 %. Myocardial infarction occurred in 4.8 % and the need for PCI reintervention in 2.4 % of cases (not influenced by the gender or the age of patients). There were 4 cases of death (all patients were older and had lower values of left ventricular ejection fraction).

**Conclusion:** The results of the current research demonstrated a high interventional success rate of the Absorb BVS implantation, followed by the early improvement of the anginal status. However, that was not translated into the favourable mid-term clinical outcomes, opening debate about the current status of Absorb BVS and the need for future refinements of stent design and implantation techniques.

**Key words:** Absorb everolimus-eluting bioresorbable vascular scaffold; Percutaneous coronary intervention; Major adverse cardiovascular events; Thrombolysis in Myocardial Infarction (TIMI) flow grade.

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## ARTICLE INFO

Received: 16 October 2021  
Revision received: 13 December 2021  
Accepted: 14 December 2021

## Introduction

Ischaemic coronary artery disease is the most important part of the cardiovascular pathology and is considered to be among the most frequent causes of the global mortality. It includes chronic (stable) ischaemic heart disease and acute coronary syndromes (ACS).<sup>1</sup>

Percutaneous coronary intervention (PCI) is one of the most frequently utilised interventions for the reestablishment of blood flow in the clogged artery. It is used to open the stenosed or occluded coronary vessel by the balloon inflation and finally to implant a coronary stent to ensure that it remains open. However, stent implantation can initiate negative effects, including plaque crushing, injury of vascular endothelium and stretching and lacerations of the vessel wall, which can lead to coronary restenosis.<sup>2</sup> The phenomenon of an elastic recoil, along with the constrictive remodelling and neointimal proliferation is involved in the mechanism of restenosis as well. Therefore, the motivation for developing different types of stents was generated in order to reduce restenosis and stent thrombosis, as well as to entirely restore the vascular function and physiology.<sup>3</sup> Dual antiplatelet therapy (DAPT) is mandatory in PCI procedures to avoid thrombotic complications and to reduce the device-related short- and long-term adverse events.<sup>4</sup> DAPT consists of the combination of acetylsalicylic acid (ASA) and an oral inhibitors of platelet P2Y<sub>12</sub> receptor for adenosine 5'-diphosphate.<sup>5</sup>

There are three types of vascular scaffolds available: bare-metal (BMS), drug eluting metallic (DES) and bioresorbable (BRS) stents. So far, the gold standard for PCI is a metallic drug eluting stent. However, the late adverse events (restenosis, stent thrombosis and neoatherosclerosis) have initiated the current research into the development of BRS stents.<sup>6</sup>

The Absorb everolimus-eluting bioresorbable vascular scaffold (BVS, Abbott Vascular) is a novel device, an alternative to DES for PCI, aimed to decrease the incidence of late adverse clinical events following the coronary stenting (restenosis and stent thrombosis). As a new generation of intracoronary devices, BVS are designed to be fully resorbable, providing adequate diameter and function of blood vessel and delivering a drug without permanent implant in the body.<sup>7</sup>

BVS comprises of crystalline backbone struts (150 µm thick) of poly-L-lactide coated with a 1:1 mixture of poly-D-L-lactide (resorbable polymer functioning as drug carrier) and the antiproliferative drug everolimus.<sup>6,8</sup> The mechanical support of the stent itself solves an initial problem of acute recoil following balloon angioplasty. Furthermore, everolimus elution reduces neointimal proliferation and stents' late bioresorption reduces the adverse events following the traditional drug eluting stents including late stent thrombosis, local inflammation, loss of coronary vasoreactivity and the need for long-term dual antiplatelet therapy.<sup>9</sup> However, in the present clinical scenario, BVS were accompanied by a high incidence of scaffold thrombosis during the first twelve months after implantation.<sup>10</sup> Further analyses have suggested that the risk of thrombosis closely correlates with the greater width and thickness of BVS struts compared to DES, associated with disturbed local blood flow and consequent platelet aggregation. Thrombotic risk is higher in the vessels with small referent diameter, as well as the ones with the small achieved final minimal lumen diameter at the end of the PCI procedure. Implantation techniques and discontinuation of DAPT increase the thrombotic risk, as well.<sup>11</sup> Accordingly, to the current recommendation, DAPT should be continued for at least one year after PCI,<sup>12</sup> what the patients were instructed to follow as well.

Major adverse cardiac events (MACE) are a composite of several adverse clinical outcomes, so that they may have broad and often overlapping definitions. Generally, MACE includes various components such as myocardial infarction, cardiac death, any-case sudden death, need for the repeated revascularisation, either percutaneous (PCI) or surgical coronary artery bypass grafting (CABG), re-hospitalisation due to cardiovascular problems, cerebrovascular insult, recurrent angina, worsening of heart failure.<sup>13-15</sup> Because of such variety of MACE definitions, the reported total MACE rates may differ in individual publications.<sup>16</sup> In the current study, MACE was defined as a composite of myocardial infarction, re-hospitalisation for the need of coronary revascularisation (PCI or CABG) and death of all causes.

The purpose of this research was to investigate Absorb BVS implantation safety, immediate efficacy and the mid-term (one-year) clinical outcomes.

## Methods

The research was conducted at the Dedinje Cardiovascular Institute, Belgrade, Serbia over the period of one year (from January 2015 to January 2016) on patients admitted for percutaneous revascularisation. A total of 42 patients with both stable and unstable angina pectoris met the inclusion criteria. PCI was performed exclusively with Absorb BVS, Abbott Vascular, Santa Clara, California, USA. Inclusion criteria for Absorb BVS implantation were the age of 18 years and above, clinical presence of angina pectoris, both sexes and the reference vessel diameter at the targeted lesion between 2.5 to 3.9 mm determined by quantitative coronary angiography (QCA). The patients who were included in this study were not the only patients who underwent implantation of Absorb BVS at the Dedinje Cardiovascular Institute.

The decision to limit the vessel size was based upon the concern about the higher rates of stent thrombosis in small vessels, what was confirmed in the later clinical studies.<sup>17</sup> Patients with acute coronary syndromes and patients with prior coronary interventions were included in the study as well. Patients with a need for a combination of drug-eluting stent and BVS in the same procedure were excluded. Throughout the interventional procedure, all patients were treated with heparin/enoxaparin.

Patients were preloaded with DAPT according to standard protocol and such treatment was continued for 12 months following the intervention (ASA 100 mg and clopidogrel 75 mg daily). Coronary vessel patency before and after stenting was assessed by the Thrombolysis in Myocardial Infarction flow (TIMI) grades, applying the standard definitions.<sup>18</sup>

The local Ethical Committee of Dedinje Cardiovascular Institute approved this research project and informed consent was obtained from each patient. Being a part of N.S. cardiology graduation thesis, this endeavour was endorsed by the Medical Faculty, University of Belgrade as well.

During the 12-month follow-up, all patients were followed-up prospectively and systematically by phone at one, six and twelve months after the intervention for clinical evaluation and were seen in the office if medically indicated as well. Event or patient-driven visits were provided as well.

The incidence of MACE onset was monitored methodically over the whole follow-up period.

For statistical analysis, patient data were extracted from the hospital information system to a Microsoft Excel database. Final data analysis was performed using SPSS software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). For descriptive statistics, continuous variables are presented as mean  $\pm$  standard deviation (SD) or as median with corresponding range, depending on the data distribution. The normality of sample distribution was analysed by the Kolmogorov-Smirnoff test. Nonparametric variables were presented as frequency distributions. For testing the hypothesis on the treatment effect Student's t-test for serial measurements, or Wilcoxon signed-rank test were used, depending on the normality of data distribution. Frequency distribution differences were assessed using Pearson's Chi-square test or Fisher's exact test, as indicated. A two-tailed probability level of  $p < 0.05$  was considered to indicate statistical significance.

## Results

### Baseline characteristics

The study included a total of 42 patients, 37 male (88.1 %) and 5 female (11.9 %). Their average age was  $57.4 \pm 11.5$  (ranging from 34 to 82 years). Patients' characteristics collected from the medical history and clinical examination are presented in Table 1.

### Procedural details

In the intention-to-treat analysis, all Absorb BVS procedures were successful, without the need for conversion to other treatment modalities. Pre-dilatation was performed in 66.7 % and post-dilatation in 57.1 % of patients. In the PCI procedure, 1 BVS stent was implanted in 83.3 %, 2 stents in 14.3 % and 3 stents in 2.4 % of cases. Concerning the stent dimensions used in the procedures, the predominant stent length was in the range of 16-25 mm (59.5 %) and the predominant diameter was in the range of 3.0-3.9 mm (64.3 %). During the intervention, Absorb BVS stent overlap was done in 14.3 % and spot stenting versus entire coverage was done in 4.8 % of treated patients. Characteristics of BVS implanted and implantation techniques are presented in Table 2.

**Table 1: Baseline patients' characteristics**

Comorbidities	n	%
<b>Gender</b>		
Male	38	90.5
Female	14	33.3
<b>Prior cardiovascular interventions</b>		
Coronary artery bypass graft (CABG)	38	7.1
Percutaneous coronary intervention (PCI)	14	40.5
<b>Acute coronary syndromes</b>		
Yes	23	54.8
No	19	45.2
<b>Angina pectoris</b>		
Stable angina	24	57.1
Unstable angina	13	31.0
<b>Left ventricular ejection fraction (LVEF)</b>		
< 25 %	4	9.5
26-45 %	16	38.1
> 46 %	22	52.4
<b>Bifurcations lesions</b>		
Yes	7	16.7
No	35	83.3

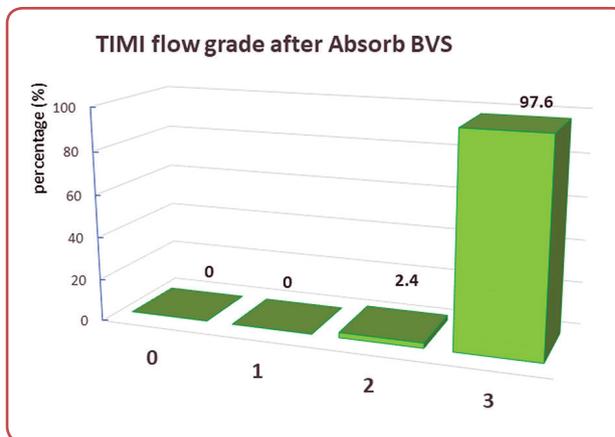
**Table 2: Characteristics of stents and implantation techniques**

Stents	n	%
<b>Number of implanted stents</b>		
1	35	83.3
2	6	14.3
3	1	2.4
<b>Stent length (mm)</b>		
to 15	6	14.3
16 - 25	25	59.5
26 and more	11	26.2
<b>Stent diameter (mm)</b>		
2.5 - 2.9	15	35.7
3.0 - 3.9	27	64.3
4.0 and more	0	0.0
<b>Intervention</b>		
Stent implanted	42	100.0
Stent predilatation	28	66.7
Stent postdilatation	24	57.1
<b>Implantation techniques</b>		
Overlap	6	14.3
Spot	2	4.8

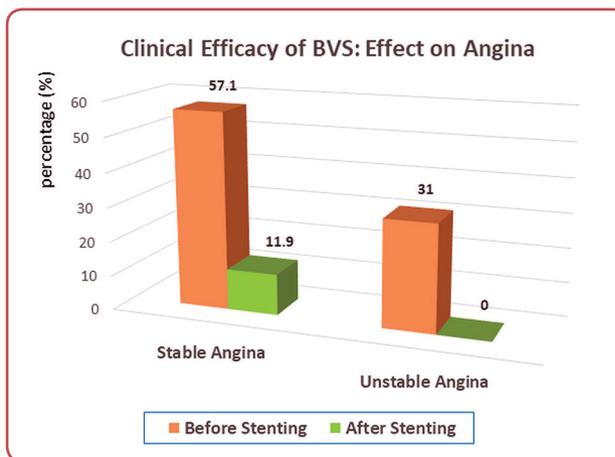
**Clinical outcomes**

Immediately after the intervention, the complete reperfusion (TIMI flow grade 3) was established in 97.6 % of patients and 100 % of them achieved TIMI flow grade ≥ 2, as shown in Figure 1. Regarding the immediate clinical efficacy of Absorb BVS stenting, the prevalence of stable angina (before and after PCI) was 57.1 % vs 11.9 %, showing statistically significant reduction ( $\chi^2$  test = 14.44,  $p < 0.001$ ). Additionally, the prevalence of unstable angina before and after Absorb BVS stenting showed a significant decrease as well: 31 % be-

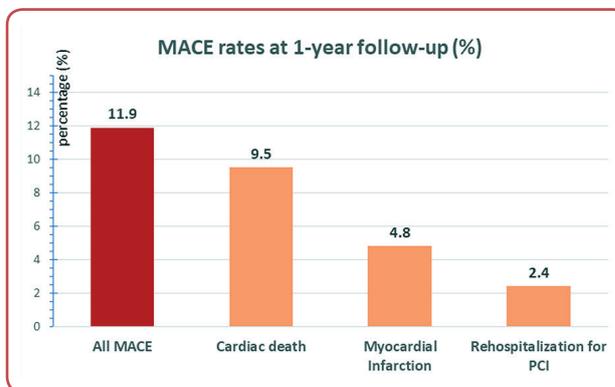
fore and 0 % after the procedure ( $p < 0.001$ ). Immediate efficacy of Absorb BVS implantation on the improvement of anginal status is presented in Figure 2.



**Figure 1: Results of Absorb BVS stenting: Distribution of achieved TIMI flow grades**



**Figure 2: Results of Absorb BVS stenting: Effect on angina pectoris**



**Figure 3: MACE rate at 1 year of follow-up after Absorb BVS stenting**

Regarding the mid-term clinical results, at the one-year follow-up, 11.9 % of patients had MACE. Out of all MACE, the most common clinical event



occurring in 9.5 % of patients was cardiac death; the next one was acute MI (4.8 %), followed by the PCI re-intervention (2.4 %). Overall incidence of MACE and its components at the 12-month follow-up is presented in Figure 3. The average age of patients with MACE was  $61.0 \pm 11.4$  and without MACE  $57.0 \pm 11.6$  years; statistically nonsignificant difference between these groups related to the age ( $t = 0.733$ ,  $p = 0.468$ ). There were no significant differences related to the patient gender between patients with and without MACE (Fisher's exact test,  $p = 0.488$ ) as well.

## Discussion

BVS, compared to metallic stents offer the potential to preserve vascular geometry and function. They provide less alteration of vessels angulation compared to metallic stents and offer a chance for the complete restoration of vascular endothelial function. At 6 to 12-month follow-up, the ABSORB BVS showed improvement in coronary configuration and myocardial function regarding the state before intervention.<sup>19</sup>

Historically, the Igaki-Tamai stent (inventors Dr Keiji Igaki and Dr Hideo Tamai) was the first fully BRS implanted in the human coronary arteries. It was made of poly-L-lactic acid (PLLA) monofilament with a zigzag helical design but without drug elution.<sup>20</sup> In the late 1990s, a long-term clinical trial (10 years) enrolled fifty patients who were treated with Igaki-Tamai stents. The stent showed a high survival rate, pointing to the efficacy and safety of the device years after implantation. The authors presented that 87 % of patients survived at ten years and 50 % of them were free from MACE. TLR rate per patient was 16 % after one and three years, 18 % after five years and 28 % after ten years. Two stent thromboses happened during the follow-up period. These stents were not widely used in clinical practice, primarily due to the absence of antiproliferative component.<sup>21</sup>

In humans, Absorb BVS 1.0 (the first-generation device) was evaluated in the ABSORB Cohort A. Clinical five-year follow-up did not show scaffold thrombosis; it showed only one case of MI with the MACE rate of 3.4 %.<sup>22,23</sup>

Absorb BVS 1.1 (the second-generation device)

was evaluated in the ABSORB Cohort B and results did not show scaffold thrombosis and cardiac death and the MACE rate was 10 % (at the three-year clinical follow-up).<sup>22,24</sup>

ABSORB EXTEND was initiated as a global prospective research trial including over 800 patients from different locations and with wider range of inclusion criteria (multiple vessels and various lesions). The results of this research showed good efficacy and safety of BVS at three-year follow-up with MACE rates of 9.2 %, TLR rate 10.6 % and scaffold thrombosis rate of 2.2 %, with 1.2 % after one-year of monitoring.<sup>25</sup>

ABSORB-II trial, with one-year follow-up (similar duration as in this study), was a prospective randomised controlled trial aimed to investigate effects of everolimus-eluting BRS comparing to everolimus-eluting DES in the treatment of *de novo* coronary lesions (however with the more selective inclusion criteria than in this study). It enrolled approximately 500 patients at 40 sites in Europe and New Zealand. Clinical endpoints included MI, coronary revascularisation, cardiac death, intervention success and anginal status estimated by the Seattle Angina Questionnaire (SAQ). After a year, cumulative rates of angina deterioration and the new angina occurrence were decreased in the BRS group (22 %) vs DES group (30 %), whereas angina status by SAQ was similar. However, in the BRS group, three patients had definite stent thrombosis compared to the DES group with no cases of thrombosis. MACE rate was 5 % vs 3 % (BRS vs DES) and the most common adverse event was MI (14 % vs 1 %, respectively). Clinically indicated TLR was 1 % vs 2 %, respectively.<sup>26,27</sup>

Stone et al (2019) published a systematic meta-analysis of clinical trials comparing Absorb BVS with DES (everolimus-eluting) encompassing 3,384 patients. Outcomes were analysed throughout five years. A five-year follow-up of BVS implantations showed an increase in rates of TLF in the BVS patients (14.9 % vs 11.6 %) and stent thrombosis (2.5 % vs 0.8 %, respectively). Monitored adverse effects (target vessel-related MI, ischaemia-driven TLR, cardiac death) occurred in 11.6 % of BVS vs 7.9 % of DES until the third year and additional 4.3 % of BVS vs 4.5 % of DES between the third and fifth year. This study has suggested that BVS device might be an admissible alternative to DES in the treatment of coronary artery disease.<sup>28</sup>

Three-year clinical outcomes of ABSORB III<sup>17</sup> trial have revealed that device-related events beyond one year continued to accrue, particularly myocardial infarction and device thrombosis. Multivariate analysis identified reference vessel diameter < 2.25, prior cardiac intervention and diabetes as predictors of the 3-year device thrombosis. Interestingly, most of the patients with BVS thrombosis were on DAPT at the time of the event.

The current study confirmed the high procedural success rate of Absorb BVS implantation with excellent short-term clinical results (driven by the improvement in anginal status). High completeness of reperfusion rates had opened the expectation on favourable clinical outcomes since it was previously shown that patients with TIMI flow grade  $\geq 2$  had better five-year clinical outcome.<sup>29,30</sup> Furthermore, a significant reduction in the prevalence of stable and unstable angina was obtained following Absorb BVS intervention.

However, 1-year clinical outcomes did not follow the initial success raising concern with Absorb BVS device *per se*, as well as with proper indications, optimal procedural techniques and the choice and duration of antiplatelet therapy following its implantation.

As a matter of fact, this study had broader inclusion criteria than most of the presented ones, which might account for some of the observed differences in clinical outcomes. Actually, in the current study, the only limitation for patient inclusion, in an essentially all-comers population was the referent vessel size at the targeted lesion less than 2.5 mm. Therefore, the high MACE rate in current research could be attributed to the complex characteristics of the included patients. At the baseline, 90.5 % of patients had dyslipidaemia, while other studies showed lower prevalence, from 7.7 %<sup>31</sup> to 77.6 %.<sup>32</sup> Likewise, acute coronary syndromes were present in 54.8 % of patients and 47.6 % of patients had prior coronary revascularisation (40.5 % PCI and 7.1 % CABG). However, in the previous studies, few patients with biomarker-positive acute coronary syndromes were enrolled and the rates of prior PCI were much lower, ranging from 1.4 % to 9.2 %.<sup>31, 32</sup> Besides, it was shown in the ABSORB III trial that prior coronary interventions were the independent predictor of device-related adverse events, particularly device thrombosis.<sup>17</sup>

Interestingly, it seems that, in this study, diabetes mellitus did not contribute to the inferior clinical outcome in these patients because its incidence of 33.3 % was comparable with other studies that showed a lower MACE rate.<sup>31-33</sup> Also, the prevalence of stable and unstable angina at the baseline (57.1 % and 31.0 %, respectively) in treated patients was similar to the available data from the ABSORB III and ABSORB EXTEND clinical trials.<sup>17, 33</sup>

Relating to the lesion characteristics in the current study, bifurcations lesions were present in 16.7 %. Lesions at coronary bifurcations represent a challenging category of PCI procedures. Hypothetically, BVSs have certain advantages (faster arterial healing and late luminal enlargement) over DES, which makes them suitable for PCI of bifurcations lesions. However, there are overt disadvantages present like struts thickness (150  $\mu\text{m}$ ), causing disturbance of the local blood flow and increasing the chance of stent thrombosis.<sup>17, 33</sup> Having this in mind, the high rate of bifurcation lesions in the current study could be another contributing factor for the high MACE rate observed.

Concerning the effects of vessel size and procedural technique of BVS implantation on the long-term clinical outcomes, majority of individual studies – including the current one – were not properly sized to evaluate it. However, in a recent large-scale analysis from the major ABSORB studies, Stone has demonstrated that vessel sizing and operator technique were strongly associated with BVS-related outcomes during a 3-year follow-up.<sup>34</sup>

ABSORB IV was a large-scale, randomised, blinded, multicentre trial powered to detect small differences in safety and effectiveness between BVS and everolimus-eluting DES related to procedure techniques and selected population. This study established that 30-day and 1-year clinical outcomes of Absorb BVS can be improved with particular attention to the type of patients and lesions treated and the scaffold implant techniques used.<sup>35</sup> However, the adverse events in this study continued to occur slightly more frequently with BVS than with DES, mainly driven by BVS thrombosis.<sup>35</sup> With great interest, it is being awaited whether the 5-year follow-up results of this large study will reveal whether the improved stenting technique will favourably affect the late outcomes.

Finally, a putative influential factor on the presented results is inclusion of numerous operators in the current study, implying a lower number of stents per individual interventional cardiologist and the consequent slowing down of the learning curve. Besides, it is worth mentioning that the institutional interim adverse events monitoring was not prespecified in the study design.

The present study was, by design, limited to a 12-month follow-up, so it is inappropriate to expand the projections of MACE events beyond one year. However, despite the initial expectations, it was documented in the novel studies that composite adverse event rates continue to accumulate beyond one up to 3-years of follow-up and more frequently than with DES, mainly due to higher rates of BVS thrombosis and myocardial infarction.<sup>17</sup> AIDA trial reported long-term clinical outcomes of Amsterdam Academic Medical Centre (AMC) registry of Absorb BVS in the patient population reflecting daily clinical practice with up to 4-year follow-up. This registry confirmed also that long-term BVS related adverse events, particularly BVS thrombosis, continued to accrue beyond two years after Absorb implantation.<sup>36</sup> These findings are consistent with the reports obtained by selective optical coherence tomography showing persisting struts even four years after BVS deployment, with the potential to precipitate very late BVS thrombosis.<sup>37</sup> It is assumed that such late BVS discontinuities with translocation of uncovered strut fragments into the vessel lumen (intraluminal scaffold dismantling) represent one of the main factors responsible for the late BVS thrombosis.<sup>17,37</sup>

To summarise, overall, based on the favourable outcomes of multiple observational research and clinical trials, Absorb BVS was approved by the US Food and Drug Administration (FDA). However, after a relatively brief period of clinical usage, the pooled data of randomised clinical trials showed increased rates of late scaffold thrombosis and MACE compared to the second-generation DES. Related to these data, the FDA issued a warning notice in 2017 and Abbott Vascular stopped selling this device consequently.<sup>38</sup>

To our knowledge, the present research is one of few published reports on the experience with BVS implantation in our region since the clinical application of these scaffolds has been limited to a short period of time, with a limited number of Absorb stents allocated to individual interventional sites.

## Conclusion

Results of the current research demonstrated a high interventional success rate of the Absorb BVS implantation, followed by the early improvement in anginal status. However, that was not translated into favourable mid-term clinical outcomes. Therefore, the later findings strongly support Abbott's decision to recall the current version of Absorb BVS and foster future research on this disruptive innovative technology focusing on the improvement of device design and deployment techniques.

Finally, as a pearl of late wisdom, we point out the importance of an attentive and critical approach when adopting new disruptive technologies in interventional cardiology, with the need for watchful interim monitoring of clinical outcomes and on-the-go readjustment of proper indications and related procedural techniques.

## Study limitations

Current research shares all drawbacks of clinical registries, particularly the broad inclusion criteria allowing inclusion of unstable patients, as well as the involvement of numerous operators with individual approaches to the implantation techniques. At the time of project implementation, presence of dedicated institutional interim adverse events monitoring was not provided as well. Finally, limited sample size and consequently small number of clinical events disabled the use of statistical models to determine uni- and multi-variate predictors of device-related events at the follow-up.

## Acknowledgements

The authors share the highest personal appreciation with all interventional cardiologists from the Dedinje Cardiovascular Institute for their kindness in sharing their work on Absorb BVS implantation to be included in the current publication.

## Conflict of interest

None.

## Abbreviations and Acronyms used

ACS - Acute coronary syndrome  
 BRS - Bioresorbable stent  
 BVS - Bioresorbable vascular scaffold  
 BMS - Bare-metal stent  
 CABG - Coronary artery bypass grafting  
 DAPT - Dual antiplatelet therapy  
 DES - Drug-eluting metallic stent  
 FDA - Food and Drug Administration  
 LVEF - Left ventricular ejection fraction  
 MACE - Major adverse cardiovascular events  
 MI - Myocardial infarction  
 PCI - Percutaneous coronary intervention  
 SAQ - Seattle Angina Questionnaire  
 TIMI - Thrombolysis in myocardial infarction  
 TLF - Target lesion failure  
 TLR - Target lesion revascularisation  
 QCA - Quantitative coronary angiography

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# Risk Factors for Coronary Heart Disease and Family Medicine – What Can be Done?

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## Abstract

**Background / Aim:** More people die each year due to cardiovascular diseases (CVDs) than from any other cause. The most common cause of ischaemic heart diseases (IHD) is atherosclerosis of the coronary arteries. Risk factors for the development of coronary heart disease (CHD) can be preventable and non-preventable. The aim of the study was to determine the frequency of individual risk factors in patients with CHD.

**Methods:** Retrospective analysis included patients with diagnoses of stable angina pectoris (AP), unstable angina pectoris and myocardial infarction - acute coronary syndrome (ACS) and ischaemic cardiomyopathy (iCMP). The prevalence of the following risk factors for IHD was analysed: hypertension, diabetes, obesity, cholesterol, smoking, family history, age and sex. Data were taken from the Register of Patients with Chronic Diseases and Risk Factors and electronic patient records.

**Results:** Of the total number of respondents older than 18, 4.95 % had CHD. Of the 178 patients with IHD, 70 (39.3 %) patients had AP, 60 (33.7 %) patients had ACS and 48 (27.0 %) patients had iCMP. Positive family history had 63.5 % of patients, 72 % were older than 66, 24.1 % were smokers and 74.2 % of patients had elevated blood cholesterol levels. Diabetes mellitus affected 29.2 % of patients, hypertension 88.8 %, and BMI  $\geq 25$  kg/m<sup>2</sup> had 70.8 % of patients. Of the total number of patients with ACS, 68.3 % were men, while higher percentage of women suffered from AP (62.9 %) ( $p = 0.002$ ). In the age below 65, CHD was more common in men ( $p = 0.007$ ). Cholesterol was elevated more often in patients with AP than iCMP ( $p = 0.001$ ). Patients with ACS were more likely to have diabetes mellitus compared to patients with AP and iCMP ( $p = 0.010$ ).

**Conclusion:** The prevalence of preventable risk factors is alarmingly high. Of particular importance is the timely detection and treatment of risk factors by family physicians and strengthening the personal responsibility of each individual in choosing their lifestyle and active involvement in the therapeutic process.

**Key words:** Ischaemic heart disease; Diabetes mellitus; Hypertension; Obesity; Dyslipidaemia; Smoking; Physical inactivity.

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## ARTICLE INFO

Received: 16 October 2021  
Revision received: 6 December 2021  
Accepted: 7 December 2021

## Introduction

Ischaemic heart disease (IHD) occurs when the heart muscle's need for blood is greater than its ability to supply it.<sup>1</sup> It occurs in various diseases

and conditions in which the need of the myocardium is tampered and/or the blood supply is reduced due to various clinical features. The symp-

tom of ischaemic myocardium is pain (lat. *Angere* - choke).<sup>2</sup>

The diagnosis of IHD in generic terms refers to all diseases that lead to myocardial ischaemia, including non-atherosclerotic diseases of the coronary arteries. As atherosclerosis is by far the most common cause of IHD today (in more than 90 % of cases), the diagnosis of IHD in specific terms is used in everyday practice to describe atherosclerotic coronary artery disease. The term coronary heart disease (CHD) is often used for the same diagnosis as IHD in specific terms.<sup>3</sup>

More people die each year due to cardiovascular disease (CVD) than from any other cause. Around 17.7 million people died of CVD in 2016, representing 31 % of all deaths. Of those deaths, about 7.4 million died of CHD and 6.7 million died of stroke.<sup>4</sup>

In Europe, CVDs are responsible for just over 4 million deaths a year, or 47 % of all deaths.<sup>5</sup> Slightly less than half of CVD deaths are caused by IHD.<sup>6,7</sup> CVDs are more common in men than in women, but this difference in frequency between the sexes is statistically balanced at the age above 70.<sup>8</sup>

The most common cause of IHD is atherosclerosis of the coronary arteries. Atherosclerosis leads to mechanical - fixed narrowing of the coronary artery, but at the same time it is the basis for the occurrence of dynamic narrowing or spasm of the coronary artery (extremely strong pathological vasoconstriction), which occurs due to endothelial dysfunction. Mechanical and dynamic coronary artery stenosis reduce blood flow in segments of the myocardium supplied by a narrowed coronary artery.<sup>9</sup> Rare forms of IHD occur when coronary arteries are not altered by an atherosclerotic process but ischaemia is due to some other cause (aortic valve disease, hypertrophic cardiomyopathy, congenital anomalies of coronary circulation).<sup>9</sup> Atherosclerotic altered coronary arteries lose their normal vasodilatory reserve, already in the early stages of the disease, before the onset of significant narrowing, which is why the coronary flow cannot be adequately increased when myocardial oxygen demand increases.<sup>2</sup>

Risk factors that contribute to the development of atherosclerosis and CHD can be variable and invariable. The main variable (and preventable)

risk factors include: hypertension, obesity, dyslipidaemia, diabetes mellitus, smoking and physical inactivity and the invariable risk factors include family history, sex and age.<sup>8,10</sup> The risk of developing CHD increases progressively with high blood pressure.<sup>11</sup> Central type of obesity is more reliable risk marker for CHD than general type of obesity, determined by body mass index.<sup>12</sup> Weight loss has a good impact on blood pressure, dyslipidaemia and glucose metabolism. The risk increases with an increase in total cholesterol and low-density lipoprotein (LDL) cholesterol and with a decrease in high-density lipoprotein (HDL) cholesterol levels.<sup>13</sup> Progression of CHD can be prevented by good glycoregulation.<sup>14</sup> Preferably, the HbA1c values should be 6.5-6.9 %. The risk of developing CHD is reduced by 50 % one year after smoking cessation.<sup>15</sup> Individually tailored physical activity should be an integral part of everyday life.<sup>16</sup>

If CHD occurred in male relatives before the age of 55 and in females before the age of 65, the person is considered to have a positive family history of CHD. CHD is more common in men up to 75 years of age.<sup>17</sup> The risk increases with age.<sup>18</sup>

CHD can be clinically manifested as angina pectoris (AP), acute myocardial infarction (AMI), mute ischaemia, cardiac arrhythmia, sudden cardiac death, heart failure due to ischaemic cardiomyopathy (iCMP). Acute coronary syndrome is one of the most common and dramatic manifestations of ischaemic heart disease.<sup>19</sup> The term acute coronary syndrome encompasses three different entities of acute manifestation of coronary heart disease: Unstable angina pectoris (UAP), Non-ST elevation myocardial infarction (NSTEMI) and ST elevation myocardial infarction (STEMI).<sup>8,20</sup> Non-ST-elevation myocardial infarction and unstable angina are commonly referred to as non-STEMI acute coronary syndromes.<sup>21</sup>

The aim of the study was to analyse the prevalence and importance of risk factors in CHD patients: hypertension, diabetes, obesity, cholesterol, smoking, family history, age and sex.

## Methods

This research was a retrospective cross-sectional study. Data from two family medicine teams in the Primary Healthcare Centre in Banja Luka

were analysed. By using data from the Register of Chronic Non-Communicable Diseases (CVD Registry) of selected teams, patients with diagnoses of stable and unstable AP, AMI and iCMP were identified.

Data on the presence of individual risk factors were taken from electronic patient records. The following parameters were recorded: hypertension, diabetes, obesity, cholesterol, smoking, positive family history, age and sex. For obesity, the value of the Body Mass Index (BMI)  $\geq 25.0$  kg/m<sup>2</sup> was taken as a risk factor.<sup>22</sup> A value higher than 5.2 mmol/L was taken as elevated cholesterol. Patients were classified into age groups as follows: 18-45, 46-55, 56-65 years and fourth group older than 65.

Data were analysed in IBM SPSS Statistic 18.0. Descriptive analysis in the form of frequencies and percentages was used to review the sample and individual variables. Category data were compared using the Chi-square test. Statistical significance was set at  $p < 0.05$ .

## Results

The total number of registered patients older than 18 in the two selected family medicine teams was 3634. The study analysed a total of 178 patients (4.95 % of the total number of patients older than 18) with diagnoses of stable and unstable AP, myocardial infarction and iCMP.

Of the 178 patients, 92 (51.7 %) were male and 86 (48.3 %) were female (Table 1). Only one patient (0.6 %) was younger than 45. There were 9 (5.1 %) patients in the age between 46-55. A significantly higher number of patients, 40 of them (22.5 %) was in the age between 56-65 and the largest number of patients was older than 66, 128 or 72.0 % (Table 1).

**Table 1:** Gender and age distribution of patients with ischaemic heart disease

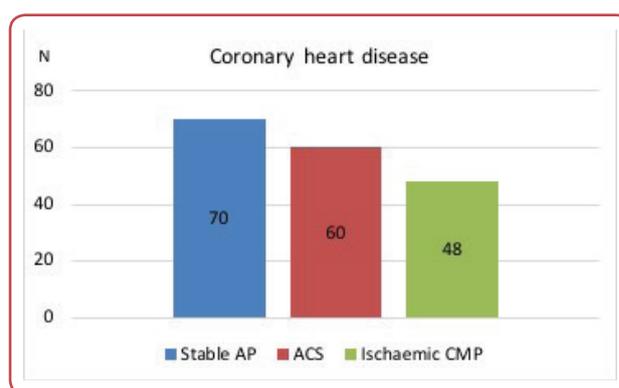
Parameter	N	%
<b>Gender</b>		
Male	92	51.69
Female	86	48.31
<b>Age (Years)</b>		
18-45	1	0.56
46-55	9	5.06
56-65	40	22.47
$\geq 66$	128	71.91
<b>Total</b>	<b>178</b>	<b>100.00</b>

Out of the total number of patients, 113 patients had a positive family history (63.5 %), 43 patients were smokers (24.1 %), and 132 patients (74.2 %) had elevated blood cholesterol values. Diabetes mellitus affected 52 patients (29.2 %), hypertension 158 (88.8 %) patients and there were 126 (70.8 %) overweight/obese patients (Table 2).

**Table 2:** Recorded risk factors in patients with ischaemic heart disease

Risk factors	N	%
<b>Family history</b>		
Positive	113	63.48
Negative	65	36.52
<b>Tobacco use</b>		
Yes	43	24.16
No	135	75.84
<b>Chol (mmol/L)</b>		
< 5.2	132	74.16
$\geq 5.2$	46	25.84
<b>Diabetes mellitus</b>		
Yes	52	29.21
No	126	70.79
<b>BMI (kg/m<sup>2</sup>)</b>		
< 25	126	70.79
$\geq 25$	52	29.21
<b>Hypertension</b>		
Yes	158	88.76
No	20	11.24
<b>Total</b>	<b>178</b>	<b>100.00</b>

Considering the type of IHD, 70 (39.3 %) patients had AP, 60 (33.7 %) patients had ACS, and 48 (27.0 %) patients had iCMP (Figure 1).



**Figure 1:** Distribution of certain types of ischaemic heart disease in the examined sample

\*AP: angina pectoris; ACS: acute coronary syndrome; CMP: cardiomyopathy;

A significant difference was found between certain types of CHD and the sex of the patient ( $\chi^2 = 12.59$ ,  $p = 0.002$ ). Of the total number of patients with ACS, 68.3 % were men, while in the case of AP, there was a higher percentage of women (62.9 %).

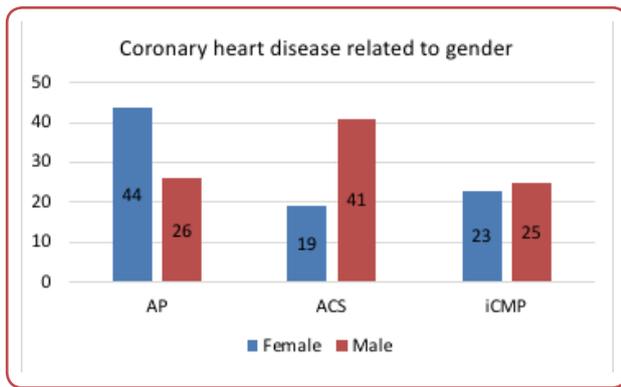


Figure 2: Distribution of the type of ischaemic heart disease by sex \*AP: angina pectoris, ACS: acute coronary syndrome, iCMP: ischaemic cardiomyopathy;

A significant difference was found between sex and age and frequency of CHD ( $\chi^2 = 11.989, p = 0.007$ ). In the age below 65, a significantly higher percentage of men than women suffered from CHD (Figure 3).

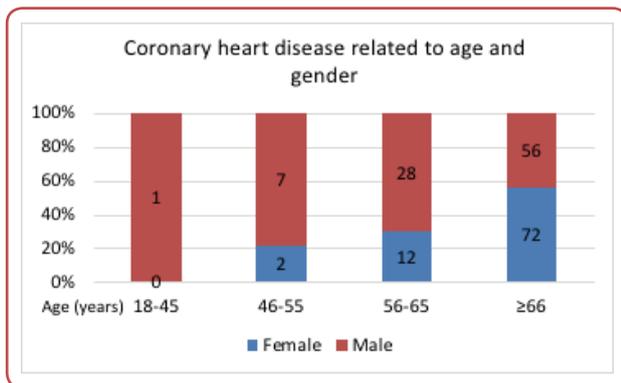


Figure 3: Incidence of ischaemic heart disease in relation to the sex and age of the patient

No significant difference was found between individual types of CHD and patients' age ( $\chi^2 = 8.791, p = 0.186$ ), a positive family history of cardiovascular disease ( $\chi^2 = 0.429, p = 0.807$ ) or a cigarette smoking ( $\chi^2 = 0.465, p = 0.793$ ).

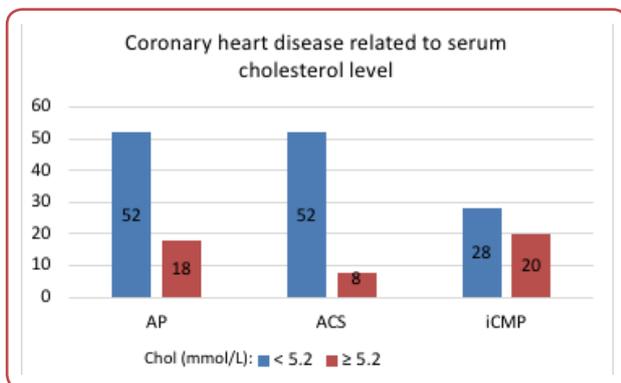


Figure 4: Distribution of ischaemic heart disease in relation to serum cholesterol values \*AP: angina pectoris, ACS: acute coronary syndrome, iCMP: ischaemic cardiomyopathy, Chol: cholesterol;

A significant difference was found between certain types of CHD and cholesterol values ( $\chi^2 = 14.980, p = 0.001$ ). In patients diagnosed with AP, 23.5 % of those with normal cholesterol were found, 13.3 % were diagnosed with ACS and 45.8 % were diagnosed with iCMP (Figure 4).

A significant difference was found between certain types of CHD and the presence of diabetes ( $\chi^2 = 9.148, p = 0.010$ ). Patients diagnosed with ACS in 43.3 % of cases also had diabetes mellitus, in contrast to patients diagnosed with AP and iCMP where diabetes as a risk factor was present in a significantly lower percentage (Figure 5).

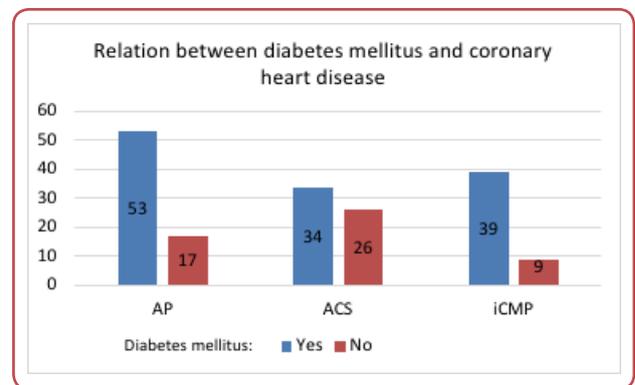


Figure 5: Distribution of ischaemic heart disease in relation to whether the patient suffered from diabetes mellitus \*AP: angina pectoris, ACS: acute coronary syndrome, iCMP: ischaemic cardiomyopathy;

No statistically significant difference was found either between individual types of CHD and hypertension ( $\chi^2 = 4.821, p = 0.090$ ), or between certain types of CHD and BMI ( $\chi^2 = 2.312, p = 0.315$ ).

## Discussion

The decline in standardised CVD mortality was observed in many European countries between the 1970s and 1990s including the most prompt and significant decline in wealthier countries, reflecting the potential for prevention of premature death and prolongation of healthy years of life. Mortality remains high in several Eastern European countries.<sup>23</sup>

CVDs are the leading cause of death in the Republic of Srpska. In the observed period from 2007-2011, the percentage of CVD in total mortality is constantly increasing (from 50.17 % in 2007 to 53.73 % in 2011).<sup>7</sup> During 2013 and 2014, there is a declining trend in CVD mortality. In the Re-



public of Srpska the CVDs cause 47.3 % of total mortality.<sup>24</sup>

The research data show that up to the age of 65, CHD affected a significantly higher percentage of men than women. This is explained by the protective effect of the female sex hormones on the occurrence of atherosclerotic heart disease. Similar results were obtained by other authors in their research.<sup>25</sup> In the study published in 2015, Mozaffarian et al showed that up to the age of 65 there is a significantly higher number of men than women suffering from heart attack and fatal cardiovascular disease, with the subsequently declining difference and with much higher number of women older than 85 being affected. Also they demonstrated a significantly higher percentage of women suffering from stable AP and men with myocardial infarction in all age groups.<sup>26</sup> Similar results were obtained in this study.

European Action on Secondary and Primary Prevention to Reduce Events resulted in three big studies: EUROASPIRE I, II and III.<sup>27-29</sup> The objective of these studies was to determine the prevalence of risk factors in coronary patients, ie how much changing lifestyle habits and educational programs are able to reduce risk factors and achieve target values according to the guidelines for cardiovascular prevention of the European Society of Cardiology. The set targets were related to smoking cessation, healthier diet, adequate physical activity, achieving BMI lower than 25 kg/m<sup>2</sup>, blood pressure lower than 140/90 mmHg, total cholesterol lower than 5 mmol/L, LDL-cholesterol lower than 3 mmol/L.<sup>27,28</sup> Following the implementation of the 2012 European recommendations for the prevention of coronary heart disease, EUROASPIRE IV was organised in 24 European countries during 2012 and 2013.<sup>23,30</sup> Between EUROASPIRE I (1996) and EUROASPIRE IV (2013), it was observed that the proportion of smokers did not change and that blood pressure control did not improve despite increased use of antihypertensive drugs, while the number of patients with (central) obesity continued to increase. On the other hand, lipid status control has improved significantly.<sup>29,31,32</sup>

The trend of incidence of risk factors for CHD in the Study of monitoring coronary patients in the Republic of Srpska (ROSCOPS) was made according to the EUROASPIRE methodology. The ROSCOPS I study was conducted in 1999-2001 in 5 centres and included 430 patients. The data indi-

cate a high prevalence of modifiable risk factors: there were 40.5 % smokers, 74.3 % hypertensive patients, 28.4 % patients with hyperlipidaemia, 24.3 % with diabetes, 25.7 % with obesity. After two years, the follow-up study of coronary patients ROSCOPS II (2002-2003), ROSCOPS III during 2006-2007, ROSCOPS IV during 2012 and ROSCOPS V during 2016 continues, which still show a high percentage of modifiable risk factors. The increase in obese patients and patients with elevated cholesterol levels is worrying.<sup>33,34</sup>

Data from this study shown that the leading risk factor were hypertension (88.8 %), followed by hypercholesterolaemia with 73.9 %, obesity 71 % and positive family history in 63.8 % of patients, while 29.2 % of patients were diagnosed with diabetes mellitus and the proportion of smokers was 24.1 % of the total number of patients. Compared to the research conducted in Croatia in the period 2007-2010 in hospitalised CHD patients, risk factors have a similar prevalence, except for smoking which in this sample showed a lower prevalence (24.1 % vs 42.2 %). The reason for the lower prevalence of smoking in this study is the assumption that a certain number of non-smokers are actually ex-smokers who stopped smoking after the illness, while in the Croatian study the data were taken during the patient's hospitalisation, ie at the time of coronary events.<sup>30</sup>

The main and independent risk factors for cardiovascular disease are cigarette smoking to any extent, high blood pressure, elevated total cholesterol and LDL-cholesterol, low HDL-cholesterol, diabetes and advanced age. The quantitative relationship between the listed risk factors and their influence on the development of cardiovascular diseases is shown by numerous studies (eg Framingham Heart Study).<sup>35</sup> The studies show that the main risk factors have an augmentative effect on the occurrence of cardiovascular diseases. Therefore, a person's overall risk of becoming ill can be estimated by multiplying the risk posed by each of the major risk factors individually.<sup>36</sup>

Tobacco smoking as an independent risk factor may result in a sevenfold increase in the risk of CHD.<sup>15</sup> The harmful effects of tobacco are proportional to the length of smoking and the amount of cigarettes smoked. On average, smokers die three years earlier than non-smokers, and with other risk factors combined, dying can be 10-15 years premature. The effect of tobacco smoking is especially pronounced if smoking is started before

the age of fifteen. Studies show that three years after smoking cessation, the risk of CHD equals the risk of non-smokers.<sup>37</sup> Thus, in EUROASPIRE IV the number of smokers at the time of the interview was 16 %, in ROSCOPS V 21 % (the interview was conducted at least 6 months after the coronary incident), while the number of smokers at the time of the coronary incident was much higher (48.6 % and 52 %, respectively). The number of smokers in this study was 24.1 %, assuming that the number of smokers at the time of the coronary incident was much higher.

High blood pressure is the so-called major risk factor for AP.<sup>38</sup> The prevalence of arterial hypertension increases with age. In addition to genetic factors, hypertension can be caused by obesity, alcohol consumption, intake of higher amounts of salt and animal fats and other factors.

Dyslipoproteinaemia represents an equally important and independent risk factor for the development and progression of the atherosclerotic process. This is especially true for the relation between LDL cholesterol increase and the HDL cholesterol concentration decrease and the increase in CHD incidence.<sup>39</sup> With a moderate increase in LDL cholesterol, in the presence of other risk factors such as smoking, hypertension or diabetes, the adverse effect of LDL on the progression of the atherosclerotic process is significantly increased. Patients with diabetes mellitus often have hypercholesterolaemia, ie increased amount of especially atherogenic LDLs. Their mortality from coronary heart disease is almost 10 times higher than in people without overt diabetes.<sup>40</sup>

Diabetes mellitus and CHD are strongly associated. Diabetes is associated with various forms of CHD, especially in women. Almost half of diabetics die from heart disease. Only a decrease in glucose tolerance poses a risk of developing coronary heart disease. Mortality from coronary heart disease in insulin-dependent diabetics is almost 10 times higher than in people without diabetes.<sup>41</sup> Comparison of the results with European and national data shows a significantly higher prevalence of elevated cholesterol, obesity, hypertension and smoking in the examined sample. The prevalence of diabetes in this sample is higher compared to EUROASPIRE IV, but slightly lower compared to the national population.

The results of this study show a high prevalence of variable risk factors, so it is obvious that phy-

sicians should insist on promoting a healthy lifestyle and improving patient awareness. It is necessary to raise the awareness of the general public about the need and importance of a healthier lifestyle and the preservation and improvement of health. Cooperation between all levels of health care in preventing and combating risk factors should be improved.

## Conclusion

The results of this study show a high prevalence of preventable risk factors for CVD. Of particular importance is the timely detection and treatment of risk factors by family physicians and strengthening the personal responsibility of each individual in choosing their lifestyle and active involvement in the therapeutic process.

## Acknowledgements

None.

## Conflict of interest

None.

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# Preparing a Rat Brain Tissue Samples for Acetylcholinesterase Activity Measurement - the MM method

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## Abstract

**Background/Aim:** Organophosphorus compounds (OP) bind to acetylcholinesterase (AChE) causing an irreversible inhibition of the enzyme. When doing *in vivo* studies of OP intoxication, to precisely measure AChE activity in the brain tissue it is necessary to remove as much blood from the brain as possible. By doing so, interference of the OPs present in the blood is avoided. Usually this demands expensive equipment, therefore, the aim of this study was to find a simple and economical method to eliminate the blood from brain blood vessels.

**Methods:** Wistar albino rats were divided into four groups named Control (C), Control washout (CW), Paraoxon (Pox) and Paraoxon washout (PoxW) group. Rats in Pox and PoxW were treated with 0.25 mg/kg paraoxon subcutaneously (sc), while C and CW received 1 mL/kg sc saline instead. The "Marinković-Maksimović" ("MM") method was performed in rats from PoxW and CW groups. Activity of AChE was measured both in erythrocyte lysate and in brain tissue using spectrophotometry.

**Results:** Macroscopic examination revealed that the elimination of blood was achieved in CW and PoxW groups. Activity of AChE in homogenised brain tissue was expectedly lower in the Pox and PoxW group, when compared to C and CW group, respectively. The CW group had a lower value of AChE activity in the brain tissue compared to C group, while activity of AChE in the PoxW group was statistically higher than in the Pox group ( $p = 0.044$ ).

**Conclusion:** The MM method provides good elimination of blood from the brain. Together with blood, present confounding factors that interfere with analysis in homogenised brain tissue, were also eliminated.

**Key words:** Organophosphorus compounds; Oximes; Acetylcholinesterase; Enzyme activity; Rat brain.

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## ARTICLE INFO

Received: 21 December 2021  
Revision received: 22 December 2021  
Accepted: 25 December 2021

## Introduction

Organophosphorus compounds (OP) are synthetic compounds first synthesised in the 1930s, that are vastly used as pesticides, but also developed as warfare nerve agents.<sup>1</sup> OP poisoning causes about 3,000,000 acute intoxications annually, 300,000 of which lead to fatalities.<sup>2</sup> They bind to the serine group of acetylcholinesterase (AChE),

form stable covalent bonds, making the enzyme inactive.<sup>3</sup> The OP-AChE bond does not spontaneously dissociate, which is why OPs are called irreversible AChE inhibitors. Inactivated AChE cannot perform its function - the breakdown of acetylcholine (ACh) in the synaptic cleft. Accumulated ACh stimulates muscarinic and nicotinic

postsynaptic receptors causing acute cholinergic effects.<sup>4</sup> OPs are mostly lipophilic substances that penetrate the blood-brain barrier (BBB) easily.<sup>5</sup> More lipophilic OPs are vastly distributed in fat tissue, from which they are gradually released to re-inhibit AChE. This leads to a prolonged inhibition of AChE and slower elimination of OPs.<sup>6</sup>

OP-AChE bonds can be reactivated by oximes. As such, they are an important therapeutic agent in the therapy of OP intoxication.<sup>5</sup> Unlike the OPs, oximes generally have a low degree of penetration through the BBB, because of which they have little to no ability to reactivate brain AChE.<sup>7</sup> Knowing that central respiratory depression is a main cause of death in OP intoxication,<sup>8,9</sup> when researching a new oxime, it is very important to determine the degree of its penetration through the BBB. Finding an oxime that is more penetrable, would mean a greater therapeutic success.

In animal models of OP intoxication, to determine the level of AChE inhibition in the central nervous system, a brain tissue is homogenised and the homogenate is analysed for AChE activity. To get accurate test results, it is necessary to remove as much blood from the brain tissue sample as possible. Otherwise, test results would be altered because of the OPs present in blood.<sup>10</sup> When searching the literature on this matter, a method of circulatory perfusion using an isotonic buffer solution and a Masterflex pump (Cole-Parmer, Chicago, IL, USA) was found.<sup>11</sup> As such equipment was not available to us at short notice, the aim of this study was to find a simple and affordable method for circulatory perfusion of Wistar albino rat brain.

## Methods

### Experimental animals

Wistar albino rats, male and female, weighing between 260 - 350 g were used. The animals were housed 4 per cage, in air-conditioned rooms with controlled temperature ( $21 \pm 1$  °C) and a 12 h light/dark cycle. They had access to food and water *ad libitum*. For this study ethical approval was given by the Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka (Decision No 18/1/20). During the

entire experiment, the “Guiding principles in the care of and use of laboratory animals” have been observed.

### Experimental protocol

To determine the efficacy of the circulatory perfusion method, rats were divided into 4 groups, 2 animals each. Groups were named Control (C), Paraoxon (Pox), Control with washout (CW) and Paraoxon with washout (PoxW). Rats in the Pox and PoxW group were treated with 0.25 mg/kg (0.25 mg/mL solution) paraoxon (Sigma Aldrich, St Louis, MO, USA) subcutaneously (sc), while C and CW groups received 1 mL/kg sc of saline instead. Half an hour later, rats were anaesthetised using 90 mg/kg ketamine and 10 mg/kg xylazine administered intraperitoneally (ip). The rat thoracic cavity was opened and blood samples were collected from the *vena cava inferior* into Lithium Heparin plasma tubes after which the animals were sacrificed via exsanguination. The “MM” method was performed in the rats from both PoxW and CW groups. Brains from both groups were excised, including *cerebrum*, *cerebellum* and the *truncus encephali* and stored at  $-20$  °C until homogenisation.

### The Marinković-Maksimović method (MM method)

To access the rat heart and major blood vessels the rats were tied to operating boards 15 x 25 cm in size. A skin and muscle incision was made just below the *processus xiphoides* using large surgical scissors. Next, bilateral incisions were made, following the medial axillary line. After separating the diaphragm from the anterior thoracic wall, the semi-attached anterior thoracic wall was clamped and put away to leave a clear operating field (Figure 1). To achieve exsanguination, *inferior vena cava* was cut just above the liver.

Prior to the cannulation of the aorta, a 10 cm long thread was pulled under the ascending aorta. Using microscissors a small diameter incision (approx. 1-2 mm) was made in the anterior wall of the ascending aorta, just above the aortic root. The tip of the cannulation tube was then inserted into the lumen of the aorta, making sure that it does not surpass the point of the first aortic arch branch (Figure 2). Using the earlier prepared tread, the tube was tied into place. The abdominal aorta was clamped, to ensure that the saline used for washout goes only to the upper half of the body. Also, to achieve a free outflow of saline



Figure 1: Access to the thoracic cavity of the rat

solution, *superior vena cava* was cut just above the heart. The upper body blood vessels were then perfused with a total of 100 mL of saline through the cannulation system, using 10 mL syringes (Figure 3). The efficiency of perfusion is observed during the procedure itself by observing the eyeballs of rats - pallor indicates the elimination of blood from the blood vessels of the eye (Figure 4).



Figure 3: Washout procedure

### Tissue homogenisation and measuring of the AChE activity

The right side *cerebrum*, *cerebellum* and *truncus encephali*, priorly frozen at - 20 °C were cut into smaller tissue fragments and weighted. Fragments were then mixed with phosphate buffer (0.1 M, pH 8.0) in a 1:20 ratio and homogenised at 10,000 rpm for 1 minute. Cold chain was main-

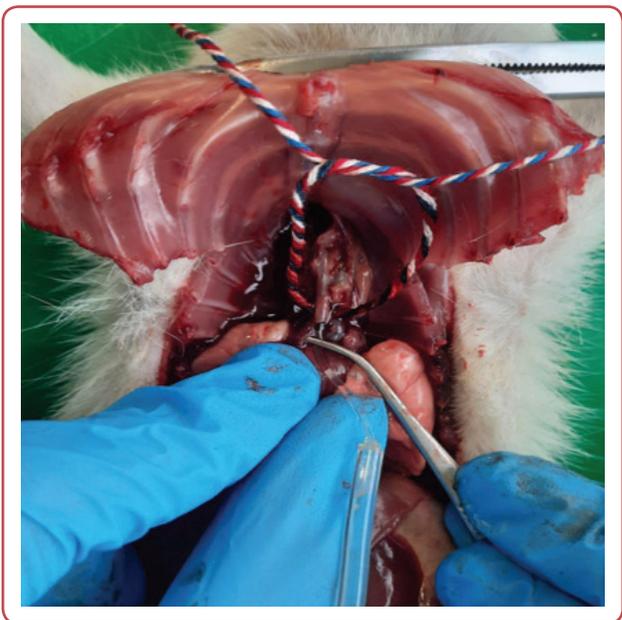


Figure 2: Cannulation of the rat aorta

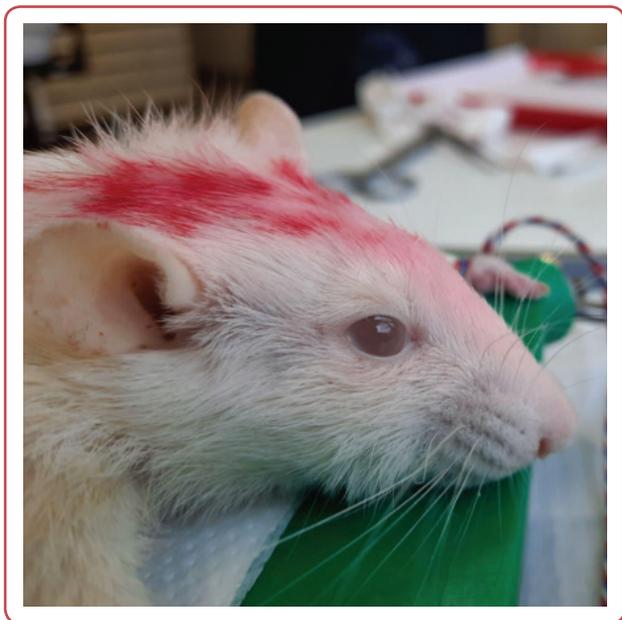


Figure 4: Iris pallor observed after implementation of the "MM" method

tained during the whole procedure. Homogenised tissue was centrifuged at 4 °C, for 15 minutes at 15,000 rpm and the supernatant was used as a sample for determining AChE activity in the brain tissue.

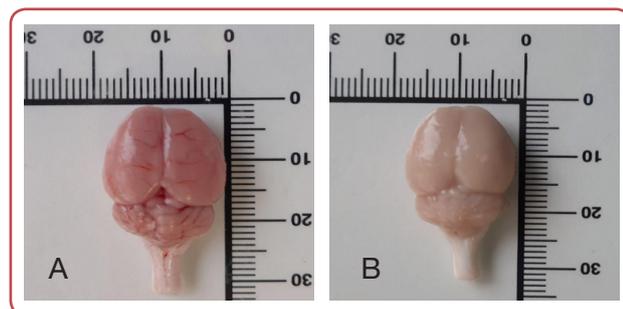
The AChE activity was measured by the Ellman colorimetric method using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) and UV Probe 2.17 software (Kyoto, Japan).<sup>12</sup> Activity was determined from the brain tissue samples and from blood samples (erythrocyte lysate). Activity of AChE in erythrocyte lysate was expressed as  $\mu\text{mol}/\text{min}/\text{mL}$ , while its activity in the brain tissue was expressed as  $\mu\text{mol}/\text{min}/\text{g}$ .

### Statistical analysis

Normality of data was analysed and confirmed by Kolmogorov-Smirnov test. Appropriate parametric test was performed (Student t-test). Data were shown as mean values with their standard deviations (SD). Statistical significance was set at  $p < 0.05$ . IBM SPSS version 23.0 for Windows software was used for data analysis.

## Results

Macroscopic examination of the brain indicated that good elimination of blood from brain tissue was achieved by washout (Figure 5).



**Figure 5:** Macroscopic results of the "MM" method. The macroscopic difference in appearance of the rat brain from the control group (A) and from the control with washout group (B)

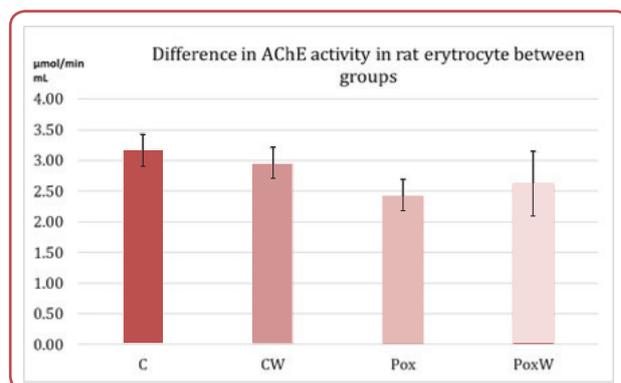
Detailed values of weight, brain weight and AChE activity for each experimental animal are given in Table 1.

No significant difference in mean values of rat brain mass between groups with and without washout was found ( $t = 2.29$ ,  $p = 0.262$ ). There was no significant difference between mean values of AChE activity in erythrocytes between C

and CW group (Student t-test:  $t = 0.88$ ,  $p = 0.472$ ), as well as between Pox and PoxW group ( $t = 0.45$ ,  $p = 0.708$ ) (Figure 6).

**Table 1:** Acetylcholinesterase (AChE) values in rat brain and erythrocytes related to treatment

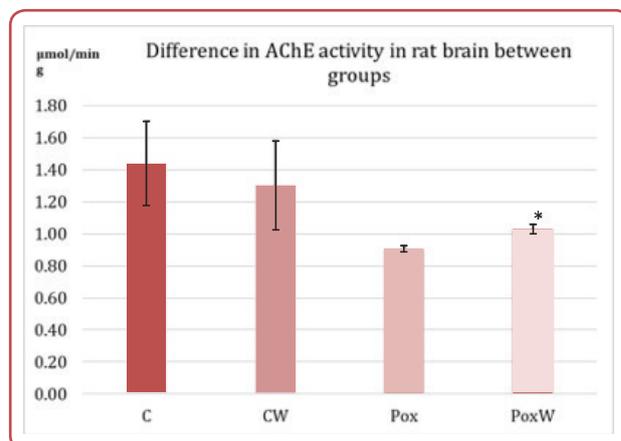
Group	Rat No	Rat mass (g)	Rat brain mass (g)	AChE activity in erythrocyte ( $\mu\text{mol}/\text{min}/\text{mL}$ )	AChE activity in brain tissue ( $\mu\text{mol}/\text{min}/\text{g}$ )
C	1	263	1.92	3.36	1.63
	2	271	1.84	2.99	1.26
CW	3	342	2.16	2.76	1.50
	4	354	2.30	3.13	1.11
Pox	5	271	2.30	2.24	0.92
	6	350	2.13	2.61	0.89
PoxW	7	307	2.02	2.24	1.05
	8	315	2.02	2.99	1.01



**Figure 6:** Acetylcholinesterase (AChE) activity in the rat erythrocyte lysate samples

C - control group; CW - control with washout group; Pox - paraoxon group; PoxW - paraoxon with washout group.

Values are presented as mean values  $\pm$  standard deviations;



**Figure 7:** Acetylcholinesterase (AChE) activity in the rat brain tissue samples

C - control group; CW - control with washout group; Pox - paraoxon group; PoxW - paraoxon with washout group;

Values are presented as mean values  $\pm$  standard deviations;

\*There was significant difference between AChE activity in rat brain between Pox and PoxW group (Student t-test:  $t = 5.00$ ,  $p = 0.044$ ).

There was no significant difference between mean values of AChE activity in rat brain between C and CW group (Student t-test:  $t = 0.52$ ,  $p = 0.655$ ), but there was significant difference between Pox and PoxW group ( $t = 5.00$ ,  $p = 0.044$ ) (Figure 7).

## Discussion

When analysing the AChE activity in brains of rats poisoned with OP and treated with oximes, the presence of blood in the brain can distort the results for several reasons, primarily due to lipophilicity of OPs and poor penetration of oximes through the BBB. Following absorption, OPs accumulate rapidly in fat, liver, kidneys and salivary glands. Lipophilic OPs are released more slowly from adipose tissue and have a milder acute but prolonged effect. When the applied oxime reactivates AChE, the leakage of lipophilic OPs from the depot re-inhibits AChE. Paraoxon (diethyl (4-nitrophenyl) phosphate) is the active metabolite of the OP insecticide parathion.<sup>13</sup> The phosphorothioates (P=S) (eg, diazinon, parathion) are more lipophilic than phosphates (P=O) (eg, dichlorvos, paraoxon). Spontaneous reactivation of dimethyl-phosphorylated AChE often occurs rapidly even without oxime therapy. There is no such expectation for AChE inhibited with diethyl phosphoryl insecticides.<sup>5</sup>

How to increase therapeutic potential of oxime, by improving their BBB penetration remains a challenge in modern toxicology. As a very selectively permeable membrane, the BBB imposes several limitations for oxime penetration to the brain tissue, such as hydrophilicity, polarity and molecule size. Consequently, pyridinium aldoximes (PyAls) such as pralidoxime (2-PAM) have a very low rate of BBB penetration,<sup>14, 15</sup> which is mostly associated with their high hydrophilicity<sup>10</sup> and permanent cationic charge. 2-PAM, which is most commonly used oxime in the treatment of OP poisonings, has a relatively low level of transport through the BBB.<sup>15-17</sup>

Respiratory failure has been identified in numerous studies as the leading cause of death in OP poisoning.<sup>18-20</sup> Houze et al<sup>21</sup> have shown that respiratory failure is most likely the result of central respiratory depression, rather than peripheral bronchoconstriction, bronchorrhoea and

respiratory muscle fatigue.<sup>8, 9</sup> Knowing that, a crucial point in therapy would be reactivation of AChE in brain tissue.<sup>15</sup> Because of that, non-quaternary organic compounds are introduced as novel antidotes in OP poisoning.<sup>22, 23</sup> Not having a quaternary group gives them a better penetrability through the BBB. Data from the literature indicate that the chlorine atoms in the oxime increase its lipophilicity.<sup>24</sup> In OP insecticides, it has been shown that oximes with a functional group at the position 4 reactivate OP insecticides most effectively. Oximes with a bis-quaternary structure are more efficient than those ones with mono-quaternary structure. It is best that linker has 3-5 C-C connections. The double bond in the linker increases both their efficiency and toxicity.<sup>25, 26</sup>

There are several ways to determine the efficiency of brain AChE reactivation by oximes, but most widely used is an indirect method. With this method the level of BBB of an oxime penetration is measured by the level of AChE reactivation in the brain tissue. To achieve precise results, it is necessary to remove blood from the brain. This way OPs present in the blood will not interfere with the results. Washing out blood from the brain by using the "MM" procedure, showed reduced detected AChE activity in homogenised brain tissue, which resulted in 0.15  $\mu\text{mol}/\text{min}/\text{g}$  difference between control groups (C and CW). This decrease was due to the removing of the AChE present in red blood cells, whose activity could be measured in the brain homogenate in the absence of washing out. In both groups treated with paraoxon AChE activity was expectedly lower when compared with controls (37.24 % drop when comparing C and Pox group and 21.37 % difference between CW and PoxW). Comparing the results between Pox and PoxW group, AChE activity has remained higher in the PoxW group that was subjected to the "MM" procedure. Evidently the washout prevented remaining paraoxon from blood to bind with AChE and thus to distort the level of AChE activity in the brain after homogenisation.

By introducing the "MM" method, few things were tried to be avoided; one of them is that OP re-arrives from the blood into the brain to re-inhibit the AChE, thus obtaining lower values of AChE activity than the real ones. On the other hand, a new oxime that would penetrate BBB better could reactivate AChE in the brain. The presence of blood in homogenates would call into question

the origin of reactivated AChE - by eliminating the blood, the obtained values of AChE activity are those from the brain and not from the blood and brain.

## Conclusion

The “MM” method provides good elimination of blood from the rat brain and thus eliminates confounding factors in the analysis of parameters from brain homogenates. The introduction of the procedure in the Centre for Biomedical Research, Faculty of Medicine, University of Banja Luka enables the universality of the procedure and the reproducibility of the results.

## Acknowledgements

We would like to thank the Centre for Biomedical Research, Medical Faculty, University of Banja Luka, for providing us with the space, equipment and experimental animals. We would also like to thank Professor Rajko Igić, for sending to us the plastic tubing without whom the method would not have been possible.

## Conflict of interest

The authors declare that there are no conflicts of interest. This study is partially funded by the Ministry of Scientific and Technological Development, Higher Education and Informational Society of the Government of the Republic of Srpska (Grant No 125 7030).

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# Independent Role of CT Chest Scan in COVID-19 Prognosis: Evidence From the Machine Learning Classification

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## Abstract

**Background:** The current coronavirus disease-19 (COVID-19) pandemic call attention to the key role informatics play in healthcare. The present study discovers an independent role of computerised tomography chest (CT) scans in prognosis of COVID-19 using classification learning algorithms.

**Methods:** In this retrospective study, 57 RT PCR positive COVID-19 patients were enrolled from SMS Medical College, Jaipur (Rajasthan, India) after approval from the Institutional Ethics Committee. A set of 21 features including clinical findings and laboratory parameters and chest CT severity score were recorded. The CT score with mild, moderate and severe categories was chosen as response variable. The dimensionality reduction of feature space was performed and classifiers including, decision trees, K-nearest neighbours, support vector machine and ensemble learning were trained with principal components. The model with highest accuracy and area under the ROC curve (AUC) was selected.

**Results:** The median age of patients was 55 years (range: 20-99 years) with 37 males. The feature space was reduced from 21 to 7 predictors, that included fever, cough, fibrin degradation products, haemoglobin, neutrophil-lymphocyte ratio, ferritin and procalcitonin. The linear support vector machine was chosen as the best classifier with 73.7 % and 0.69 accuracy and AUC for severe CT chest score, respectively. The variance contributed by first three principal components were 97.5 %, 2.4 % and 0.0 %, respectively.

**Conclusion:** In view of low degree of relationships between predictors and chest CT scan severity score category as interpreted from accuracy and AUC it can be concluded that chest CT scan has an independent role in the prognosis of COVID-19 patients.

**Key words:** Classification; COVID-19; Chest CT scan; Machine learning; Pandemic.

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## ARTICLE INFO

Received: 17 October 2021  
Revision received: 23 November 2021  
Accepted: 23 November 2021

## Introduction

Globally, there have been 76,023,488 confirmed cases and 1,694,128 deaths due to COVID-19 as on 22 December 2020.<sup>1</sup> A large number of mathematical models and machine learning methods have been proposed to characterise the various aspects of pandemic.<sup>2</sup> The machine learning algorithms have been used in automated analysis of

CT images during COVID-19 pandemic to reduce the effort of clinicians.<sup>3-5</sup> The World Health Organization recommends RT PCR test for the diagnosis of COVID-19.<sup>1</sup> The low sensitivity of RT PCR and high sensitivity of chest computerised tomography (CT) for COVID-19 make chest CT a useful tool in the diagnosis.<sup>6</sup> A number of studies showed

relationship between disease severity and chest CT severity score in COVID-19. Similarly, studies showed relationship of clinical and laboratory parameters with disease severity.

In addition to usefulness of CT chest in diagnosis, its key role in prognostication and further management of COVID-19 patients has been proposed. Furthermore, CT scan is important in the patient triage and from logistics point of view. The objective of the present study was to evaluate independent role of CT chest in the prognosis of COVID-19 patients utilising various machine learning classifiers.

## Methods

A retrospective cross-sectional study was planned to discover independent role of CT chest in COVID-19 prognosis. A total of 57 RT-PCR SARS CoV-2 positive patients were enrolled from SMS Medical College, Jaipur (Rajasthan, India) after obtaining approval from the institutional Ethics Committee. The clinical findings and laboratory parameters along with CT chest score of COVID-19 patients were recorded. The relationship between CT score category with clinical and laboratory parameters was evaluated using classifiers while selecting CT score category as response variable. The CT chest has definite role in prognosis of COVID-19, but this study was undertaken to assess the independent role of CT chest in prognosis of COVID-19 as compared to clinical and laboratory parameters.

### Feature selection

A set of 21 features included age, sex, history of diabetes mellitus, history of hypertension, complaints of fever, cough, shortness of breath, sore throat, haemoglobin (Hb) in grams %, total leucocyte count (TLC) in 1,000 cells per  $\text{mm}^3$ , platelet count (PC) in 100,000 per  $\text{mm}^3$ , Differential Neutrophil Count (DNC) in percent, Differential Lymphocyte Count (DLC) in percent, neutrophil-lymphocyte count ratio (NLR), fibrin degradation products (FDP) in  $\text{mg/mL}$ , D-dimer in  $\text{ng/mL}$  FEU, activated partial thromboplastin time (APTT) in seconds, prothrombin time (PT) in seconds, international normalised ratio (INR), procalcitonin in  $\text{ng/mL}$  and ferritin in  $\text{ng/mL}$ . The CT scoring was based on lobe involvement, as suggested by Li et al.<sup>14</sup> A score was assigned for each lobe on the basis of its involvement: score 0 for 0 % involvement, score 1 for less than 5 % involvement, score 2 for 5 % to 25 % involvement, score

3 for 26 % to 49 % involvement, score 4 for 50 % to 75 % involvement and score 5 for greater than 75 % involvement. There was a score of 0 to 5 for each lobe, with a total possible score lie between 0 to 25.<sup>7</sup> The CT score was converted from ordinal to categorical response variable so machine learning classification can be applied. The CT score from 0 to 11 was considered as mild, from 12 to 17 as moderate and from 18 to 25 as severe.

### Dimensionality reduction

The features and response variable were imported in the classification learner app.<sup>8</sup> The classifiers were trained with individual predictors and accuracy and area under the ROC curve (AUC) of each model was recorded. Among them, 10 most accurate predictors were selected. Correlation analysis and parallel coordinate plot (PCP) was used for further feature selection and visualisation. A total of 7 features were selected from 10 features (Figure 1). To further reduce the dimensionality of feature space, principal component analysis (PCA) was performed. In view of 57 observations, considering 10 observations per feature, 5 features were used to train the classifiers with 5-fold cross validation.<sup>9</sup>

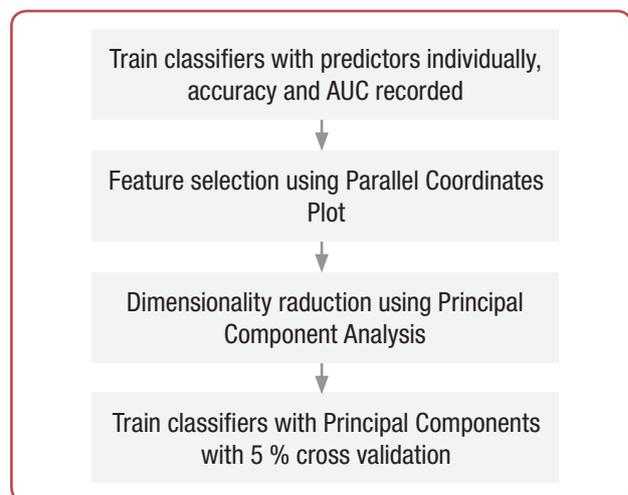


Figure 1: Flowchart showing steps performed to reduce dimensionality of feature space

### Classifiers and training

The classifiers used in the application include decision trees, support vector machine (SVM), K nearest neighbours and ensemble learning classifiers. These classifiers used different methodology for classification.<sup>10</sup> The decision trees include complex tree, medium tree and simple tree classifiers. The SVM include linear SVM, quadratic SVM, cubic SVM, fine Gaussian SVM, medium Gaussian SVM and coarse Gaussian SVM classifiers. The ensemble classifiers include boosted trees, bagged trees and RUS boosted trees classifiers.

### Statistical analysis

The accuracy and AUC for various classification models were calculated using classification learner app in MATLAB 2016a (MATLAB Team, 2020).<sup>9</sup> A low degree of relationship between CT score and predictors can be interpreted as more likelihood of independent role of CT scan and vice-versa.

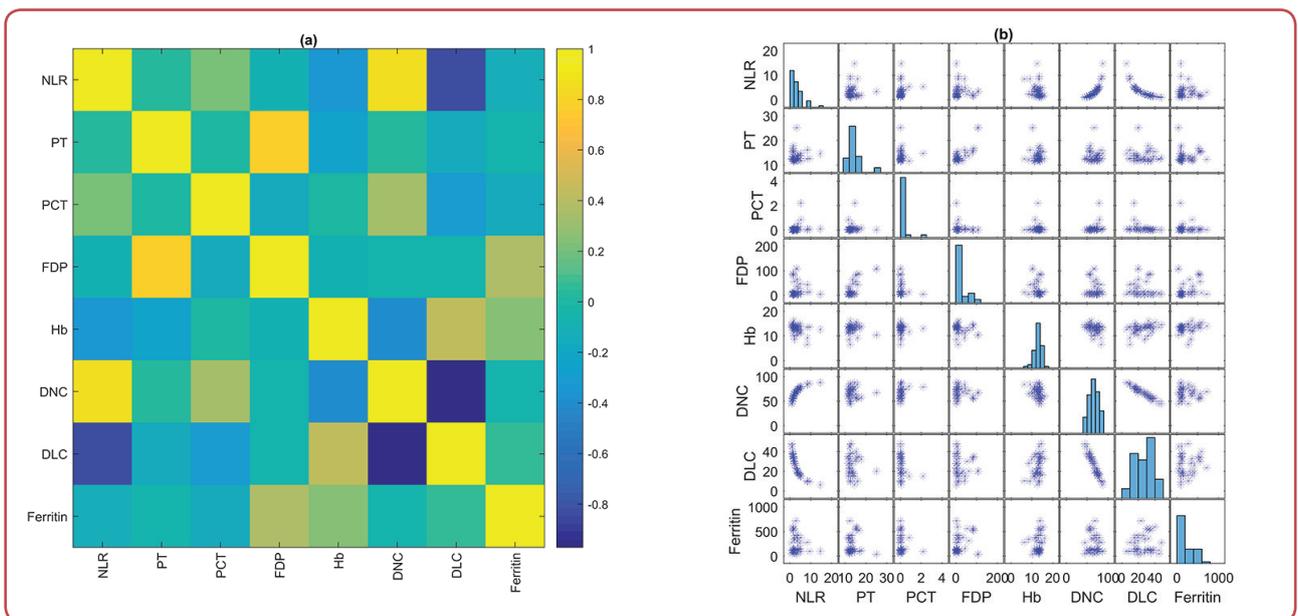
## Results

The individual features (number of features = 21) of 57 patients (median: 55 years, range 20-99 years; 37 males) with COVID-19 were trained with CT score as response variable. The top ten predictors with highest accuracy were selected. These include NLR, fever, PT, procalcitonin, cough, FDP, Hb, DNC, DLC and ferritin (Table 1). Correlation analysis showed that PT was highly correlated with FDP ( $r = 0.78$ ;  $p < 0.001$ ); DNC was highly correlated with NLR ( $r = 0.85$ ;  $p < 0.001$ ) and, DLC ( $r = -0.85$ ;  $p < 0.001$ ) showed high correlation with NLR and DNC ( $r = -0.98$ ;  $p < 0.001$ ). From the above features PT, DNC and DLC were removed. The relationships can be visualised in parallel coordinate plots (Figure 2 and 3). Thus, two qualitative features, fever and cough and five quantitative features including FDP, Hb, NLR, ferritin and procalcitonin were selected. As there were 2 categorical features (as PCA is not applicable to categorical variables), 3 principal components were chosen manually to get a total of 5

**Table 1:** The decision tree, support vector machines (SMV), K-nearest neighbour (KNN) and ensemble classifiers were trained with individual features. The classifier model with highest accuracy and area under the curve (AUC) for each feature is shown

Variable	Classifier	AUC	Accuracy (%)
NLR	Ensemble Bagged Trees	0.87	75.40
Fever	Fine KNN and Ensemble Subspace KNN	0.50	64.90
PT	Fine Gaussian SVM	0.80	64.90
Procalcitonin	Fine Gaussian SVM	0.74	64.90
Cough	Fine KNN and Ensemble Subspace KNN	0.50	63.20
FDP	Medium Gaussian SVM	0.64	63.20
	Ensemble Boosted Trees	0.58	63.20
DNC	Complex and Medium Tree	0.61	63.20
DLC	Ensemble Bagged Trees	0.70	63.20
Ferritin	Complex and Medium Tree	0.69	63.20
Age	Cubic and Medium KNN	0.63	61.40
APPT	Simple Tree	0.68	61.40
INR	Quadratic SVM	0.70	61.40
TLC	Fine Gaussian SVM	0.47	61.40
Shortness of Breath	Ensemble RUS Boosted Trees	0.72	59.60
PC	Fine KNN	0.60	59.60
Sex	Cubic SVM	0.56	57.90
Sore Throat	Cubic SVM	0.70	57.90
History of Hypertension	Coarse Gaussian SVM	0.63	57.90
History of Diabetes Mellitus	Cubic SVM	0.51	57.90
d-Dimer	Coarse KNN	0.44	57.90

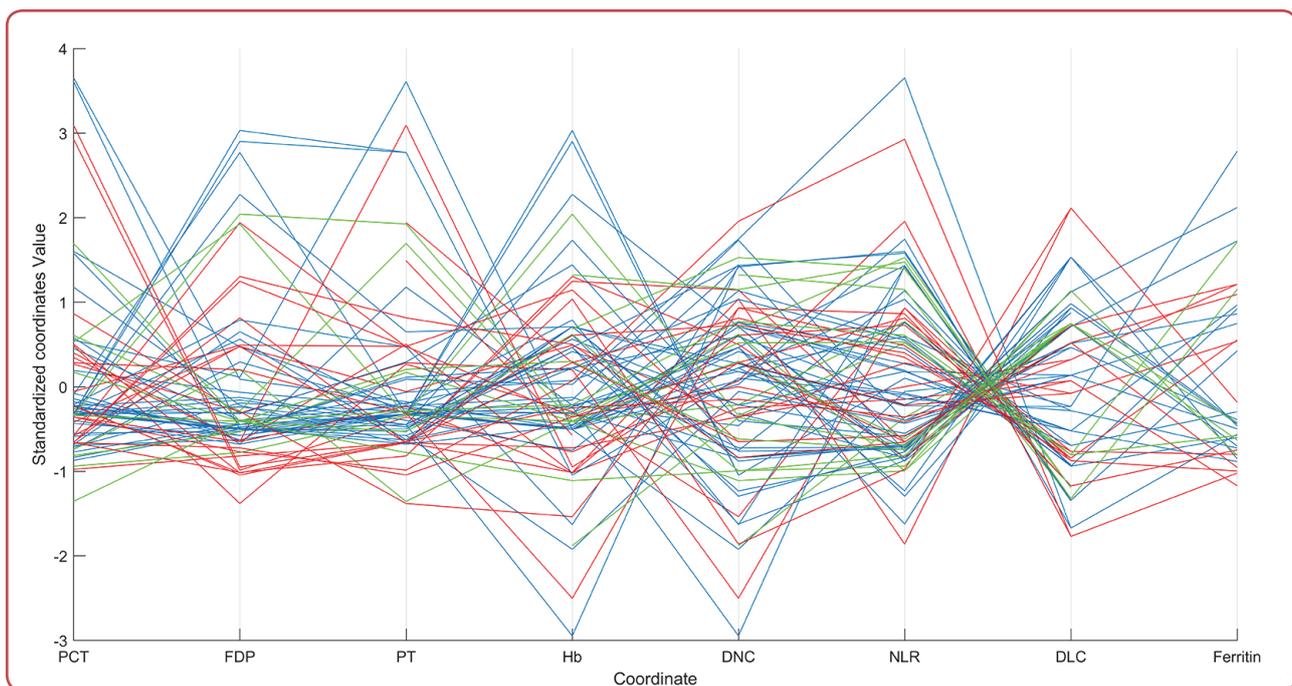
PCT: procalcitonin; FDP: fibrin degradation products; PT: prothrombin time; Hb: haemoglobin; DNC: differential neutrophil count; NLR: neutrophil-lymphocyte ratio; DLC: differential lymphocyte count.



**Figure 2:** Correlation analysis (a) Heat map shows positive correlation between FDP and PT; DNC and NLR; negative correlation between DLC and NLR; DLC and DNC (b) scatter map shows correlation among selected features.

PCT: procalcitonin; FDP: fibrin degradation products; PT: prothrombin time; Hb: haemoglobin; DNC: differential neutrophil count; NLR: neutrophil-lymphocyte ratio; DLC: differential lymphocyte count.





**Figure 3:** Shows parallel coordinate plots to visualise relationship among various features. NLR and DLC features has shown inverse relationship. PCT: procalcitonin; FDP: fibrin degradation products; PT: prothrombin time; Hb: haemoglobin; DNC: differential neutrophil count; NLR: neutrophil-lymphocyte ratio; DLC: differential lymphocyte count.

features to train the models. The best model was linear support vector machine with 73.7 % and 0.69 accuracy and AUC for severe CT chest level respectively. The variance contributed by three principal components were 97.5 %, 2.4 % and 0.0 %, respectively.

## Discussion

As per World Health Organization, a confirmed case of COVID-19 is defined as a patient with RT-PCR test-positive for SARS CoV-2, irrespective of clinical signs and symptoms. The pooled estimate of sensitivity of RT-PCR tests for SARS CoV-2 is 89 % (95 % CI: 81 - 94 %). Thus, one or more negative results do not rule out COVID-19.<sup>11</sup> To compensate the shortcomings of the low sensitivity and time-consuming process of RT-PCR, chest CT examination has an auxiliary and key role in the diagnosis and subsequent management of COVID-19 patients.

First, CT chest is more sensitive than RT-PCR test as concluded from various studies. A meta-analysis included six studies comprising a total of 1431 patients who were mainly symptomatic and at high risk for COVID-19, reported a chest CT pooled sensitivity of 94.6 % (95 % CI: 91.9 - 96.4 %) and a pooled specificity of 46.0 % (95 %

CI: 31.9 - 60.7 %) in the detection of COVID-19.<sup>6,12</sup> Similarly, Caruso showed sensitivity, specificity and accuracy of CT were 97 % (95 % CI: 88 - 99 %), 56 % (95 % CI: 45 - 66 %) and 72 % (95 % CI: 64 - 78 %), respectively.<sup>13</sup> The misdiagnosis rate of CT chest scan to diagnose COVID-19 is quite low when taking reverse transcriptase polymerase chain reaction as gold standard (3.9 %).<sup>14</sup> The CT chest scan has a key role in diagnosis of COVID-19.<sup>15,16</sup> In more than 70 % of RT-PCR test positive cases of SARS CoV-2, CT chest findings include ground-glass opacities, vascular enlargement, bilateral abnormalities, lower lobe involvement and posterior predilection. However, about 10 % to 70 % patients revealed consolidation (51.5 %), linear opacity (40.7 %), septal thickening and/ or reticulation (49.6 %), crazy-paving pattern (34.9 %), air bronchogram (40.2 %), pleural thickening (34.7 %), halo sign (34.5 %), bronchiectasis (24.2 %), nodules (19.8 %), bronchial wall thickening (14.3 %) and reversed halo sign (11.1 %). The uncommon findings include pleural effusion (5.2 %), lymphadenopathy (5.1 %), tree-in-bud sign (4.1 %), central lesion distribution (3.6 %), pericardial effusion (2.7 %) and cavitating lung lesions (0.7 %).<sup>17</sup>

Second, CT chest can be used as a follow-up tool to monitor the disease evolution and evaluate the severity of COVID-19 patients for its invasiveness and objectivity. Furthermore, CT can predict the prognosis.<sup>18</sup> The present study emphasises the in-

dependent role of CT in prognosis of COVID-19 patients. The use of a chest CT severity score may be useful for standardised assessment of the degree of pulmonary involvement in COVID-19 for prognostication purposes.<sup>19</sup> Based on the CT chest score, disease severity can be classified into mild, moderate and severe. Though fever (80 %, 74 - 87 %) and cough (53 %, 33 - 72 %) are prevalent symptoms in COVID-19, there was no correlation with disease severity as evaluated in the present study.<sup>20</sup> In case of laboratory parameters, except NLR no other parameter was significantly related to CT chest score. However, Xiong showed relationship between CT severity and C-reactive protein, erythrocyte sedimentation rate and lactate dehydrogenase.<sup>21</sup> In his study, Durhan found no relationship between COVID-19 disease severity and fever ( $p = 0.82$ ), dry cough ( $p = 0.46$ ), diabetes ( $p = 0.60$ ), hypertension ( $p = 0.29$ ), haemoglobin ( $p = 0.92$ ). Though he showed significant correlation between CT severity score and age ( $p = 0.001$ ), sex ( $p = 0.002$ ), neutrophil count ( $p = 0.03$ ), lymphocyte count ( $p = 0.01$ ), NLR ( $p = 0.004$ ), platelet count ( $p = 0.03$ ), ferritin ( $p < 0.001$ ), CRP ( $p < 0.001$ ), procalcitonin ( $p < 0.001$ ) and D-dimer ( $p < 0.001$ ).<sup>22</sup> Hana et al found platelet count is not associated with COVID-19 severity.<sup>23</sup> In a retrospective study of 313 patients, ferritin and CRP levels were significantly higher in patients with severe CT findings compared to the patients with mild and moderate CT findings ( $p < 0.05$ ).<sup>24</sup> The chest CT score had positive associations with total leukocyte count, CRP, ESR, procalcitonin and a negative association with lymphocyte count.<sup>25</sup> In a similar study, correlation analysis showed that the CT chest score was significantly correlated with lymphocyte count, monocyte count, C-reactive protein, procalcitonin, days from illness onset and body temperature ( $p < 0.05$ ).<sup>26</sup> In view of low degree of relationships between predictors and CT score category it can be concluded that chest CT scan has independent role in prognosis of COVID-19.

In addition to above, the Fleischner Society recommends CT imaging: (a) to establish a baseline pulmonary status; (b) to facilitate risk stratification in patients with comorbidities and (c) in patients with moderate to severe symptoms of COVID-19.<sup>27</sup> The current COVID-19 pandemic has highlighted the essential role of chest CT examination in patient triage in the emergency departments, allowing them to be referred to "COVID" or "non-COVID" wards.<sup>28</sup> Rubin et al emphasised the use of CT chest in diagnosis of COVID-19 from logistic point of view.<sup>27</sup>

## Conclusion

Despite inconsistencies in relationships between CT chest score and other parameters, most of the relationships are not clinically significant and an independent role of CT chest in the prognosis and further management of COVID-19 patients is proposed. Furthermore, due to the low sensitivity of RT PCR, CT chest scan is recommended for disease management.

## Limitations of the study

The sample size of the study is low. The study includes the routine clinical and laboratory parameters, however, independence of CT chest scan with other laboratory parameters needs to be tested.

## Ethical Statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Ethical Committee of the SMS Medical College, Jaipur (No 429 dated 2020 Jun 26) and the individual consent for this retrospective analysis was waived.

## Acknowledgements

The contributors to the article acknowledge the Principal, SMS Medical College and Attached Hospitals, Jaipur (Rajasthan, India) and Department of Medicine for availability of data and their ongoing support against the menace of the global pandemic of COVID-19.

## Conflict of interest

None.

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# Esmolol as an Adjunct to General Balanced Anaesthesia in Neurosurgery

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## Abstract

**Background / Aim:** In surgery, and especially in the neurosurgical operations, maintenance of cardiovascular stability during and in the phase of the immediate postoperative recovery is of vital importance. The aim of this study was to investigate the effects of continuous esmolol infusion on the values of cardiovascular parameters and quality of the emergence from anaesthesia in neurosurgical patients.

**Methods:** A total of 40 patients of both sexes scheduled for elective supratentorial surgery were randomly assigned to two groups. Esmolol group received intravenous (iv) infusion of esmolol dissolved in 5 % glucose solution (during the first 5 min at a rate of 0.3 mg/kg/min and thereafter at a rate of 0.1 mg/kg/min), while the ones from the control group received a 5 % glucose solution without esmolol at the same volume and rate. Cardiovascular parameters were registered at critical phases of anaesthesia and operation (induction, intubation, placement of Mayfield frame, craniotomy, skull closure, extubation). Recovery after anaesthesia was assessed based on times of eye opening on command, spontaneous eye opening and regaining of full orientation.

**Results:** Values of systolic blood pressure and heart rate were significantly lower in the esmolol than in the control group of patients. Although the durations of anaesthesia did not differ, patients from the esmolol group required significantly less opioids and isoflurane and recovered after the anaesthesia significantly faster than the patients in the control group.

**Conclusion:** Ultrashort-acting beta-adrenergic receptor antagonist esmolol, administered as a continuous iv infusion, assures better cardiovascular stability and smoother emergence from the balanced inhalation general anaesthesia than the control glucose infusion in elective neurosurgical patients.

**Key words:** Beta-adrenergic receptor antagonists; Esmolol; General anaesthesia; Hypertension; Tachycardia.

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### ARTICLE INFO

Received: 20 December 2021  
Revision received: 28 December 2021  
Accepted: 29 December 2021

## Introduction

Surgical trauma activates numerous neurohumoral mechanisms, including increased secretion of catecholamines, followed by hypertension and tachycardia.<sup>1</sup> Modern anaesthetic techniques tend to use a combination of various drugs with the aim to prevent or at least alleviate such reflexes. Usually, this combination includes premedication

with benzodiazepines, intubation under the use of short-acting neuromuscular relaxants, induction to anaesthesia with barbiturates, maintenance inhalation and/or intravenous anaesthetics, opioid analgesics, use of competitive neuromuscular blockers and, at the end, of atropine and neostigmine for the decurarisation purposes.<sup>2</sup>

Esmolol, as a rapid-acting and titratable beta-adrenergic receptor antagonist, turned out to be an important addition to this concept of general balanced anaesthesia.<sup>3</sup> It was used to treat the episodes of hypertension and tachycardia, first in cardiac surgery<sup>4,5</sup> and thereafter in all other similar indications,<sup>6,7</sup> including the electroconvulsive therapy.<sup>8</sup>

There are some critical phases of anaesthesia and operation where a proper cardiovascular control is needed. In neurosurgery specifically, these moments include induction, tracheal intubation, placement of the Mayfield frame, craniotomy, skull closure, skin suture and extubation.<sup>9,10</sup> Fast-track emergence from neuro-anaesthesia in order to early detect the possible neurological sequelae is usually accompanied by the increased sympathetic activity, hypertension, tachycardia and increased cerebral perfusion pressure, which all put patients at risk for the development of the postoperative haemorrhage and cerebral haematoma.<sup>11</sup> For these reasons, the aim of this study was to ascertain the effects of the continuous esmolol infusion on the cardiovascular parameters and other indicators of the quality of anaesthesia in patients scheduled for elective supratentorial surgery.

## Methods

A total of 40 American Society of Anesthesiologists (ASA) I or II category patients aged 18-80 years and scheduled for elective supratentorial surgery were enrolled in this clinical study, after signing an informed consent. The study had previously been approved by the local ethics committee. Patients were randomly assigned to two groups by using the block randomisation.

All of them were subjected to standard premedication and general anaesthesia technique. The only difference was in the fact that the patients from the esmolol group received intravenous (iv) infusion of esmolol dissolved in 5 % glucose solution (during the first 5 min at a rate of 0.3 mg/kg/min and thereafter at a rate of 0.1 mg/kg/min), while the ones from the control group received a 5 % glucose solution without esmolol at the same volume and rate.

Esmolol 5 g was dissolved in a bottle containing 500 mL of 5 % glucose solution, yielding the final concentration of 10 mg/mL. The speed of the iv infusion in both groups was 0.03 mL/kg/min during the first 5 min and 0.01 mL/kg/min thereafter. The speed of the maintenance iv infusion of esmolol was set at 0.1 mg/kg/min, since it had been earlier revealed that hypertension rarely occurs at the infusion rates below 0.15 mg/kg/min<sup>12</sup> and because it had been ascertained in a separate dose-finding clinical study that the esmolol infusion rate that did not affect the baseline cardiovascular parameters by more than 10 % was 0.118 mg/kg/min.<sup>13</sup>

Premedication technique in all the patients consisted of diazepam 10 mg intramuscularly (im) 30-45 min before induction into anaesthesia with thiopentone 3-5 mg/kg iv and fentanyl 1.5 µg/kg. Tracheal intubation was facilitated with suxamethonium 1-2 mg/kg iv. Long-lasting neuromuscular blockade was maintained with pancuronium bromide 0.07 mg/kg iv. In case of need, incremental iv boluses of pancuronium bromide 0.01 mg/kg were administered. Nitrous oxide and oxygen (2:1) were used by inhalation for anaesthesia maintenance. At the end of operation, atropine 0.5 mg and neostigmine 1.5 mg were used for the neutralisation of the neuromuscular block.

An increase in systolic blood pressure or heart rate by more than 20 % of the pre-induction values was treated with an iv bolus of fentanyl 1.5 µg/kg alone, or with droperidol at the ratio 1:50 (in a form of Thalamonal®). In case that a bolus of fentanyl or Thalamonal® would not be sufficient to treat an episode of hypertension or tachycardia, isoflurane 0.5 % was added into the inhalation mixture. An iv bolus of atropine 0.5 mg was injected to treat episodes of bradycardia, defined as decrease in heart rate by more than 20 % of the pre-induction values.

Quality of anaesthesia was estimated by the attending anaesthesiologist by using the following scale: 1 – bad, 2 – good and 3 – excellent.

Parametric tests – Student t test and ANOVA – and non-parametric test Chi-square were used for statistical analysis. For these purposes the Statistical Package for Social Sciences (SPSS) version 18.0 was utilised.

## Results

The two studied groups of patients were equal (20 patients each) and not significantly different from each other regarding their demographic characteristics and mean pre-induction values of cardiovascular parameters (Table 1) in a form of Thalamonal).

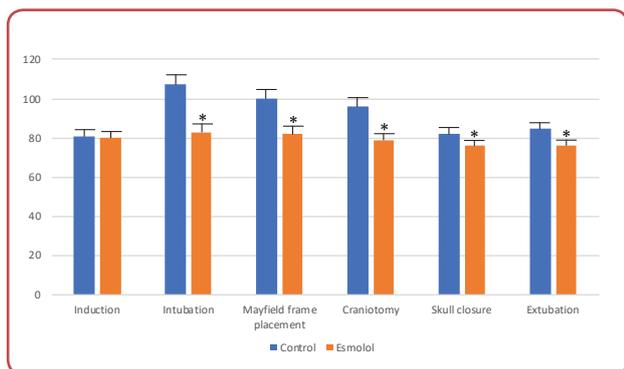
**Table 1:** Demographic data and preinduction values of the cardiovascular parameters in control patients (n = 20) and patients infused with esmolol (n = 20) during the neurosurgical operations under the general balanced anaesthesia

Parameter (unit)	Control (mean ± SE)	Esmolol (mean ± SE)	Statistical significance
Age (years)	49.58 ± 3.62	46.75 ± 5.12	ns
Body mass (kg)	81.17 ± 3.55	76.83 ± 3.20	ns
Gender: Male/Female	12 vs 8	12 vs 8	ns
Systolic blood pressure	142.08 ± 5.42	153.92 ± 9.12	ns
Diastolic blood pressure	92.50 ± 1.90	89.42 ± 3.00	ns
Heart rate	85.50 ± 2.55	92.15 ± 4.47	ns

SE - standard error of the mean; ns - not significant

Values of cardiovascular parameters - systolic blood pressure, diastolic blood pressure and heart rate - in critical phases of anaesthesia and operation are shown in Figures 1, 2 and 3, respectively.

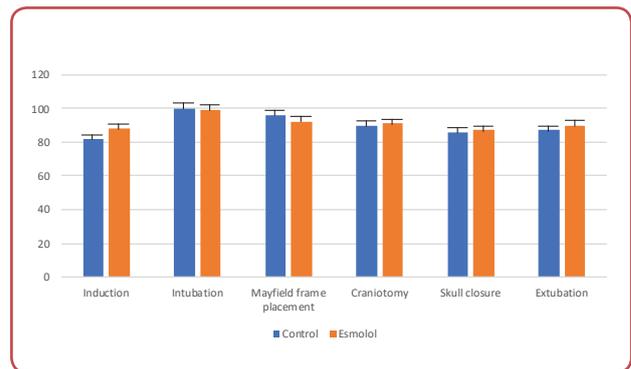
It is obvious from the Figure 1 that in all the critical phases of anaesthesia and operation, with the exception of the intubation phase, esmolol group of patients had significantly lower values than the patients from the control group.



**Figure 1:** Systolic blood pressure in various phases of anaesthesia and operation in control patients (n = 20) and patients infused with esmolol (n = 20) during the neurosurgical operations under the general balanced anaesthesia\*

\*Values are expressed as percentages of pre-induction values (mean ± standard error of the mean)

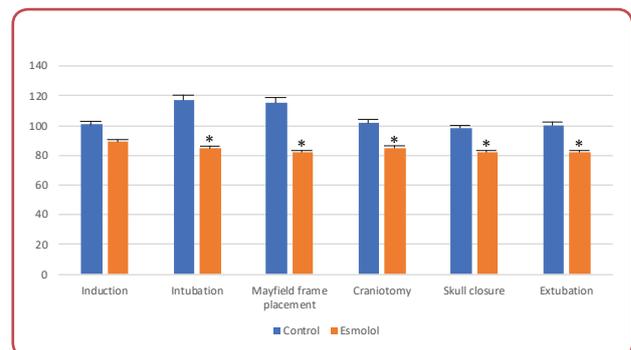
There were no significant differences between the values of the diastolic pressure between the control and esmolol group of patients (Figure 2).



**Figure 2:** Diastolic blood pressure in various phases of anaesthesia and operation in control patients (n = 20) and patients infused with esmolol (n = 20) during the neurosurgical operations under the general balanced anaesthesia\*

\*Values are expressed as percentages of pre-induction values (mean ± standard error of the mean)

Regarding the heart rate, it was obvious that patients from the esmolol group had not only significantly lower values than the control patients throughout the anaesthesia and operation, but also had very stable heart rate values in all the critical phases (Figure 3).



**Figure 3:** Heart rate in various phases of anaesthesia and operation in control patients (n = 20) and patients infused with esmolol (n = 20) during the neurosurgical operations under the general balanced anaesthesia\*

\*Values are expressed as percentages of pre-induction values (mean ± standard error of the mean)

**Table 2:** Consumption of medicines in control patients (n = 20) and patients infused with esmolol (n = 20) during the neurosurgical operations under the general balanced anaesthesia

Drug (unit)	Control (mean ± SE)	Esmolol (mean ± SE)	Statistical significance
Fentanyl (mg)	1.30 ± 0.14	0.49 ± 0.09	p < 0.01
Droperidol (mg)	23.13 ± 2.99	7.08 ± 1.34	p < 0.01
Atropine (mg)	1.08 ± 0.08	0.92 ± 0.08	ns
Pancuronium (mg)	16.50 ± 1.67	11.00 ± 1.18	p < 0.05
Isoflurane (% of patients)	50.00	10.00	p < 0.05

SE - standard error of the mean; ns - not significant

All the medicaments, including fentanyl and isoflurane, were used in significantly larger quantities in patients from the control group, in com-



parison with the patients treated with esmolol (Table 2). The only exception was use of atropine that did not differ between the groups.

**Table 3:** Effect of esmolol on speed and quality of postoperative recovery in control patients (n = 20) and patients infused with esmolol (n = 20) during the neurosurgical operations under the general balanced anaesthesia

Parameter (unit)	Control (mean ± SE)	Esmolol (mean ± SE)	Statistical significance
Duration of anaesthesia (min)	174.17 ± 16.62	170.00 ± 18.64	ns
Opening of eyes on command (min)	16.33 ± 1.94	4.40 ± 1.21	p < 0.01
Spontaneous opening of eyes (min)	25.20 ± 3.09	10.70 ± 2.70	p < 0.01
Full orientation (min)	29.83 ± 3.34	16.00 ± 3.85	p < 0.05
Extubation possible (% of patients)	50.00	90.00	p < 0.05
Evaluation of quality of anaesthesia	1.50 ± 0.15	2.17 ± 0.17	p < 0.01

SE - standard error of the mean; ns - not significant

Patients in the esmolol group had a tendency to recover from anaesthesia quicker than the patients from the control group (Table 3).

At the same time, the mean duration of operation in both groups was similar and yet extubation in the operation theatre was possible in almost all patients in the esmolol group, while this was the case in only half of the control patients.

## Discussion

The results of this study show that the continuous esmolol infusion assured cardiovascular stability enabling a decreased use of additional opioids and anaesthetics, which in turn resulted in better post-anaesthesia recovery.

These results are in general agreement with the results of other clinical trials. Indeed, injection and/or iv infusion of esmolol alleviated increases in blood pressure and heart rate induced by tracheal intubation<sup>9</sup> and extubation in neurosurgical patients.<sup>10-17</sup> Especially this effect at the end of the neurosurgical operation, after the extubation of trachea and in the immediate post-anaesthesia recovery period seems to be vitally important in neurosurgery, since esmolol blocks the beta<sub>1</sub>-adrenergic receptors and prevents the sympathetic overdrive-induced cerebral hyperaemia.<sup>11, 18-20</sup>

Since opioid analgesics control painful stimuli and thus blunt the sympathetic nervous system reflexes, use of a beta-blocker like esmolol assures the similar outcome, however without additional doses of opioids or inhalation anaesthetics. The present study showed a significant opioid-sparing effect of esmolol and so did some other clinical trials.<sup>21-23</sup> Various publications even report on the decreased need for the administration of intravenous and inhalation anaesthetics as a result of esmolol iv bolus or infusion.<sup>24, 25</sup> In the present study similar result was obtained, since there was a significantly lower need for isoflurane addition in the esmolol group, as compared to the control group of patients.

Although similar effects of esmolol were obtained as regards to the systolic blood pressure and heart rate, its effect on the values of heart rate were more accentuated than the ones on the blood pressure, which is in accordance with the findings of Ornstein et al, who found that the half-time for the 14 %-decrease in heart rate was only 1.2 min, as compared to the half time of 17.8 min for the 25 %-decrease in mean arterial blood pressure.<sup>26</sup>

Besides esmolol, some other agents were tried with the same aim to control reflex sympathomimetic reactions resulting from laryngoscopy and intubation<sup>27</sup> or from tracheal extubation.<sup>28</sup> It seems that esmolol should be preferred over nitroglycerin<sup>29</sup> or lidocaine,<sup>30</sup> while dexmedetomidine<sup>27</sup> or nicardipine<sup>28</sup> could be more efficient than esmolol.

## Conclusion

Ultrashort-acting beta-adrenergic receptor antagonist esmolol, administered as a continuous iv infusion, assures better cardiovascular stability and smoother emergence from the balanced inhalation general anaesthesia than the control glucose infusion in elective neurosurgical patients.

## Acknowledgements

None.

## Conflict of interest

None.

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# Association Between Oxygen Saturation, Neutrophil-Lymphocyte Ratio and D-dimer With Mortality Based on Clinical Manifestation of COVID-19 Patients

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## Abstract

**Background / Aim:** Coronavirus 2019 (COVID-19) infection is caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). It has become an emergency condition for global public health. Oxygen saturation has important role for diagnosing the patient in the hospital. The neutrophil-lymphocyte ratio (NLR) is a marker for the viral inflammatory reaction to confirm the viral or bacterial infection. The D-dimer is also known as biomarker for severity and prognosis of COVID-19. The aim of the study was to investigate the risk factors of mortality in COVID-19 patients based on oxygen saturation, neutrophil-lymphocyte ratio and D-dimer.

**Methods:** Data has been obtained from confirmed cases of COVID-19 in Abdul Wahab Sjahranie Hospital from October until November 2020. They were collected and analysed with Chi-squared test by SPSS 22.0 software.

**Results:** There were 60 patients with SARS-CoV-2 infection in this study, 48 (80 %) patients survived and 12 (20 %) deceased. Peripheral oxygen saturation < 90 % had 13 (21.7 %) and ≥ 90 % had 47 (78.3 %) patients. There were 13 (21.7 %) patients with NLR value < 3.13 and ≥ 3.13 47 (78.3 %) patients. D-dimer value less than 0.5 had 9 (15 %) and ≥ 0.5 had 51 (85 %) patients. The results of statistical analysis have showed that there were relationship between oxygen saturation ( $p = 0.002$ ) and survival rate of COVID patients.

**Conclusion:** According to the research that has been conducted, there was correlation between oxygen saturation and mortality of COVID-19 patient. It could be used as biomarker to improve the management of COVID-19 patients.

**Key words:** Oxygen saturation; Neutrophil lymphocyte ratio; D-dimer; Clinical manifestations of COVID-19.

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## ARTICLE INFO

Received: 28 October 2021  
Revision received: 4 December 2021  
Accepted: 5 December 2021

## Introduction

Coronavirus disease 2019 (COVID-19), was discovered in Wuhan, China, in December 2019. It was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).<sup>1</sup> Since December 2019 to January 2020, number of cases have increased rapidly, reported of 44 cases. In

less than a month, this disease has spread in other countries such as Thailand, Japan and South Korea. This virus could be transmitted from human to human widely. Latest case on 13 August 2020, WHO announced there were 20 million confirmed cases and 737,417 cases people died of COVID-19

worldwide; while in Indonesia 1,026,954 million cases has been determined with 132,138 confirmed positive cases and 5,968 (4.5 %) cases with lethal outcome.<sup>2</sup> The pathogenesis of SARS-CoV-2 is still unknown. It will infect alveoli with receptors binding and make entrance to cell. Glycoprotein that contained in envelope spike, virus will bind to cellular receptor as Angiotensin-converting enzyme 2 (ACE-2) on SARS-CoV-2. SARS-CoV-2 will do duplication of genetic material and synthesising proteins in cells, then it will appear on the surface cell as new virion.<sup>3</sup>

Based on existing data, comorbid disease such as hypertension and diabetic mellitus, as well as male gender, active tobacco smoking are risk factors of SARS-CoV-2 infection. It is caused by ACE-2 receptors that increase rapidly.<sup>4</sup> Others risk factors are having contact, including living in the same household with COVID-19 patient and travel history to affected area. If living in one environment but no contact within two meters radius is also considered as low risk. It has been determined by Centres for Disease Control and Prevention (CDC).<sup>5</sup> There are asymptomatic, mild, moderate and severe infection that could be found in severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). There are several clinical manifestations of this virus including fever, cough and shortness of breath, watery diarrhoea, myalgia, severe lymphopenia, prolonged clotting profiles, heart disease and sudden death.<sup>6,7</sup> The incidence rate of coronavirus has increased greatly in various countries, so WHO has declared it as pandemic on 30 January 2020.<sup>8</sup> Statistics has shown 14 % of pneumonia-related COVID-19 cases are severe and 5 % of infected patients require intensive care.<sup>9</sup>

This situation is very high risk nationally as well as globally as long as vaccine implementation is still in progress. In Indonesia, public health emergency management is carried out through quarantine both at the entrance and region. Indonesia has taken policy for making large social restriction, based on epidemiological considerations, the magnitude of threat, effectiveness, resources support, technical operations, political considerations, economics, social and cultural.<sup>10</sup> Based on the laboratory results, there are biomarkers that could represent inflammation also mortality such as oxygen saturation, neutrophil lymphocyte ratio (NLR) and D-dimer.

## Methods

### Design of the study

This study was cross-sectional observational analytical study of 60 patients that were treated at Abdul Wahab Sjahranie Hospital, Indonesia from October to November 2020.

### Study subjects

These subjects were patients who were under supervision for COVID-19. Inclusion criteria were: (1) confirmed COVID-19 infection based on real time reverse transcriptase polymerase chain reaction (RT-PCR) from throat swab sample; (2) typical symptoms of pneumonia such as fever, cough, shortness of breath, etc; and (3) the severity of pneumonia classified as moderate or severe.

### Diagnostic procedures

The diagnosis of COVID-19 was based on the Coronavirus Disease Prevention and Control Guideline of the Directorate General for Disease Prevention and Control, Indonesia.<sup>10</sup> All subjects have done blood tests, chest X-rays and PCR swab tests.

### Data measurement

Peripheral oxygen saturation (SpO<sub>2</sub>) was divided into < 90 % and ≥ 90 %.<sup>10</sup> The NLR is systemic inflammatory marker of the infectious disease obtained by dividing the absolute number of neutrophil by the absolute lymphocyte count. The NLR cut-off point of was divided into two groups at < 3.13 and ≥ 3.13 based on research.<sup>11</sup> D-dimer value less than 0.5 µg/mL was considered normal.<sup>12</sup>

### Statistical analysis

The data showed normal distribution. Chi-square was used to analyse the association between oxygen saturation, NLR and D-dimer with mortality. P-value < 0.05 was set as statistically significant. SPSS 22.0 software was used.

### Ethical clearance

Ethical authorisation was obtained from the Health Research Ethics Committee, Abdul Wahab Sjahranie Hospital under No 253/KEPK-AWS/X/2020.

## Results

In this study, medical records from COVID-19 patients at Abdul Wahab Sjahranie Hospital were used.

The total sample were 60. From this study, 48 (80 %) patients survived and 12 (20 %) deceased.

**Table 1:** Characteristics and status of patients infected with SARS-CoV-2

Gender	All patients (n = 60)	Survived (n = 48)	Deceased (n = 12)
Male	38 (63.3 %)	28 (65.1 %)	10 (34.9 %)
Female	22 (36.6 %)	17 (39.5 %)	5 (60.5 %)

SpO<sub>2</sub> < 90 % had 13 (21.7 %) and ≥ 90 % had 47 (78.3%) patients had significance mortality rate based on clinical manifestation (p = 0.002).

**Table 2:** Oxygen saturation findings of patients infected with SARS-CoV-2

Oxygen saturation	Deceased (n = 12)	Survived (n = 48)	p value	Mean	Standard Deviation
< 90 %	7 (58.3 %)	6 (12.5 %)	0.002	1.78	0.41
≥ 90 %	5 (41.7 %)	42 (87.5 %)			

There were 13 (21.7 %) patients with NLR value < 3.13 and ≥ 3.13 47 (78.3 %) patients. There was no statistical significance in mortality rate between groups, with p = 0.488 (p > 0.05 %).

**Table 3:** Neutrophil-lymphocyte ratio of patients infected with SARS-CoV-2

Neutrophil-lymphocyte ratio	Deceased (n = 12)	Survived (n = 48)	p value	Mean	Standard Deviation
≥ 3.13	13 (30.2 %)	47 (69.8 %)	0.488	1.21	0.41
< 3.13	2 (16.7 %)	11 (22.9 %)			

**Table 3:** D-dimer values of patients infected with SARS-CoV-2

D-dimer	Deceased (n = 12)	Survived (n = 48)	p value	Mean	Standard Deviation
≥ 0.5	12 (100 %)	39 (81.3 %)	0.113	1.15	0.36
< 0.5	0 (0 %)	9 (18.8 %)			

The value of D-dimer ≥ 0.5 was in 51 (85 %) and < 0.5 was in 9 (15 %) patients. There was no statistical significance in mortality rate between groups, with p = 0.113 (p > 0.05).

## Discussion

Based on the Coronavirus Disease Prevention and Control (COVID-19) Guideline of the General Directorate of Disease Prevention and Control, Indonesia, there are some criteria to categorise COVID-19. First is asymptomatic COVID-19 (patient show no health problem). Second, mild clinical manifestations without complications (patients with non-specific symptoms such as fever, cough and headache). Patients usually recover after one week. The third is a moderate, including symptoms such as mild pneumonia, but no sign of severe pneumonia. Fourth, severe pneumonia (patients with respiratory infection who present with one of symptoms: respiratory rate more than 30 times per minute and oxygen saturation less than 90 %). Fifth is a critical illness (patients get worst after one week, with manifested acute respiratory distress syndrome - ARDS).<sup>10</sup>

There are 3 criteria of ARDS: mild ARDS if 200 mmHg < PaO<sub>2</sub>/FiO<sub>2</sub> ≤ 300 mmHg; moderate ARDS if 100 mmHg < PaO<sub>2</sub>/FiO<sub>2</sub> ≤ 200 mmHg with PEEP ≥ 5 cm H<sub>2</sub>O, or without ventilation; severe ARDS if PaO<sub>2</sub>/FiO<sub>2</sub> ≤ 100 mmHg with PEEP ≥ 5 cmH<sub>2</sub>O, or without ventilation. Critical illness could cause septic shock, multi organ failure, including kidney failure or heart failure and death. The patient's final conditions that confirmed as COVID-19 pneumonia indicated that 48 (80 %) patients survived and 12 (20 %) patients deceased. SpO<sub>2</sub> had important role in mortality ratio. It also could be correlated with the final prognosis.<sup>10</sup>

The role of pathogenesis and progression of ACE-2 is target receptor for viral entry that can cause hypoxia. There is decrease of amount oxygen level especially in arterial blood. ACE-2 is an enzyme that can attach to the outer surface membrane in several organs, such as lungs, kidney, heart, arteries and intestines. ACE-2 will catalyse the conversion of angiotensin-2 (vasoconstrictor) to angiotensin 1-7 (vasodilator) to protect lung injury. Excessive ACE-2 expression in human will increase the severity of COVID-19 infection.<sup>13</sup> In human body, ACE-2 has important roles in expressing the surface of pulmonary alveolar epithelial cells and enterocytes in arterial as well as venous endothelial cells.<sup>14</sup> Another consideration is the phenomenon of hypoxic pulmonary vasoconstriction. Clinical observations of several patients with COVID-19 have infiltrate, endothelitis of pulmonary vasculature and microthrombi

from pulmonary pathology of COVID-19 that can make airway obstruction, low oxygen level and death.<sup>15</sup>

Neutrophils have a role as one of the human immune cells. When pathogenic microorganism penetrate the body, immune cells will do the defence regulation.<sup>16</sup> When the body's immunity is compromised, the risk of infection can increase.<sup>17</sup> Lymphocytes are one of leukocyte cell that have function in an immune system. Lymphocyte help to defence and protect body from bacterial and viral infection. If the lymphocyte level decreases (lymphopenia), it could make the infection become worse.<sup>18</sup>

Neutrophil lymphocyte ratio (NLR) is one of the parameters to look in prognosis of infection and inflammation. Increased NLR indicates a poor clinical prognosis.<sup>19</sup> It may be related to the cytokine release storm that can change clinical condition from moderate to severe clinical manifestations. If there is a new virus with no memory in the immune system and also has high patho-

genicity, it tends to release cytokines out of control which activate more white blood cell. There is also research that that state that NLR is not related to the mortality rate. Therefore, it could be used as an early warning signal and important step to give treatment for COVID-19 patients.<sup>20</sup>

D-dimer is a fibrin degradation product occurring as a result of the fibrinolysis process. D-dimer is a blood test used to detect parts of thrombus in blood stream. Higher D-dimer concentrations could be produced by a blood clot. In this research, D-dimer value greater than 0.5 µg/mL were found, which caused the endothelial cell dysfunction. It impacts severity grade of COVID-19 patients.<sup>21</sup> D-dimer also related to hypoxia. Hypoxia associated with COVID-19 could cause vasoconstriction and low blood flow that contribute to epithelial injury and cause microthrombosis.<sup>22</sup> Patients with severe cases are usually older and have underlying conditions, which were risk factors of hypercoagulation or thrombosis.<sup>23</sup>

## Conclusion

COVID-19 can cause hypoxaemia as result of damage to the lungs from inflammatory reactions to the viral infection of the respiratory tract. The NLR and D-dimer have been the most promising predictors of moderate-severe incidence from COVID-19 pneumonia. An early diagnose will be beneficial to patient classification management.

## Acknowledgements

None.

## Conflict of interest

None.

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# Proprioception Recovery After Anterior Cruciate Ligament Reconstruction – Isokinetic Versus Dynamic Exercises

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## Abstract

**Background / Aim:** Proprioception recovery is one of the main postoperative rehabilitation goals after the anterior cruciate ligament (ACL) reconstruction. The aim of this study was to examine the level of proprioception recovery in patients 9 months after the ACL reconstruction using hamstring graft.

**Methods:** A prospective study followed 70 male subjects (mean age  $27.36 \pm 5.94$ ) divided into two groups depending on the type of proprioceptive exercise applied. Group A patients ( $n = 35$ ) underwent proprioceptive training on a Biodex 4 Pro System isokinetic dynamometer for 10 minutes 5 times per week. In group B, patients underwent proprioception exercises in the gym for 10 minutes 5 times per week. The degree of recovery of proprioception was measured on an isokinetic dynamometer preoperatively and 9 months after ACL reconstruction. Flexion angles in the operated knee of 15, 30 and 45 degrees were monitored. The Chi-square test was used for statistical analyses. The value of  $p < 0.05$  was considered significant.

**Results:** A significant difference was found in the recovery of proprioception in patients depending on the type of training applied. Statistically significant recovery of proprioception was observed in patients from both groups 9 months after ACL reconstruction, but it was better in patients of group A ( $p < 0.05$ ).

**Conclusion:** Applying proprioceptive training on an isokinetic dynamometer achieves better proprioceptive recovery in patients 9 months after ACL reconstruction versus dynamic exercises in the gym.

**Key words:** Proprioception; Rehabilitation; Anterior cruciate ligament reconstruction.

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## ARTICLE INFO

Received: 5 December 2021  
Revision received: 7 December 2021  
Accepted: 7 December 2021

## Introduction

Proprioception is one of the key elements in the complex process of maintaining balance.<sup>1</sup> Having in mind the histological structure of the anterior cruciate ligament (ACL), it is clear that its rupture leads to damage of fast and slow mechanoreceptors and consequently, to damage of proprioception.<sup>2</sup> Surgical treatment of patients after the anterior cruciate ligament reconstruction restores static stability, but achieving dynamic stability is the task of postoperative rehabilitation. If the restoration of proprioception is not achieved, the

patient is at risk of re-injuries to the structures of the operated but also injuries of the contralateral knee.<sup>3</sup> During the postoperative rehabilitation of patients after ACL reconstruction proprioceptive re-education exercises are one of the constituent elements of the rehabilitation protocol. Proprioception training is conducted in all phases of postoperative rehabilitation in these patients, but the emphasis on this segment is given from the third postoperative month until its completion. There are different types of proprioceptive

training. When choosing them, the rule from simpler to more complex is followed, exercises in closed and open kinetic chain, dynamic and isokinetic.<sup>4</sup> The state of proprioception can be evaluated by various methods.<sup>5, 6</sup> Measurement of an isokinetic system is one of the objective ways to assess its recovery.<sup>7</sup>

In this study, the degree of proprioception recovery was investigated in patients 9 months after ACL reconstruction with a hamstring graft using two types of therapeutic exercises, isokinetic and dynamic.

## Methods

This prospective clinical study comprised a total of 70 recreational athletes, men, divided into two groups depending on the type of proprioceptive exercise applied during rehabilitation after arthroscopic ACL reconstruction with a hamstring graft. All patients were operated by the same surgical team, all had unilateral, isolated ACL rupture; all were operated on in the phase of chronic ACL insufficiency with the same surgical technique and were of the same level of physical activity (football players, recreational athletes who performed sports activities twice a week for 90 minutes). Non-inclusion criteria were: female gender, joint injuries of other structures of the same knee or injuries and operations on the contralateral knee and injuries and surgical interventions on other ankles. Postoperative rehabilitation in the first three months was performed without differences in the type of therapeutic exercises of proprioceptive re-education. After three months from the operation, all patients were evaluated for neuromuscular performance on the Biodex 4 Pro System isokinetic dynamometer in order to be prescribed with targeted exercises to increase the muscle strength, endurance and proprioception. Patients were then divided into two groups (group A and group B) according to the available type of proprioceptive exercise determined by the place of residence. Group A patients ( $n = 35$ ) underwent proprioceptive training on a Biodex 4 Pro System isokinetic dynamometer for 10 minutes 5 times per week. In group B, patients underwent proprioception exercises in the gym for 10 minutes 5 times per week. These patients performed the above type of therapeutic exercises at the Department of Physical Medicine and Rehabilitation "Dr Miroslav Zotović" Banja

Luka, which is only public health institution in the Republic of Srpska that has the isokinetic system.

After a six-minute warm-up on a stationary bike, all patients began performing isokinetic exercises to strengthen the quadriceps and hamstrings of the operated leg for 20 minutes. They then continued with proprioception exercises for 10 minutes. The starting position for conducting proprioceptive re-education exercises was a sitting position on a dynamometer chair with the torso, upper leg and a lower leg fixed with stabilisation straps for the chair back and for dynamometer extensions. The torso was placed at an angle of  $90^{\circ}$  in relation to the upper leg, as well as the upper leg in relation to the lower leg. The lower leg was fixed with stabilisation straps to continue the dynamometer in the area of the fibula head and 3 cm above the malleolus. The range of motion in the knee was from  $0-90^{\circ}$  ( $0$  represented the initial position - full extension in the knee and the final position is flexion in the knee of  $90^{\circ}$ ).<sup>8</sup> The patient was then asked to first recognise and keep the lower leg at the indicated flexion angles with his eyes open and then closed. In group B, patients performed proprioception exercises for 10 minutes 5 times a week during the rehabilitation treatment at the gym in the basic rehabilitation centres (CBRs) in place of residence. Proprioceptive training in these patients consisted of exercises on unstable surfaces (balls, balance plates and balancers). Exercise on the isokinetic system was not available due to the lack of equipment of local CBRs for this technology. Recovery of proprioception was measured on an isokinetic dynamometer 3 and 9 months after ACL reconstruction. Recognition of flexion angles in the operated knee of  $15^{\circ}$ ,  $30^{\circ}$  and  $45^{\circ}$  were monitored. The Chi-square test was used for statistical analyses. The value of  $p < 0.05$  was considered significant.

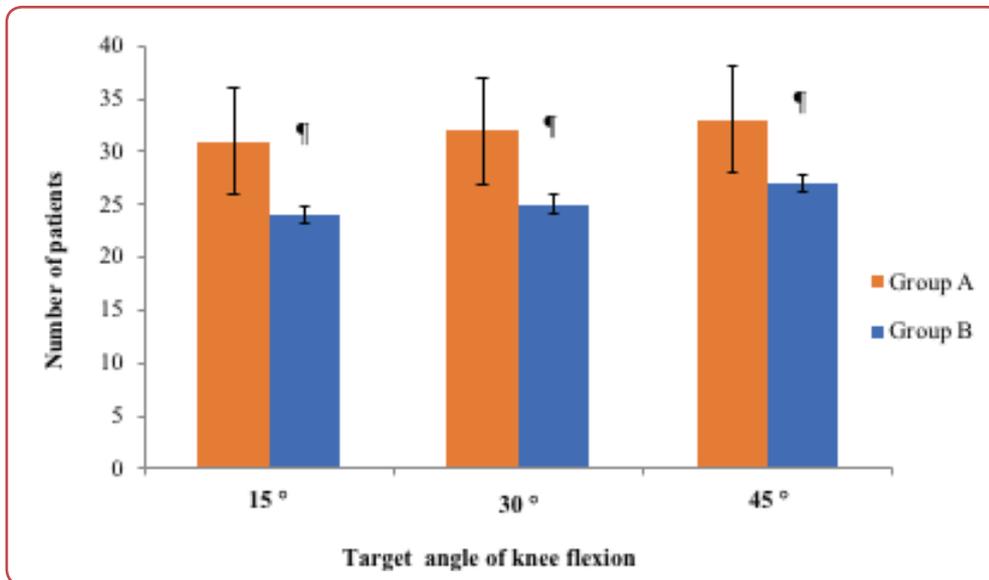
## Results

The sample consisted of 70 men, recreational athletes, with an average age of  $27.36 \pm 5.36$  years (Table 1).

Significant recovery of proprioception was observed 9 months after ACL reconstruction in patients from both study groups, but its recovery was statistically significantly better in patients from the group A (Figure 1).

**Table 1:** Demographic characteristics of patients by groups

Group	Number of patients	Age (Mean ± SD)	Sport-specific activity (football) (% of total sample)	Sport level /recreatives (% of total sample)
Group A	35	26.31 ± 4.72	100	100
Group B	35	28.42 ± 6.01	100	100



**Figure 1:** Differences in achieving target knee flexion angles on isokinetic testing in patients 9 months after anterior cruciate ligament (ACL) reconstruction using hamstrings graft between patients in Group A and Group B. Isokinetic vs dynamic proprioception exercises. ¶  $p < 0.05$

## Discussion

Proprioception is a feeling for the position of one part of the body in relation to others and the ability to detect movement in the joint (kinaesthesia). It is an important part of the somatosensory nervous system.<sup>9</sup> The ACL is known to contain mechanoreceptors, including Golgi tendon organs, Pacinian corpuscles and Ruffini nerve endings.<sup>10</sup> These receptors form part of the reflex arc in which anterior tibial translation results in contraction of the muscles of the posterior tibial lobe representing the protective mechanism. This contraction occurs at the level of the reflex arc. Therefore, it can be expected that the rupture of the ACL will lead to the rupture of the reflex arc because the rupture had eliminated the first element of the reflex arc, the receptor.<sup>11</sup>

Modern rehabilitation protocols cannot be imagined without the element of proprioceptive training as one of the key elements of the restoration of the dynamic stability of the knee after ligamentoplasty of the ACL. Many authors have monitored

the recovery of proprioception in patients after ACL reconstruction.<sup>12-18</sup> The speed of recovery was determined, among other things, by the type of proprioceptive re-education exercises used. However, the different results regarding the recovery time of proprioception in these patients can be explained by differences in the characteristics of the examined sample in terms of age, sex and level of physical activity. It also depends on: the type of surgical technique applied, the time that elapsed from the injury to the operation, the period of monitoring the patient, different methods of measuring the degree of recovery.<sup>19</sup> There were also studies that compared the level of proprioception of operated patients in different postoperative periods in comparison to healthy subjects.<sup>20</sup>

Kinesitherapy is a basic component of postoperative rehabilitation protocols in patients after ACL reconstruction. A therapeutic exercise or kinesitherapy program is a physical activity prescribed on the basis of evidence-based medicine with the



goal of restoring or altering a specific function in a joint, muscle, or kinetic chain created after an injury. These specific functions can be increasing the range of motion in the joint, strengthening muscle strength, increasing muscle endurance and improving neuromuscular control.<sup>21, 22</sup> Isokinetic exercises primarily belong to the dynamic type of exercise during which movement occurs in the joint or part of the body. Isokinetic exercise means that it is performed at constant speed (ranging from 1 / sec to 1000 / sec) with variable resistance. Variable resistance means that this is the only way of exercising during which the muscle can be maximally loaded through the entire range of motion. This is of particular importance because it avoids fatigue and overload, which represents absolute safety for the patient as he is never exposed to resistance that he cannot overcome. The isokinetic exercise contains three main components: acceleration, deceleration and load range.<sup>23</sup>

It is clear that during these exercises, especially when it comes to acceleration and deceleration, in addition to muscle strengthening exercises, patient also conducts targeted proprioceptive training. Proprioceptive re-education exercises include a competent feedback mechanism of audio-visual signals that provide the patient with information regarding the confirmation or failure of the target flexion angle in the examined knee. Thus, the application of proprioceptive re-education exercises on the isokinetic system also includes the vestibular, sensory and motor mechanisms needed to maintain body balance. This could be one of the reasons for better recovery of proprioception in patients from group A. On the other hand, patients from group B conducted a kinesiotherapy program based on static and dynamic exercises to strengthen the thigh muscles with variable speed and different loads. The challenges of this exercise are overload, faster fatigue and the occurrence of reflex quadriceps inhibition during which proprioceptive training is absolutely useless because the central sensory control mechanisms in this situation do not receive information about the condition of the joint and/or body on the periphery. This results in the absence of motor control of the thigh muscles, which significantly affects the recovery of proprioception.<sup>24</sup> It is probable that with the advancement of technology in the future it can be expected that the appearance of isokinetic systems that will be able to measure the parameters of proprioceptive recovery after ligamentoplasty of the ACL of the knee with great sensitivity and specificity. It is also to be hoped that the quality of

patient rehabilitation after ACL reconstruction in the CBRs in the Republic of Srpska and Bosnia and Herzegovina will improve in the future through improved infrastructural conditions, equipment supply and staff education.

## Conclusion

Proprioceptive training on the isokinetic system gives better recovery of patients. The recovery is achieved 9 months after ACL reconstruction using a hamstring graft compared to dynamic proprioception exercises. These encouraging initial results need to be confirmed on a larger sample and over a longer follow-up period.

## Acknowledgements

None.

## Conflict of interest

None.

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# Lipid-Reducing Effects of Sulphate-Sulphide Mineral Water in Patients With Knee Osteoarthritis

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## Abstract

**Background / Aim:** The positive effect of thermal mineral waters on human health has been known for a long time. Many pathophysiological mechanisms of action of balneotherapy are not specified. Patients with gonarthrosis often have elevated values of serum lipids. This study aimed to examine the effect of drinking sulphate-sulphide thermo mineral water on the lipid status of patients with gonarthrosis.

**Methods:** A prospective clinical study followed 60 patients, both sexes, mean age  $65.02 \pm 1.03$ , with gonarthrosis. All inpatient underwent physical treatment with topical application of sulphate-sulphide mineral water. Patients of group A (N = 30) had the use of this mineral water as an additional therapy by drinking, unlike patients of group B (N = 30) who drank plain water. The level of serum lipids of these patients was monitored at admission and 4 weeks after. The variance analysis test (ANOVA) with a level of statistical significance  $p < 0.001$  was used for statistical analysis.

**Results:** A significant reduction in the levels of all lipid fractions in the serum of patients with gonarthrosis was found 4 weeks after the completion of inpatient physical treatment in both study groups. This decrease was statistically significant in patients of group A ( $p < 0.001$ ).

**Conclusion:** Drinking sulphate-sulphide mineral water in patients with gonarthrosis shows a positive effect on the reduction of serum lipid levels in the short-term follow-up period.

**Key words:** Balneotherapy; Serum; Lipids; Osteoarthritis; Knee.

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## ARTICLE INFO

Received: 5 December 2021

Revision received: 7 December 2021

Accepted: 7 December 2021

## Introduction

Osteoarthritis (OA) is the most common joint disease and one of the main causes of pain and disability. It is a mildly progressive, degenerative and chronic disease that occurs as a result of joint cartilage damage. There is growing evidence that OA is also a "metabolic disorder" associated with insulin resistance, abdominal obesity, high cholesterol, high blood pressure and coagulation disorders.<sup>1-3</sup> The level of total fatty acids and arachidonic acid within chondrocytes noticeably increases with severe cartilage damage.<sup>4</sup> In addition, the expression of genes that regulate cho-

lesterol uptake into chondrocytes of damaged cartilage is reduced, which also contributes to lipid deposition.<sup>5</sup> Some studies have linked elevated blood lipids and osteoarthritic changes.<sup>6</sup> The use of bicarbonate and sulphate-sulphide mineral water by drinking under controlled conditions could lower blood lipid levels.<sup>7-9</sup> The exact mechanism of antilipemic action of these mineral waters is unknown. The increase in bile acid excretion is related to the alkaline nature of mineral waters and the osmotic effect. The release of pancreatic enzymes and bile acids increases with

increasing pH under the influence of mineral water, which leads to reduced uptake of cholesterol in the small intestine and its increased excretion in the faeces. The loss of bile acids through faeces is increased which could stimulate the liver to produce more bile acids from cholesterol. Consequently, the number of hepatic LDL cholesterol receptors increases, leading to a decrease in serum LDL cholesterol.<sup>10</sup> This study aimed to examine the effect of internal application (drinking) of sulphide-sulphate mineral water on the lipid status of patients with gonarthrosis.

## Methods

The study was conducted as an open, controlled, prospective study of 60 patients with gonarthrosis at the Hospital for Physical Medicine and Rehabilitation "Mlječanica" in Kozarska Dubica. All respondents gave their voluntary consent to participate in the trial and signed informed consent. The Ethics Committee of the "Mlječanica" Hospital has approved this study. The microbiological correctness of the mineral drinking water was confirmed by the PHI Institute of Public Health Banja Luka No M-3206-V-4-2015 (Table 1).<sup>11</sup> Criteria for inclusion in the study were knee pain most days of the previous month and radiological confirmation of knee osteoarthritis. The study non-inclusion criteria were: significant changes in blood pressure, cardiac arrhythmias, cardiac decompensation, neurological disorders, complications of the gastrointestinal tract (vomiting, diarrhoea, abdominal cramps) and personal reasons of the subjects. The sample was divided into two groups (group A and group B) of 30 respondents each. All patients were given balneo-physical treatment every day, six days a week (the seventh day is a break from treatment) for the 4 weeks. The total of 24 treatments included: transcutaneous electrical nerve stimulation (TENS) on the affected knee exercises to increase the range of motion in the knee and strengthen the quadriceps and hamstrings and locally applied baths of sulphate-sulphide mineral water temperature 31-33 ° C for 20 minutes. Subjects from group A drank 100 mL of sulphate-sulphide mineral water three times a day, just before meals and patients from group B drank three times 100 mL of plain tap water three times a day before meals. The outcome was evaluated on the first and 28th day of rehabilitation. The following serum laboratory parameters were measured on each subject: to-

**Table 1:** Physical-chemical analysis of sulphurous mineral water "Mlječanica"

Compound		mg/L	mmol/L	m/val/L	m. val %
Anions	Hydrocarbon	577.06	9.460	9.460	18
	Chloride	15.80	0.440	0.440	1
	Sulfate	2840.00	21.250	42.500	81
	Phosphate	0.01	0.000	0.000	0
Total mineralisation (mg/L) 2932.87					
Cations	Sodium	165.00	7.174	7.174	14
	Potassium	7.20	0.185	0.185	0
	Calcium	436.86	10.900	21.800	41
	Magnesium	257.79	10.600	21.200	40
	Strontium	11.20	0.127	0.225	1
	Ammonium	35.00	2	1.940	4
Total mineralisation (mg/L) 913.06; m. val: mineral value					

tal cholesterol, triglycerides, HDL cholesterol and LDL-cholesterol. The concentration of total cholesterol and triglycerides in the serum was determined by standard enzymatic methods with the help of commercial tests from the company Human. The concentration of HDL-cholesterol was determined in the supernatant after precipitation of all other lipoproteins with the help of a precipitating agent for the semi-micro method PREC-b and the concentration of LDL-cholesterol by the precipitation method. The variance analysis test (ANOVA) with a level of  $p < 0.001$  was considered statistically significant.

## Results

A total of 60 patients (19 males and 41 females, mean age  $65.02 \pm 1.03$ ) participated in the study, 30 in each group. Both groups were homogenous regarding age, gender and affected knee (Table 2).

**Table 2:** Demographic characteristics of the subjects

Group	Group A	Group B
No of subjects	30	30
Mean age	65.70	66.50
Sex	M = 9; F = 21	M = 10; F = 20
<b>Comorbidities</b>		
Hypertension	22	24 * $p = 0.542$
Varicose veins	3	4 * $p = 1.000$
Affected knee	L = 16; R = 14	L = 18; R = 12

\*Fisher test:  $p > 0.05$ ; M = male; F = female; L = left; R = right;

Decrease of serum lipids had obtained in patients in both groups. This decrease was statistically significant in patients in group A (treated with sulphate-sulphide mineral water)  $p < 0.001$  (Table 3).

**Table 3:** Differences in serum lipid values in patients of groups A and B (admission/discharge)

Lipid values	Treatment (water)	Water					p value
		Mean	SD	Median	Perc. 25	Perc. 75	
Total cholesterol (admission)	regular	7.01	1.11	6.75	6.12	7.97	0.700 <sup>a</sup>
	mineral	6.91	0.85	6.74	6.54	7.26	
Total cholesterol (discharge)	regular	6.00	0.87	5.91	5.43	6.43	< 0.001 <sup>a</sup>
	mineral	5.20	0.62	5.30	4.80	5.80	
Δ Total cholesterol	regular	-1.01	1.02	-0.71	-1.77	-0.27	0.001 <sup>a</sup>
	mineral	-1.70	1.02	-1.80	-2.35	-0.71	
Triglycerides (admission)	regular	2.67	1.33	2.76	1.82	2.97	0.539 <sup>b</sup>
	mineral	2.84	1.21	2.52	1.97	3.30	
Triglycerides (discharge)	regular	2.41	1.13	2.37	1.74	2.72	0.007 <sup>b</sup>
	mineral	1.80	0.88	1.77	1.50	1.98	
Δ Triglycerides	regular	-0.27	0.85	-0.19	-0.40	0.06	< 0.001 <sup>b</sup>
	mineral	-1.04	0.91	-1.04	-1.51	-0.27	
HDL (admission)	regular	1.43	0.60	1.37	1.02	1.78	0.703 <sup>a</sup>
	mineral	1.49	0.67	1.29	1.00	1.85	
HDL (discharge)	regular	1.47	0.60	1.34	1.02	1.89	0.005 <sup>a</sup>
	mineral	1.94	0.64	1.90	1.40	2.45	
ΔHDL	regular	0.04	0.37	0.04	-0.07	0.20	0.001 <sup>a</sup>
	mineral	0.45	0.53	0.28	0.05	0.90	
LDL (admission)	regular	5.21	1.28	5.22	4.00	6.20	0.228 <sup>a</sup>
	mineral	4.83	1.18	4.53	3.70	5.71	
LDL (discharge)	regular	4.55	0.96	4.22	3.80	5.00	< 0.001 <sup>a</sup>
	mineral	3.56	0.48	3.52	3.10	3.91	
ΔLDL	regular	-0.66	1.08	-0.32	-0.98	-0.01	0.028 <sup>a</sup>
	mineral	-1.26	1.00	-1.15	-2.13	-0.32	

a - T test for independent samples; b - Mann Whitney U test; red colour: statistical significance; perc: percentile;

## Discussion

At first glance, patients with gonarthrosis would not be closely associated with elevated serum lipids. However, these patients are mostly older, less physically active and adipose, the connection of these clinical entities can be seen. Patients from both study groups had elevated serum lipid fractions at baseline. There are studies showing lipid deposition within damaged cartilage. The expression of genes that regulate the intake of cholesterol in chondrocytes is reduced, which in turn contributes to increased lipid deposition in chondrocytes.<sup>5</sup> In a 2009 study of 148 middle-aged women without clinical signs of gonarthrosis and with reference cholesterol and triglyceride values, Davies et al found an increased number of women with osteoarthritis who had low and elevated serum lipids after two years.<sup>6</sup> The treatment of patients with dyslipidaemias is complex and requires a multidisciplinary approach. Physiological treatment of patients with gonarthrosis can affect the level of reduction of serum lipids in several ways. One is the reduction of body weight, the other is the increase of physical activity through training, but the effect of balneotherapy is not reduced.<sup>12-15</sup> The mechanisms of action of sulphate-sulphide mineral waters on

the level of serum lipids have been insufficiently investigated. The mechanisms that could explain this effect are increased excretion of bile acids and pancreatic enzymes, alkalinity of mineral waters and osmotic effect. Cholesterol uptake in the small intestine is reduced and its excretion is increased. This leads to a decrease in the liver's cholesterol reserve which withdraws LDL cholesterol from the serum and thus leads to its reduction.<sup>16</sup>

Positive effects of sulphate-sulphide mineral water have been found in patients with constipation and gastritis.<sup>17</sup> Positive effects of local application of these mineral waters have also been reported in patients with hypertension and after cerebrovascular insult.<sup>18-20</sup>

Our research can undoubtedly be considered pioneering because no research has been done so far on the impact of mineral water in patients with dyslipidaemia. Balneotherapy as a cheap and affordable therapy could, if its positive effects are proven, be a significant modality of non-pharmacological treatment of these patients.

The results obtained in this study need to be interpreted from several angles. Namely, the effect of mineral water on BMI was not examined due to a short follow-up period of 4 weeks. The effect of drug therapy was minimised because all patients due to pain took only Paracetamol in a maximum dose of 3 g per day due to knee pain. Patients in the study groups did not receive statin therapy.

As all patients were on a reduction diet during all 28 days, the effect of the diet was reduced to a minimum. Therefore, a significant decrease in serum lipids in patients in group A can only be attributed to the effect of drinking sulphate-sulphide mineral water. However, a short follow-up period and a small sample are the disadvantages of this study.

## Conclusion

Drinking sulphate-sulphide mineral water leads to a decrease in serum lipids in patients with gonarthrosis in the short-term follow-up period.

## Acknowledgements

None.

## Conflict of interest

None.

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# The Venous Contribution to Cardiovascular Performance: From Systemic Veins to Left Ventricular Function - a Review

Tonino Bombardini<sup>1, 2, 3</sup>

## Abstract

The venous system contains  $\approx 70\%$  of the total blood volume and is responsible in heart failure for key symptoms of congestion. It is active: it can increase or relax its tone with physiologic or pharmacologic stimuli. It is heterogeneous, behaves as a two-compartment model, compliant (splanchnic veins) and non-compliant (nonsplanchnic veins). It is dynamic in health and disease: in heart failure the vascular capacitance (storage space) is decreased and can result in volume redistribution from the abdominal compartment to the thoracic compartment (heart and lungs), which increases pulmonary pressures and precipitates pulmonary congestion. A noninvasive assessment of venous function, at rest and dynamically during stress, is warranted. The systemic haemodynamic congestion is assessed with inferior vena cava diameter and collapsibility. The pulmonary congestion is assessed with B-lines and pleural effusion. The contribution of left ventricular filling is assessed with end-diastolic volume, integrated with left ventricular function.

**Key words:** Venous return; Ventricular function; Central venous pressure; Mean systemic pressure.

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### ARTICLE INFO

Received: 25 November 2021

Accepted: 15 December 2021

## Introduction

The venous system contains approximately  $\approx 70\%$  of total blood volume and veins are 30 times more compliant than the arteries. Therefore, changes in blood volume within the veins are associated with relatively small changes in venous pressure.<sup>1, 2</sup> They are called capacitance vessels and serve as a reservoir of blood. The venous system is active: it can increase or relax its tone with physiologic or pharmacologic stimuli. It is heterogeneous: the venous system behaves as a two-compartment model, compliant (mainly splanchnic veins) and noncompliant (nonsplanchnic veins). The compliance of small venules and veins is almost 40 times greater than that of arterial vessels.<sup>3-5</sup> Thus, the total compliance of the circulation is dominated by the compliance of the systemic veins and venules. In humans, cardiac

output (CO) is strongly governed by the amount of venous return, ie, blood flowing into the right atrium. According to the general equation of flow, three factors determine venous return: the right atrial pressure, the vascular resistance and the mean systemic pressure (MSP). Although the right atrial pressure and the venous resistance to flow are well considered by cardiologists, the MSP is less known, yet it is important, since MSP is the driving pressure competing against right atrial pressure to create a gradient that promotes a forward flow.

The concept of MSP dates back to the late 1800s when Bayliss and Starling surmised that if the circulation was transiently halted, arterial pressure would fall and venous pressure would rise.<sup>3, 4</sup> The

pressure in the entire system during cardiac standstill would equilibrate at what they termed MSP. MSP must be independent of Mean Arterial Pressure (MAP) because it could be defined in the absence of cardiac pump function.

### Mean systemic pressure: its measurement and meaning

Let us imagine the heart is stopped for a relatively short period of time. Blood will not be flowing from the heart and toward the heart and pressure will be the same in all parts of the circulatory system. Such a pressure is called MSP.<sup>6,7</sup> In dogs and presumably in healthy humans, MSP is approximately between 7 and 12 mmHg, whereas central venous pressure (CVP) is approximately 2-3 mmHg.<sup>8-13</sup> Thus, the gradient for venous return (VR) is somewhere between 5 and 10 mmHg and therefore the change in CVP of just by a few mmHg can have a considerable effect on venous return.<sup>11</sup>

## Pathophysiological basis

Although the cardiovascular circuit is a two-compartment model comprising both systemic and pulmonary circuits, > 60 % of the blood volume held in veins is in the systemic venous circulation with three-fourths of that in small veins and venules.<sup>1,2</sup> The pulmonary veins contain only a small blood volume. For these reasons, the physiology of venous return can be described, in practical terms, as the physiology of venous return to the right atrium.

The left heart plays a major role in the regulation of CO. Three of the four determinants of left heart CO, that is, preload, heart rate and contractility, are intrinsically cardiac-related indices.<sup>14,15</sup> Cardiac function plays only an indirect role in the governance of the fourth determinant of CO, the venous return.<sup>12</sup> Under normal conditions and at rest the Frank-Starling mechanism primarily provides fine adjustment to cardiac function by making sure that the same volume that fills the ventricles on each beat leaves them.<sup>3</sup> During exercise, heart rate normally increases two- to three-fold, contractility three- to four-fold and systolic blood pressure by  $\geq 50$  %, while systemic vascular resistance decreases. LV end-diastolic volume (EDV) initially increase (increase in venous return) to sustain the increase in stroke volume through the Frank-Starling mechanism and later fall at high heart rates. CO during mild exercise is achieved by

**Table 1:** Distribution of blood and pressures ranges for indicative aggregate data in the various components of the circulatory system at rest and during exercise or heart failure<sup>2, 42, 46, 47, 49</sup>

Distribution of blood	REST CO $\approx$ 5 L/min	EX Health CO $\approx$ 20 L/min	HF CO $\approx$ 4 L/min
<b>Systemic circulation</b>			
Veins	60 %	12 %	45 %
Arteries	25 %	63 %	12 %
Muscle	8 %	58%	3 %
Kidney	10 %	2 %	3 %
Skin	2 %	1 %	3 %
Brain	5 %	2 %	3 %
<b>Pulmonary circulation</b>			
Heart	6 %	11 %	23 %
Total % blood volume distribution	100 %	100 %	100 %
Pressure range (mmHg)	REST CO $\approx$ 5 L/min	EX Health CO $\approx$ 20 L/min	HF CO $\approx$ 4 L/min
Mean systemic venous pressure	7 – 12	7 – 12	6 – 18
Central venous pressure	3 (0 – 5)	3 (0 – 5)	4 – 16
RV pressure	systolic	15 – 30	20 – 45
	diastolic	3 – 8	3 – 8
Pulmonary artery	systolic	15 – 30	25 – 45
	diastolic	8 – 12	14 – 22
Pulmonary wedge pressure	3 (0 – 5)	3 (0 – 5)	4 – 16
LV pressure	systolic	100 – 140	170 – 220
	diastolic	3 – 12	5 – 14

CO: Cardiac output; EX: Exercise; HF: Heart failure; LV: Left ventricle; RV: Right ventricle;

an augmentation of both stroke volume and heart rate,<sup>16</sup> whereas the further increases in output during strenuous exercise result primarily from an increase in heart rate.<sup>17</sup> In healthy individuals, during peak aerobic exercise there is very little change in right atrial pressure with the very large increases in CO<sup>7</sup> and more venous return.<sup>6,10</sup> An increase in sympathetic discharge during exercise leads to splanchnic arterial vasoconstriction leading to a decrease in flow, pressure and volume within the splanchnic veins and an increase in venous return, while splanchnic blood flow decreases from 1,500 mL/min to 350 mL/min. Altogether, these adaptations would have increased CO by 110 %. In heart failure (HF) at rest, as a result of neurohormonal imbalance, the venous vascular capacitance (“storage-space”) is decreased and acute sympathetic nerve activation can result in acute volume redistribution from the abdominal compartment to the thoracic compartment (heart and lungs), which increases intra-cardiac pressures and precipitates HF symptoms. The splanchnic vascular compartment may be a key player in the volume dysregulation in acute and chronic HF, even in the absence of increases of total body fluid

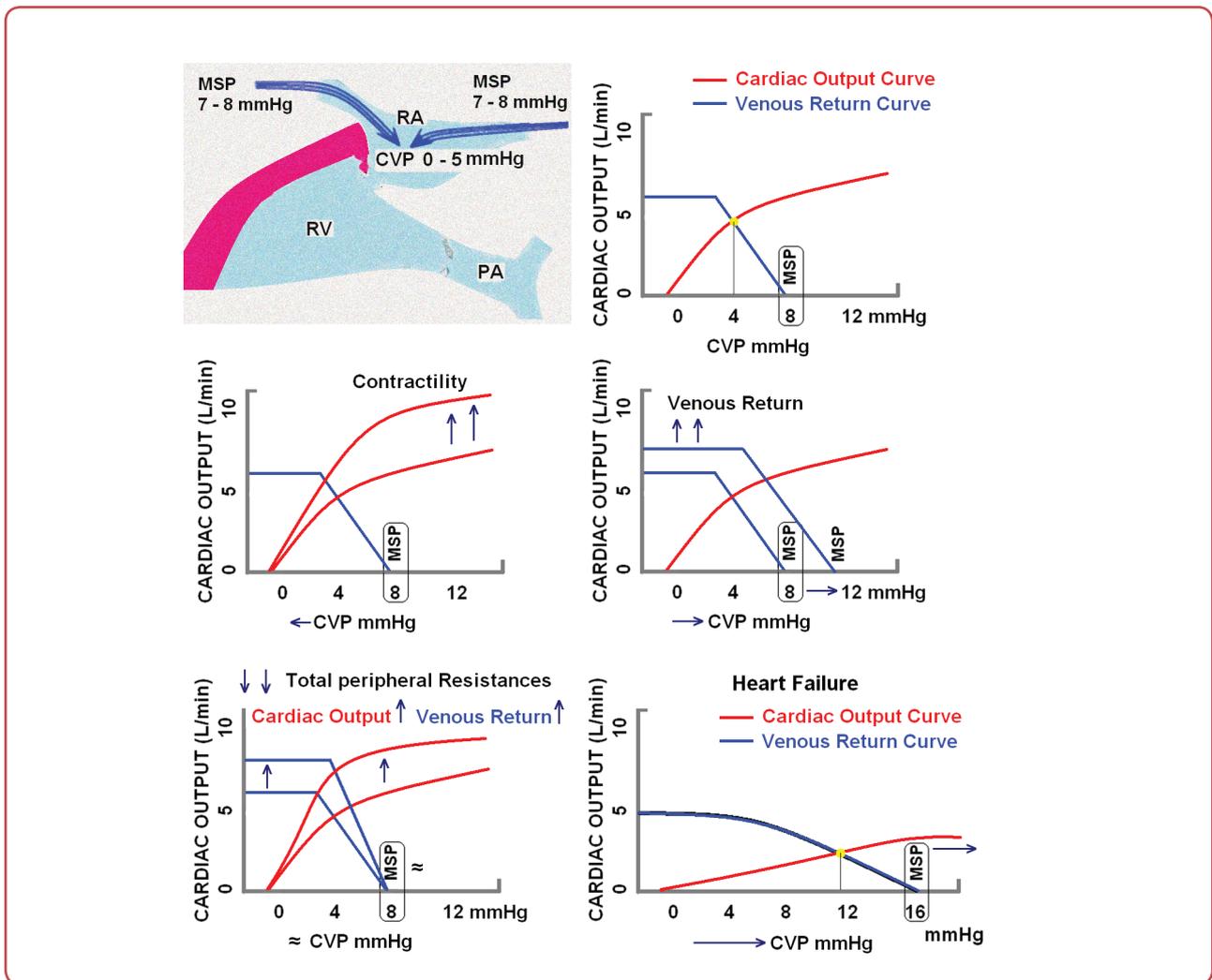


Figure 1: Venous return, cardiac output, operating point of the heart.<sup>8, 10, 20, 37</sup>

**Left upper panel:** Mean systemic pressure (MSP) is a pressure that drives the blood inside the veins to full the right atrium (RA). MSP must be higher than pressure inside the right atrium or central venous pressure (CVP). In the normal subject MSP is 7-8 mmHg and CVP is between 0 and 5 mmHg. When MSP increases more blood comes back to right atrium; when the MSP decreases less blood comes back to right atrium.

RV: Right ventricle; PA: Pulmonary artery.

**Right upper panel:** Relationship between CVP and Cardiac Output (CO). CO = red curve. Venous return = blue line and it is higher when the CVP pressure is = 0; at increasing CVP venous return starts decreasing until a point where return becomes 0: at this point CVP equals MSP and zero blood is coming back to right atrium. The point where venous return curve intercepts the x-axis identifies the MSP, normally = 7-8 mmHg. The point where venous return and CO curves cross each other shows the operating point of the heart (yellow circle): CO and venous return are equal at this point: the heart is pumping out all the blood that is coming back as venous return. A straight line down the operating point measures the CVP value = 4 mmHg = normal.

**Left middle panel:** Increased contractility (arrows) increases CO, without pre-load changes. Changing the contractility does not change the MSP and mildly decreases the CVP.

**Right middle panel:** If the volume of vein blood increases (arrows), venous return increases: the operating point shifts up and on the right: the CO increases but also the CVP and the MSP increase to 6 and 12 mmHg respectively.

**Left lower panel:** When total peripheral resistances decrease (arrows) venous return, CO and the operating point are shifted up: without changes of CVP and MSP. That's occurs during exercise with dilation of arterioles in the skeletal muscles.

**Right lower panel:** The venous engorgement of congestive HF: reduced gradient between MSP and CVP, both increased with resulting decreasing venous return. The steady-state operating point (yellow circle) shifts down and CO is blunted despite increased preload.

CO: Cardiac output; CVP: Central venous pressure; MSP: Mean systemic pressure; PA: Pulmonary artery; RA: Right atrium; RV: Right ventricle.

volume.<sup>13</sup> In cardiac failure, the heart is the limiting factor in the circulation and will determine the upper limit of CO. However, in subjects without HF venous return can be seen as the limiting factor, because the heart can only pump out the amount of blood it receives.

### Practical considerations

Pulmonary compliance and volume shifts between systemic and pulmonary circuits.

Normally, the mean pressure in the pulmonary artery is about 15 mmHg at rest. Increased blood pressure in the capillaries of the lung causes pulmonary hypertension, leading to interstitial oedema if the mean pressure increases to above 20 mmHg and to pulmonary oedema at mean pressures above 25 mmHg. Total pulmonary compliance is only one-seventh of total systemic vascular compliance<sup>5</sup> and the pulmonary circuit does not contain a lot of volume that can be shifted to the systemic circulation. It also cannot take up a lot of volume without causing a large increase in pulmonary venous pressure and a major disturbance to pulmonary gas exchange. Even maximal sympathetic stimulation results in only a small shift from the pulmonary circuit to the systemic circulation.<sup>15, 18</sup> Accumulation of volume in the pulmonary vasculature is especially a problem in patients with marked left ventricular diastolic dysfunction. The higher left ventricular filling pressure increases pulmonary capillary filtration and leads to pulmonary oedema and respiratory failure. If volume is removed to treat the respiratory failure, CO decreases and the kidneys fail. If volume is then added to improve renal perfusion, respiratory failure occurs.

### Venous return

The heart does not actively suck blood. The pressure gradient between MSP and CVP determines venous return.<sup>19</sup>

The greater the difference between MSP and CVP, the greater the venous return will be. Guyton et al<sup>20</sup> showed in their experiments in dogs that when CVP is elevated, CO and venous return are reduced. When CVP is increased further and further, venous return declines until it ultimately ceases. The value that CVP reaches at zero flow is the MSP. On the other hand, with decreasing CVP, venous return increases. The pressure gradient between MSP and CVP is the driving force for venous return and consequently CO.

## Diagnostic testing

### Measurements of venous pressure

Measurement of CVP is a critical component of the complete haemodynamic assessment of a patient. CVP is considered equivalent to right atrial pressure (RAP) when the *vena cava* is continuous with the right atrium.<sup>21</sup> CVP reflects the amount of blood returning to the heart and the ability of the heart to pump the blood back into the arterial system. Central venous catheterisation is the gold standard measurement of CVP and RAP. Noninvasive techniques to estimate CVP play a crucial role in promoting a more widespread CVP evaluation in clinical practice.

The inferior *vena cava* (IVC) is a compliant vessel whose size and shape vary with changes in CVP and intravascular volume. Therefore, sonographic measurement of the IVC represents an effective and noninvasive method of estimating CVP.<sup>22-24</sup> For simplicity and uniformity of reporting, specific values of RAP, rather than ranges, should be used in the determination of systolic pulmonary artery pressure. IVC diameter < 2.1 cm that collapses > 50 % with a sniff suggests normal RAP of 3 mmHg (range, 0–5 mmHg), whereas IVC diameter > 2.1 cm that collapses < 50 % with a sniff suggests high RAP of 15 mmHg (range, 10–20 mmHg). In scenarios in which IVC diameter and collapse do not fit this paradigm, an intermediate value of 8 mmHg (range, 5–10 mmHg) may be used. This estimate should be used in estimation of the pulmonary artery pressure on the basis of the tricuspid regurgitant jet velocity, rather than assuming a constant RAP for all patients.<sup>25, 26</sup> More difficult is the measure of MSP ie, the pressure that drives the blood inside the veins to full the right atrium. This means that MSP must be higher than pressure inside the right atrium otherwise they would not move blood towards right atrium. In a healthy person MSP is around 7-12 mmHg and the right atrial pressure is somewhere between 0 and 5 mmHg.

### Mean systemic venous pressure using inspiratory holds

Mean systemic venous pressure can be estimated with a method based on the haemodynamic effects of mechanical ventilation: mechanical ventilation with positive airway pressures increases intrathoracic pressures, causing an increase in CVP and a decrease in venous return and CO. With the use of inspiratory holds (inspirato-

ry hold = a ventilating manoeuvre in which the delivered volume of gas is held in the lung for a while before expiration) haemodynamic steady-state conditions were met to assure that venous return and CO were equal. CVP and pulmonary artery flow were measured at the end of each inspiratory hold. Pinsky confirmed this validation in dogs, measuring mean systemic venous pressure during ventricular fibrillation and estimating mean systemic venous pressure by extrapolation of the relationship between stroke volume

and CVP.<sup>27, 28</sup> Measurement of MSP at the bedside allows the physician to gain knowledge about other haemodynamic parameters such as resistance for venous return, compliance and stressed volume. This allows to describe whether a specific vasoactive agent affects primarily the arterial or the venous side of the circulation.

The pulmonary wedge pressure or PWP, or cross-sectional pressure is the pressure measured by wedging a pulmonary catheter with an

**Table 2: Invasive vs noninvasive measurement of blood volume distribution<sup>2, 24, 47, 49</sup>**

Total blood volume (TBV) 100 %	Distribution of TBV	Invasive/ Radionuclide	Noninvasive/ Radiation free
Systemic circulation 85 %	Veins 60 %	Radionuclide Plethysmography	Bioimpedance
	Arteries 25 %	Thorax and abdomen gamma camera	Ultrasound- Doppler
	- Muscle	Thermo dilution; Green dye infusion; 133 Xenon; PET	Ultrasound-doppler; MRI; Laser-doppler; Electromagnetic flow
	- Kidney	133 Xenon radionuclide	Ultrasound-doppler; Magnetic resonance
	- Skin	Radioactive isotopes; Xenon-133 clearance	Plethysmography; Laser doppler
Pulmonary circulation 9 % (TPBV)	Pulmonary arteries 30-40 % of TPBV	Electromagnetic pulmonary artery flow; Thermo-dilution	Encoding phase subtraction MRI
	Pulmonary capillaries 20 % of TPBV	Breath-holding method	Contrast bubble echocardiography
	Pulmonary veins 40-50 % of TPBV	Venous return catheter (LA dye curve)	MRI; Ultrasound-doppler
Heart 6 %		Contrast angiography; Radionuclide imaging	Transthoracic echocardiography

LA: Left atrium; MRI: Magnetic resonance imaging; PET: Positron emission tomography; TBV: Total blood volume; TBV = Plasma volume/1-haematocrit; TPBV: Total pulmonary blood volume; TPBV = Cardiac index x mean transit time from pulmonary artery to left atrium;

**Table 3: Invasive vs noninvasive measurement of pressure ranges<sup>24, 19, 41, 46, 47</sup>**

Pressure		Invasive	Noninvasive
Mean systemic pressure		Circulatory arrest (cardiac surgery, ICD implant)	Inspiratory holds when a patient is on a mechanical ventilator
Central venous pressure		Right atrium catheter	Inferior vena cava diameter Ø; Ultrasound
Right ventricular pressure	systolic	Swan-Ganz catheter	Tricuspid regurgitation peak velocity; Doppler-ultrasound
	diastolic	Swan-Ganz catheter	Pulmonary regurgitation peak velocity; Doppler-ultrasound
Pulmonary artery pressure	systolic	Swan-Ganz catheter	Tricuspid regurgitation peak velocity + RA pressure
	diastolic	Swan-Ganz catheter	Pulmonary regurgitation end-dia- stolic velocity + RA pressure
Pulmonary wedge pressure		Inflated balloon into a small pulmonary arterial branch	(1.24 x E/è) + 1.9 doppler- ultrasound
LV pressure	systolic	LV catheter	Brachial SBP x 0.9
	diastolic	LV catheter	EDV (E/è)

EDV: End-diastolic volume; ICD: Implantable cardioverter defibrillator; LV: Left ventricular; RA: Right atrium; SBP: Systolic blood pressure;



inflated balloon into a small pulmonary arterial branch.<sup>29, 30</sup> It estimates the left atrial pressure. Physiologically, distinctions can be drawn among pulmonary artery pressure, pulmonary capillary wedge pressure, pulmonary venous pressure and left atrial pressure, but not all of these can be measured in a clinical context. Because of the compliance of pulmonary circulation, it provides an indirect measure of the left atrial pressure. Physiological pressure is 2–15 mmHg.

### Plasma volume in patients with chronic heart failure

In HF, at rest, as a result of neurohormonal imbalance, the venous vascular capacitance (“storage and pulmonary lung congestion-space”) is decreased and acute sympathetic nerve activation can result in acute volume redistribution from the abdominal compartment to the thoracic compartment (heart and lungs), in the presence or even in the absence of increased of total body fluid volume.<sup>13, 18</sup> This could result in the acute translocation of as much as 1 L of fluid without a net change in body weight. Here, vasodilator therapy would be more appropriate than aggressive diuretic intervention. In both cases, increased blood volume in the pulmonary compartment should be assessed by B-Lines (see further).<sup>31</sup> Body weight-driven diuretic therapy can lead to treating weight increase without lung congestion with adverse effects on kidney function and to miss treatment of lung congestion present in spite of lack of weight changes and clinical signs of congestion.

### Ultrasound lung comets (B-lines)

In pulmonary congestion, the presence of both air and water generates a peculiar acoustic fingerprint. Lung ultrasound shows B-lines, comet-like signals arising from a hyper-echoic pleural line with a to-and-from movement synchronised with respiration. Increasing extravascular lung water accumulation sub-pleural oedema with multiple B-lines, or a white lung pattern (alveolar pulmonary oedema) with coalescing B-lines.<sup>31</sup> The number and spatial extent of B-lines on the antero-lateral chest allows a semi-quantitative estimation of extravascular lung water. The simplified 4-site scan is much less time-consuming and equally sensitive than the 28-site scan. With the simplified 4-site scan only 4 sites are scanned on the third intercostal space, symmetrically on the right and left hemithorax, from mid-axillary

to anterior axillary and from anterior axillary to mid-clavicular line. Each of the 4 sites is scored from 0 (normal horizontal A-lines pattern in a black lung) to 10 (coalescent vertical B-lines with white lung). B-lines score is the sum of the 4 sites and ranges from normal values ( $\leq 1$ ) to mildly (2 to 4), moderately (5 to 9) and severely abnormal ( $\geq 10$ ) values. The higher the B-lines score at rest and during stress, the more profound the functional impairment and the worse the outcome in patients with known or suspected coronary artery disease and/or HF. Wet B-lines are made by water and decreased by diuretics, which cannot modify dry B-lines made by connective tissue. Cardiologists can achieve much diagnostic gain with little investment of technology, training and time. B-lines represent ‘the shape of lung water’. They allow noninvasive detection, in real time, of even subclinical forms of pulmonary oedema with a low cost, radiation-free approach. They allow a better way to follow congestion and titrate diuretics than simple weight control, which cannot separate fluid accumulation elsewhere in the body and pulmonary congestion which is the real target of diuretic therapy. In fact, a lung-ultrasound guided therapy in heart failure provides a clear symptom and possibly survival benefit compared to a standard therapy based on body weight as shown by randomised controlled trials.<sup>31-34</sup> Still, the whole story cannot be told by B-lines alone, since pulmonary congestion can arise from body volume overload or fluid redistribution from splanchnic to systemic circulation.

### Pulmonary congestion with and without body (wet) weight increase

Obviously, diuretic therapy is the mainstream in presence of body volume overload. In the second case how to redistribute volume from heart-pulmonary circulation to splanchnic reservoir? In the first-in-man study,<sup>18</sup> the authors aimed to modulate the splanchnic sympathetic tone through a minimally invasive regional nerve block, which resulted in a marked reduction in intra-cardiac filling pressures and increase in CO, primarily driven by a significant reduction in systemic vascular resistances and improved vascular capacitance (increasing volume redistribution to the splanchnic volume) more effective than depletion of total circulation volume through diuretics. Not all volume overload is the same and the measurement of intravascular volume identifies heterogeneity to guide tailored therapy.<sup>35</sup>

## From vein to left ventricular preload

### The venous side of the pulmonary circulation

The Frank-Starling relationship generally has been examined with filling pressure as the index of preload, resulting in a curvilinear function that plateaus at higher filling pressures: and difficult to quantify.<sup>36,37</sup> In the 1960s Eugene Braunwald and colleagues evaluated the Starling law in human patients. This group sewed metallic markers into ventricles and measured ventricular volume by cineradiography. Braunwald used the large beat-to-beat variation in filling during atrial fibrillation to show that stroke volume varies as a function of left ventricular end diastolic volume.<sup>38</sup> It was seen that the mechanical activity of the left ventricle during each contraction could be related to the left ventricular end-diastolic segment length, just preceding the onset of the contraction under consideration. Similarly, he showed that right ventricular systolic excursion varied with ventricular filling during Valsalva maneuver. The data demonstrated that filling and stretch of the ventricles increase the stroke volume. All of the preceding experiments confirmed that the heart has the intrinsic ability to accommodate changes in venous preload, both in the systemic (right atrium) and in the pulmonary (left atrium) venous conduit; increasing diastolic volume and stretch increase the force of contraction. Elevated cardiac pressure only increases the force of contraction and stroke volume if the experimental conditions permit the filling pressure to be an accurate proxy for end-diastolic volume.<sup>39,40</sup>

## Clinical models

### The Starling mechanism in clinical practice

The relative flatness of human myocardial resting length-tension curves with small degrees of stretching is of importance in limiting the rise of ventricular end-diastolic pressure and therefore of atrial and venous pressures, for any given augmentation of myocardial length. Conversely, the relative steepness of the curve relating resting tension to active tension indicates that substantial augmentation of the force developed by the ventricle during systole is possible with relatively

small increases in resting tension. The investigations of Braunwald demonstrated the Frank-Starling principle in the intact human right and left ventricle, ie, the dependence of the mechanical activity of the heart on the ventricular end-diastolic volume and the fundamental importance of ventricular end-diastolic volume, instead of pressure, in the regulation of the contractile activity of the human heart.<sup>20, 30</sup> Starling's text, in fact, does not suggest that right atrial pressure is an independent variable that controls stroke volume or myocardial work. The actual independent variable of his experiments was the amount he opened the valve that resisted flow into the right atrium: for the return of blood to the heart under a set of defined circumstances. Clinicians should also consider using CVP measurement instead of volume or flow because it can be obtained non-invasively. However, an isolated measurement of CVP, like any other single haemodynamic variable, cannot describe the state of the circulation.

## Perspectives

### A) The concept of preload-recruitable stroke work

The traditional teaching of cardiac physiology has focused almost exclusively on the left side of the heart. However, this focus ignores the critical role of the right heart and venous system in regulating venous return in states of haemodynamic compromise and shock.<sup>41</sup> As regarding the traditional pressure related assessment of diastolic venous return to ventricles, the conventional approach has been to ignore ventricular fibre length and to use  $E/e'$  as a surrogate of LV filling pressures.<sup>42</sup> Unfortunately,  $E/e'$  is only poorly correlated to invasively measured LV filling pressures at rest and, even more, during stress and is not readable in the majority of patients during stress.<sup>42</sup> A more feasible index of LV preload is LV EDV, which can be usefully combined with  $E/e'$  when available and with B-lines to gain insight into diastolic function. The relationship between stroke work (y-axis) and preload expressed by EDV (x-axis) is linear. A flat slope, that is a low preload-recruitable stroke work relationship, indicates that increased preload produces relatively little increase in stroke work because of the reduced contractility.<sup>40</sup> The relationship takes both preload and afterload into account and is applicable in a wide variety of cardiac diseases. During stress, it is the slope of the

relationship that shows various degrees of LV dysfunction, with steeper slope indicating better contractile reserve (higher function with lower volume) and flat slope indicating worse contractile reserve (lower functional reserve with higher ventricular volumes).<sup>43, 44</sup> Changes in EDV reflect relaxation capacity of the myocardium and adequacy of upstream venous return, in the same way as changes in ESV reflect contraction capacity of the myocardium and downstream afterload changes.

### B) Testing venous dysregulation

Venous function cannot be easily measured and no surprise if it has been overlooked until now. It is possible that venous dysregulation will be increasingly recognised as a significant pathogenetic player and a potential therapeutic target in some cardiovascular conditions, including HF,<sup>13, 18</sup> vaso-vagal syndrome or cardiac arrest.<sup>45</sup> In view of the better understanding of the role of venous system in several cardiovascular conditions a set of noninvasive, simple, widely available examinations should be developed and validated. As always, also the parameters of venous interest are more informative when tested both at baseline and during stress, since alterations occur in resting conditions can show-up under dynamic real life loading conditions.<sup>46-48</sup> At an early stage of disease, the function is not altered at rest but the possibility to increase function during stress can be already impaired. This is true for instance for left ventricular contractile reserve testing the myocardium, on the basis of its contraction capability, or coronary flow reserve testing the coronary microcirculation on the basis of vasodilatory capacity. It is our educated guess that the same is true for the venous system, but at present we miss a toolbox of parameters, stresses and normal values for venous stress echocardiography.<sup>16, 24, 42</sup>

### C) Therapeutic applications in HF patients

Recent papers<sup>13, 18</sup> highlighted how the abdominal vascular compartment is the main storage of intravascular blood volume and decreased abdominal vascular capacitance has been proposed as a major contributor to the complex pathophysiology of HF. In HF, as a result of a neurohormonal imbalance, the vascular capacitance (storage space) is decreased and acute sympathetic nerve activation can result in acute volume redistribution from the abdominal compartment to the thoracic compartment (heart and lungs), which increases intracardiac pressures and precipitates

HF dyspnoea symptoms. The abdominal compartment may redistribute the splanchnic volume to the thoracic volume, playing a key role in the volume dysregulation in acute and chronic HF, even in the absence of increases of total body fluid volume.<sup>49, 50</sup> In a first-in-man study, Fudim et al<sup>18</sup> aimed to modulate the splanchnic sympathetic tone, which resulted in a marked reduction in intra-cardiac filling pressures and increase in CO, primarily driven by a significant reduction in systemic vascular resistances and increasing volume redistribution to the splanchnic volume, more effective than depletion of total circulation volume through diuretics.

## Conclusions

- Preload, heart rate and contractility, are three determinants of cardiac output.
- The heart plays only an indirect role in the fourth determinant, the venous return.
- The veins contain  $\approx 70\%$  of blood and are responsible of congestion in heart failure.
- Volume redistribution to splanchnic veins is effective in congestive heart failure.
- A noninvasive assessment of venous function, at rest and dynamically is warranted.

## Acknowledgements

### Legal Notices and Disclaimer

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## Conflict of interest

None.

## Funding

"This research received no grant from any funding agency in the public, commercial or not-for-profit sectors."

## Authors' contributions

TB had the original idea and drafted the manuscript and approved the submitted version.

## List of abbreviations

CO = Cardiac output  
 CVP = Central venous pressure  
 EDV = End-diastolic volume  
 HF = Heart failure  
 IVC = Inferior vena cava  
 MAP = Mean arterial pressure  
 MSP = Mean systemic pressure  
 PWP = Pulmonary wedge pressure  
 RAP = Right atrial pressure  
 VR = Venous return

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# Minimally Invasive Coronary Surgery

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## Abstract

Minimally invasive options for coronary artery bypass graft (CABG) surgery progressed dramatically in the last decades. Minimally invasive CABG surgery is presented through these forms: minimally invasive direct coronary artery bypass (MIDCAB), endoscopic atraumatic coronary artery bypass (EndoACAB), robot-assisted direct coronary artery bypass (RADCAB), total endoscopic coronary artery bypass (TECAB), and hybrid coronary revascularisation (HCR). Unfortunately, these are still limited only to the specialised centres across the world and have not been accepted by the majority of cardiac surgeons. A surgeon who is starting to practice minimally invasive CABG surgery needs to be ready for long duration of the interventions, higher rate of conversions to sternotomy and significant learning curve. Excellent results that have been published on the subject of minimally invasive revascularisation methods support the potential of these alternative approaches to evolve in the near future.

**Key words:** Minimally invasive heart surgery; Coronary surgery; Midcab; Endocab; Radcab; Tecab; Hybrid revascularisation.

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## ARTICLE INFO

Submitted: 4 October 2021  
Accepted: 2 November 2021

## Introduction

Cardiac surgeons invested a great amount of effort in development of minimally invasive methods for the last couple of decades. The advance in modern technologies supported those efforts, and therefore enabled performing coronary artery bypass graft (CABG), valvular, great vessels and other cardiac surgery procedures, without total median sternotomy (TMS) – traditional, utterly invasive approach. The cooperation between cardiac surgeons and interventional cardiologists led to novel minimally invasive methods which benefit with the best from both worlds – surgical technique and precision and catheterisation-based approach.

Minimally invasive options for CABG surgery (miniCABG) progressed dramatically in the last decade. The topic of this paper will be all of those who do not require TMS. Although partial sternotomy (ministernotomy) was described in the past, it is fairly uncommon comparing to approaches

which completely preserve skeletal integrity of chest wall. Only the later will be discussed.

Minimally invasive CABG surgery is presented through these forms:

- Minimally invasive direct coronary artery bypass (MIDCAB)
- Endoscopic atraumatic coronary artery bypass (EndoACAB)
- Robot-assisted direct coronary artery bypass (RADCAB)
- Total endoscopic coronary artery bypass (TECAB)
- Hybrid coronary revascularisation (HCR)

Although the majority of cases are left anterior descending with left internal mammary artery (LAD-LIMA) surgery, more and more multi-vessel disease treatment are presented.

## “Off Pump” Coronary Surgery

Although it was developed using TMS, CABG surgery without using extracorporeal circulation (ECC) plays a big role in minimally invasive surgery. By avoiding ECC, patient is relieved from its harmful consequences, with outcome comparable to traditional CABG surgery in experiences centres.<sup>1,2</sup> While majority of minimally invasive procedures still utilise ECC, there are those who do not, especially single-vessel disease treated with LAD-LIMA. As surgeons are getting familiar with coronary stabilisers and beating heart fixation, they are making a step towards safer transition to smaller incisions and comfort sacrifices. Therefore, “off pump” surgery is utterly important in development of minimally invasive CABG procedures.

## Patient Selection

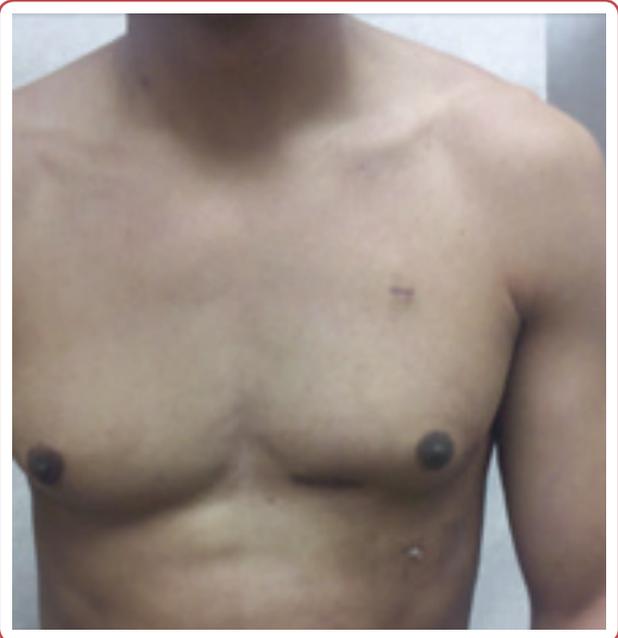
Same as always when novel technologies are introduced, careful patient selection and preoperative preparation are the key to success. All these minimally invasive CABG procedures provide worse exposition comparing to traditional CABG. Therefore, thorough coronarography analysis it is mandatory to access anastomosis site, the graft length needed, is coronary artery epicardially or intramyocardially placed, what is its calibre, lesion extension, etc. It could be quite difficult to determine how deep in the heart wall the coronary artery is placed, but some discrete signs could be of help: the more superficial, epicardial arteries are more mobile during contractions and more tortuous; while the more deep ones, intramyocardial (especially LAD) are straight and seem as they “dive in” and “dive out” on their way to the apex of the heart. Later cases can be technically much more demanding in minimally invasive surgery as it does not always allow visualisation of the entire LAD. Exploration of the anterior heart wall in these approaches is completely different experience to standard TMS CABG, so LAD and its branches must be carefully analysed on coronarography prior to surgery, to avoid bypassing the wrong vessel.

The restricted space adds another level of complexity to minimally invasive CABG procedures. The bigger the intrathoracic space is, endoscopic and robotic instrument manoeuvring is easier. Excess of subcutaneous fat tissue can limit the

manoeuvring ability, too. Therefore, it is clear that morbidly obese and small patients are a group with significant limitations for using these technologies.

## Indications and Contraindications

The critics of minimally invasive CABG procedures are making a question of safety being asked first. Opposing to TMS, approaching aorta and right atrial appendage for ECC cannulation is not possible. Utilising femoral vessels could lead to risk of embolic events due to retrograde perfusion. Making anastomosis using endoscopic and robotic instruments is by far more demanding than with the open approach. MiniCABG procedures take more time, therefore pro et contra balancing of faster recovery and aesthetics (Figure 1) versus risks of demanding technique is essential. Coronary patients are usually present with the following comorbidities: left ventricular dysfunction, peripheral arterial and venous disease, chronic obstructive pulmonary disease (COPD), renal insufficiency, etc. All of this could lead to negative outcome even with traditional TMS CABG procedures, so it is clear that a special attention is needed when planning a miniCABG.



*Figure 1: Aesthetic advantage of minimally invasive CABG. Two incisions for ports and left anterolateral thoracotomy of less than 5 cm*

In conclusion, patients undergoing miniCABG surgery could be divided into two subgroups: relatively healthy patients who wish to avoid TMS, but aim for long term result of surgical revascularisation; and elderly patients and those with severe comorbidities to whom TMS carries too much of a risk, but are not treatable with catheterisation methods and medications only. Indications for standard and miniCABG are the same, but there are several contraindications for performing miniCABG:

- Absolute - clinical: haemodynamic instability, ischaemic arrhythmias, acute coronary event, urgent conditions, cardiogenic shock;
- Relative - angiographic: intramyocardial position of coronary vessel, small diameter of coronary vessel, severely calcified coronary vessel, occluded coronary vessel without sufficient collateral filling;
- Relative - comorbidities: morbid obesity, severe respiratory insufficiency, severe peripheral vascular disease (for femoral ECC cannulation), severe left ventricular dysfunction (LVEF < 30 %), severe dilatation of left ventricle, previous TMS, previous surgery in left hemithorax, chest wall deformities, previous radiotherapy of left hemithorax, pulmonary hypertension.

## Minimally Invasive Direct Coronary Artery Bypass (MIDCAB)

MIDCAB was first introduced in the early nineties, and gained its popularity as alternative solution to single-vessel revascularisation LAD-LIMA via TMS.<sup>3,4</sup> It is performed through left anterolateral thoracotomy in the fifth intercostal space with 5 to 8 cm skin incision and decompression of left lung using double-lumen endotracheal tube or bronchial blocker. All of the miniCABG procedures are performed by selective ventilation of the right lung or bilateral ventilation with small volume. Special retraction devices are used to elevate the anterior chest wall and to harvest LIMA graft by direct vision. When the graft is prepared and pericardiotomy performed, procedure could be carried on either with or without utilising ECC ("off pump"). If the decision is made to put a patient on ECC, peripheral cannulation must be per-

formed because cannulation of ascending aorta is practically impossible. On the other hand, if "off pump" is the option of choice, some kind of stabilisers must be used to make an anastomosis on the beating heart. Anastomosing is done in standard manner, technically identical to TMS approach.

There are several centres that published excellent results of this procedure.<sup>5-14</sup> Although the mostly chosen option for the treatment of single-vessel LAD disease is percutaneous coronary intervention (PCI), even more so after introducing of drug eluting stents (DES), MIDCAB still holds advantage for that case in majority of analysed parameters.<sup>15,16</sup>

There are also published cases of MIDCAB treatment of multi-vessel coronary disease, with and without ECC use.<sup>17</sup> Even though results were great, multi-vessel MIDCAB did not make it to everyday use in many centres. The reason is probably technical demands and great risk of complications because of that. Besides, postoperative pain levels must be taken in consideration (thoracotomy syndrome).<sup>17,18</sup> Also, a question of possibility of complete LIMA harvesting with this technique is being asked, because reaching the apex of hemithorax is impossible through fifth intercostal space incision. This could lead to coronary steal syndrome by a patent LIMA side branch.

## Endoscopic Atraumatic Coronary Artery Bypass (EndoACAB)

This procedure introduced endoscopic techniques to CABG surgery. The patient is positioned in right lateral decubitus to expose left hemithorax, and 10 to 12 mm camera is inserted through the fourth or fifth intercostal space (at the mid-level of chest bone), two fingers width laterally of medio-clavicular line or by anterior axillary line. After flooding the pleural cavity with 10 to 15 mmHg of CO<sub>2</sub>, two 5 mm ports are inserted parallel to camera, two intercostal spaces above and below it. The most common configuration is 2-4-6 and 3-5-7 (intercostal spaces). Harvesting of LIMA graft is performed with endoscopic instruments (Figure 2). After pericardiotomy and systemic heparinisation, a long spinal needle

is pierced outward the chest wall, to guide the skin incision site. Then, CO<sub>2</sub> is released from the wound so the heart and lung could return to position, and 3 to 4 cm incision anterolateral thoracotomy is made, followed by extraction of endoscopy ports. LIMA graft is introduced to operation field and anastomosed to LAD (usually with the “off pump” method) (Figure 3).

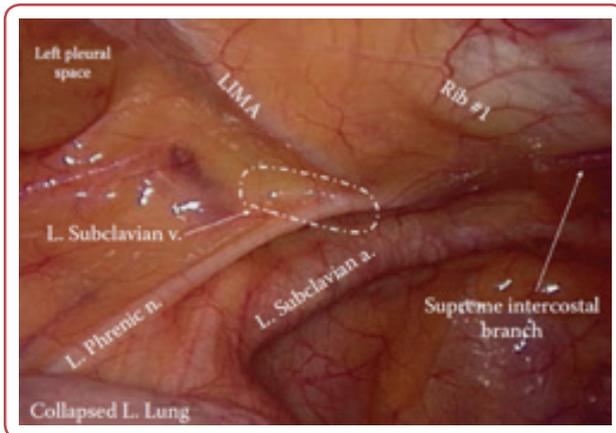


Figure 2: Left hemithorax after lung deflation, exposing LIMA and surrounding structures

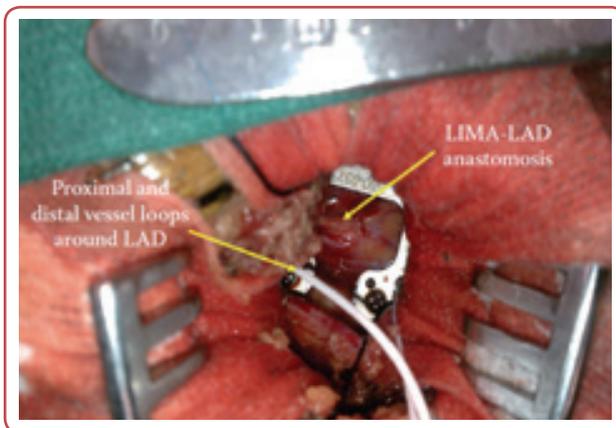


Figure 3: Making LAD-LIMA anastomosis through 3 cm incision anterolateral thoracotomy

EndoACAB made good results as a single procedure (LAD-LIMA), and as a part of the hybrid coronary revascularisation (HCR), too.<sup>19-22</sup> A long learning curve is also expected with this mini-CABG procedure, because endoscopic LIMA harvesting is technically quite demanding, just as making an anastomosis with 3 to 4 cm skin incision thoracotomy.

## Robot-Assisted Direct Coronary Artery Bypass (RADCAB)

RADCAB (Figure 4) represents the next step in the evolution of miniCABG surgery techniques. This procedure combines technological advances of robotised telemanipulation with direct anastomosing. Da Vinci system (Figure 5) provides a combination of high-definition image visualisation with 3D-flexible instruments, which enables complex manipulations in the operative field. RADCAB largely resembles EndoACAB procedure. After LIMA is harvested with the Da Vinci system, pericardiotomy is performed. Thoracotomy location is determined by spinal needle under endoscopic guidance, after which the anastomosis is made with standard instruments and stabilators.

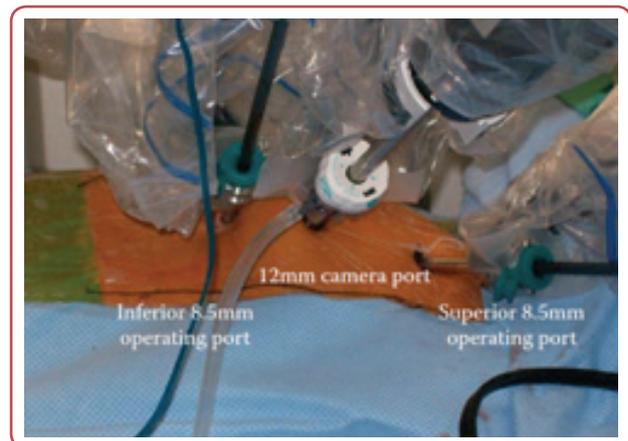


Figure 4: Ports position in RADCAB procedure



Figure 5: The Da Vinci surgical system

Although significant prospective studies that compare RAD CAB with conventional CABG surgery have not yet been performed, a few minor studies showed safety and feasibility of this method.<sup>8,23-25</sup> RAD CAB provides greater flexibility and visibility in comparison to endoscopic methods, however, the lack of tactile feedback with manipulation represents one important disadvantage. Few centres used this method as a step in the transition towards TECAB.

## Totally Endoscopic Coronary Artery Bypass (TECAB)

TECAB represents the least invasive, but also technically the most complex procedure for heart revascularisation through closed chest. This procedure is performed in only a couple of highly specialised centres across the world, and can be performed with ECC, as well as “off pump”. ECC cannulation is performed through femoral or axillar blood vessels. LIMA/RIMA is harvested with robot-assistance and pericardiotomy is performed before ECC initiation. Heart is excluded from circulation with the use of endo-aortic occluder (introduced directly through axillar or femoral approach) and then cardioplegic solution is administered anterogradely. Anastomosing is performed with robot-assistance. Da Vinci system of the latest generation has a fourth arm that can be used for stabilators application (in “off pump” TECAB). It must be mentioned that “off pump” TECAB technique is reserved only for revascularisation of LAD and its diagonal branches. For inferior and lateral wall interventions, ECC initiation and lung deflation are necessary, by which haemodynamic instability caused by heart manipulation is avoided.

There still have not been any prospective or observational studies to compare TECAB with traditional CABG surgery through medial sternotomy, nor with other minimally invasive CABG techniques. Nevertheless, early results of the few centres performing TECAB are encouraging. These are mainly LAD-LIMA interventions, although there are also cases of multivessel coronary artery disease management with this method.<sup>26-31</sup>

TECAB is considered the technically most chal-

lenging minimally invasive cardiac surgical intervention. In contrast to robot-assisted mitral surgery which uses larger dimension suture material and is sutured through the thick mitral annulus, suturing of coronary anastomoses is extremely delicate process. Therefore, learning curve is present. There is no room for flaws, considering the high risk of complications. The learning curve is notable when the extended timing of the interventions is taken into consideration.<sup>32</sup> The most experienced centres report a duration of LAD-LIMA TECAB procedure of 295 minutes (4.9 h) in average, while TECAB in ECC with bilateral IMA revascularisation requires 502 minutes (8.4 h) in average.<sup>33</sup> Obviously, this raised a question of the long-term efficiency of TECAB procedure versus standard CABG surgery.<sup>34</sup>

The price of Da Vinci system and the consumable supplies for its use in minimally invasive procedures need to be considered, as well as the extended duration of the operations. All of these represent a certain limit in comparison to standard CABG surgery. In order for wider acceptance of RAD CAB and TECAB technologies and techniques, the balance between procedure costs on one side, and the reduction of hospital stay and expected complications on the other side, needs to be established.<sup>13</sup>

## Hybrid Coronary Revascularisation (HCR)

HCR represents a treatment strategy which involves the experience of both cardiac surgeons and invasive cardiologists. This method mostly includes minimally invasive LAD-LIMA revascularisation in combination with PCI with DES on non-LAD coronary arteries. This type of multivessel coronary artery disease management drew the attention of both cardiac surgery and cardiology communities, since they agree that LIMA represents the most effective and long-lasting solution for LAD management. On the other hand, LIMA-LAD anastomosis is probably most credible for CABG longevity.<sup>35</sup> The reported incidence of venous (saphenous) grafts failure and the lower degree of DES restenosis raised a question: “Which is the optimal method for management of lesions on non-LAD blood vessels (venous graft or DES)?”<sup>36-38</sup>

HCR offers the advantages of both options: longevity of LAD-LIMA graft with minimally invasive surgery and percutaneous treatment with the use of DES for non-LAD blood vessels. Furthermore, this approach confines the disadvantages of the both methods: the invasiveness of the traditional CABG surgery and the use of unstable venous grafts on one hand, as well as short-lasting management of proximal LAD lesions with PCI on the other hand are avoided.

In terms of the interventions timing, HCR can be performed in three ways: PCI first; surgery first, and simultaneously in hybrid operating room (Figure 6). If the surgery is initially performed, operating under double antiplatelet therapy due to stents is avoided. PCI is primarily performed in patients with acute coronary syndrome when the culprit lesion is not on LAD. The existence of the hybrid operating room is the ideal scenario, because it provides the highest comfort for patient, shorter hospital time, and avoids ischaemic complications in the period between two HCR stages. Moreover, the patency of LIMA-LAD graft can be confirmed with graftography in the hybrid operating room, so a potentially inadequate anastomosis can be corrected. These benefits come with a price for coordinating double teams, as well as risk of perioperative bleeding related to the loading dose of antiplatelet therapy.



*Figure 5: Hybrid operating teatre*

Careful patient selection is necessary for optimal results. An ideal candidate is the one with proximal LAD lesion and focal lesions on ACD and/or Cx, which would be treated percutaneously anyway, if there was no LAD lesion present. More complex lesions, such as calcified coronary arteries, bifurcational lesions, chronically occluded lesions on non-LAD arteries, joined with proximal LAD lesions, are best treated with traditional

CABG surgery. The ideal population for undergoing HCR from anatomic and clinical point of view has not been defined. This method can be used for treatment of distal “left main” stenoses, as well as distal “left main” stenoses that propagate into the bifurcation, by making LAD-LIMA anastomosis, after which DES is implanted into “left main” towards Cx. Proximal and medial “left main” lesions are not suitable for HCR due to competitive flow development of MMR cannot be a final decision without a strong backup of long-term epidemiological data.

## Conclusion

Minimally invasive CABG techniques continue their evolution to the present day. Unfortunately, these are still limited only to the specialised centres across the world and have not been accepted by the majority of cardiac surgeons. MIDCAB is the most common procedure, while RADCAB and TECAB are the least common, however, their use tends to get higher. Although robot-surgery enables excellent visualisation and exposition in small spaces, implementing these new techniques requires not only significant financial resources, but also dedication of a whole team in adopting new skills.

A surgeon who is starting to practice miniCABG surgery needs to be ready for long duration of the interventions, higher rate of conversions to sternotomy, and significant learning curve. Conversion should not be considered as a failure under any condition, as the quality of anastomosis must never be compromised by avoiding it!

In order for surgical community to adopt these technologies, it is necessary to achieve a few goals: quality and longevity of the solutions must be confirmed by more centres; operation timing needs to be reduced to the level that is comparable to standard CABG methods; the higher financial cost needs to be balanced with lower postoperative expenses, including shorter stay in the intensive care unit, shorter duration of the postoperative mechanical ventilation, faster recovery and shorter hospital stay; close cooperation with cardiologists, who will accept and refer patients to these treatment methods, must be established. Great results that have been published on the subject of minimally invasive revascularisation methods support the potential of these alternative approaches to evolve in the near future.

## Acknowledgements

None.

## Conflict of interest

None.

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# Cutaneous Side Effects During Therapy With Erlotinib - Case Report

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## Abstract

Erlotinib is an antineoplastic drug used in the treatment of non-small cell lung cancer and pancreatic cancer. It is a potent, selective inhibitor of tyrosine kinase, a receptor for epidermal growth factor receptor (EGFR). Cutaneous side effects such as acneiform eruption, xerosis, telangiectasia, hair and nail changes are common. A case of a 70-year-old patient who developed unusual cutaneous side effects after 6 years of treatment with erlotinib was presented.

**Key words:** Erlotinib; Tyrosine kinase inhibitors; Dermato-toxicity.

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### ARTICLE INFO

Received: 13 December 2021

Revision received: 26 December 2021

Accepted: 26 December 2021

## Introduction

In order to increase the survival rate of oncology patients and preserve their quality of life, various therapeutic modalities have been developed, such as targeted therapy, immuno-oncotherapy and hormone therapy. In this way, patients receive personalised therapy based on the unique genetic profile of their cancer. One of the targeted therapies is erlotinib therapy (Tarceva, Roche) which is directed towards the epidermal growth factor receptor (EGFR).<sup>1</sup>

Erlotinib is an antineoplastic drug used in the treatment of non-small cell lung cancer and pancreatic cancer. It is a potent, selective inhibitor of tyrosine kinase, a receptor for EGFR. EGFR, also known as ERBB1 / HER1 is a 170 kD transmembrane glycoprotein dependent on intracellular tyrosine kinase activity that is overexpressed in different types of malignant cells. Erlotinib induces receptor phosphorylation and activates a specific set of cytoplasmic signalling molecules that reversibly block the downstream signalling pathway of the surface protein- EGFR, leading to a slowdown in the development of malignant cells

and their enhanced apoptosis. It is metabolised via CYP3A4 and excreted via the bile.<sup>1-3</sup>

However, targeted therapy has not led to a reduction in the incidence of dermato-toxicity compared to standard chemotherapy, which remains a major problem as it leads to a reduced quality of life for these patients.<sup>4</sup>

Since EGFR is also expressed on the surface of normal cells such as basal keratinocytes of the epidermis, keratinocytes of the outer coat of a hair and on the surface of cells of the sebaceous glands, a number of side effects are expected to occur on the skin when applying this therapy. The most common of these are acneiform eruptions on the skin of the face, neck and upper torso, pruritus, ichthyosis, telangiectasias, changes in the nails such as paronychia.<sup>2,5,6</sup>

The following is a case report of a patient in whom the side effects of erlotinib occurred after long-term use of this drug in the treatment of lung cancer.

## Case history

A 70-year-old patient had come for her first check-up with a dermatologist in August 2020, due to extensive changes in the scalp that had first occurred 10 days before the examination. She stated that she has been treated for lung cancer for the past six years and that she uses Tarceva, reduced dose of 150 mg for lung cancer. Apart from severe itching and dry skin, she has not had any side effects from this therapy so far. After the insight into the available medical documentation in the information system of the University Clinical Centre of the Republic of Srpska, it was found that in 2014 lung adenocarcinoma with pleural metastases (pT1aNxM1, stage IV, ECOG 1) was confirmed in the patient and mutation of EGFR gene on exon 19 was detected. After the video-assisted thoracoscopic surgery (VATS) - atypical resection of the sixth segment of the lower lobe of the right lung - in 2014, the patient received chemotherapy - two doses of cisplatin plus vinorelbine - and one dose of cisplatin plus gemcitabine, after which erlotinib monotherapy in a reduced dose of 150 mg was introduced. The patient did not receive radiotherapy.

At the time of clinical examination on the scalp in the frontal part there were present numerous individual and in some places multiple confluent pustules as well as papulopustules and on the remaining part of the scalp the multiple pustules which were almost completely covered with greenish-yellow crust and individual excoriated

crusts (Figure 1 and 2). Individual excoriations were present on the rest of the skin. Subjectively, the patient complained of intense itching, pain, and burning.

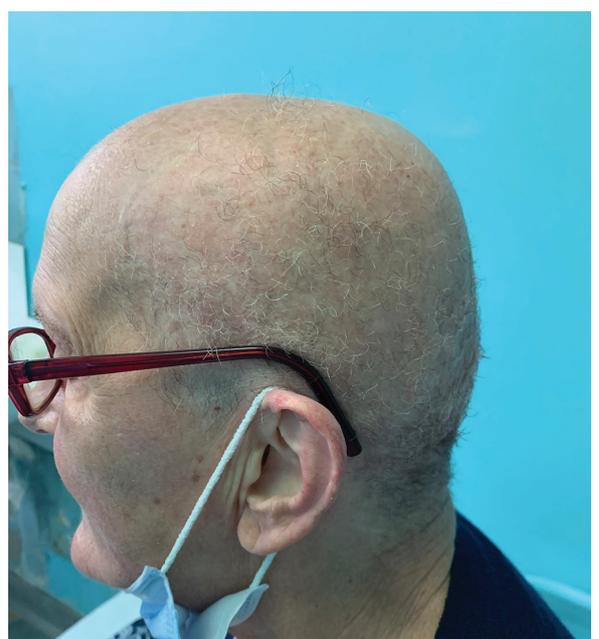
After taking a swab of the skin lesion of the scalp, the patient underwent local therapy. She used



*Figure 2: Numerous individual and multiple confluent pustules and papulopustules on the scalp covered with greenish-yellow crust*



*Figure 1: Numerous individual and multiple confluent pustules and papulopustules on the scalp covered with greenish-yellow crust*



*Figure 3: Complete regression of skin lesions*



Figure 4: Complete regression of skin lesions

paraffin packs to remove the squama, as recommended, 2 to 3 hours before washing her hair and kerato-reducing shampoo with salicylic acid for washing her hair. Locally, she applied a 2 %-aqueous solution of eosin twice a day to the scalp, then gentamicin ointment 2-3 times a day and in the evening under occlusion. *Staphylococcus aureus* was isolated by swab and then systemic antibiotic therapy was performed according to the antibiogram, 1000 mg amoxicillin and clavulanic acid twice a day for seven days.

After 20 days at the follow-up examination there was a good regression of cutaneous changes, without any new fresh changes and crusts. The patient continued to use topical therapy of 2 %-aqueous solution of eosin and chloramphenicol. At the second follow-up examination, a complete regression of skin changes was present (Figures 3 and 4). Exclusion of erlotinib from the therapy has not been considered.

## Discussion

More than 50 % of the patients who use EGFR inhibitors in their therapy develop some of the side effects on the skin.<sup>7</sup> Acneiform eruptions are one of the most common side effects when applying EGFR inhibitors and they are a consequence of disorders in the proliferation, differentiation, migration of hair follicle cells.<sup>8</sup> It usually occurs two weeks after the application of therapy, in contrast

to the case shown, where the eruption occurred after six years of continuous use of erlotinib.<sup>9</sup>

It remains unclear why this side effect occurred in the presented patient only after six years of continuous application of this therapy, in contrast to the conditions described in the available literature. Acneiform eruptions caused by erlotinib are similar to those induced by other drugs such as corticosteroids, anticonvulsants, vitamin B12 and B6, which are manifested by inflammatory papules and pustules.<sup>10</sup>

The incidence of acneiform eruptions in clinical trials varies from 33 to 79 %.<sup>7</sup> Since skin rash has been confirmed to be associated with a better clinical response to applied therapy, skin rash can be used as a biomarker for a therapeutic response to EGFR inhibitors.<sup>11,12</sup> However, the relationship between the occurrence of skin rash and the clinical response to applied therapy has not been elucidated.

Julian and Iwamoto<sup>13</sup> proposed a mechanism of rash formation which, in addition to apoptosis of EGFR-expressing cells, is based on the interruption of the synthesis of CD45 protein expressed on the surface of hematopoietic cells. After being distributed to the bone marrow and skin, erlotinib binds to EGFR on keratinocytes, leading to their apoptosis. Apoptosis triggers an inflammatory process that leads to an increase in neutrophil production in the bone marrow with decreased CD45 expression. Due to the reduced expression, neutrophils do not have the ability to migrate towards the inflammatory process. Thus altered neutrophils will be directed towards the capillaries of the hair follicles and increase the concentration of neutrophils in these regions. Inadequate neutrophil responses and constant apoptosis of keratinocytes due to neutrophilic infiltration intensify the inflammatory process and stimulate further neutrophil production in the bone marrow.<sup>13</sup>

## Conclusion

Dermatotoxicity is still one of the common side effects within different therapeutic modalities in the treatment of oncological conditions that can occur at any stage of treatment. Since these conditions reduce the quality of life of oncology patients, active involvement of a dermatologist during treatment is also required.

## Acknowledgements

None.

## Conflict of interest

None.

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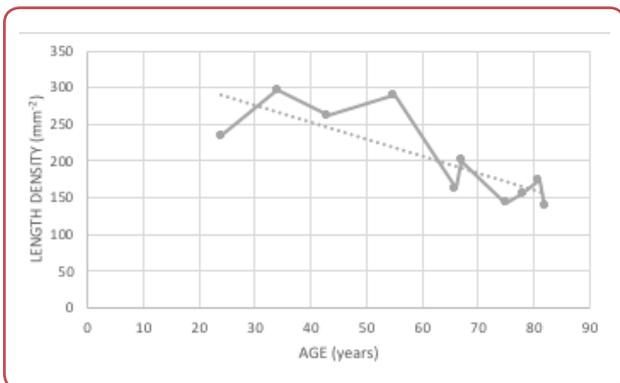
# Stereological Analysis of the Vascular Network in Superior Colliculi

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## Erratum

Sladojević et al. Scr Med 2020;51(4):230. ■

*Instead of the Figure 3 that was erroneously substituted with a replica of the Figure 2, now a true Figure 3 is given.*



*Figure 3: Changes of length density with aging*



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