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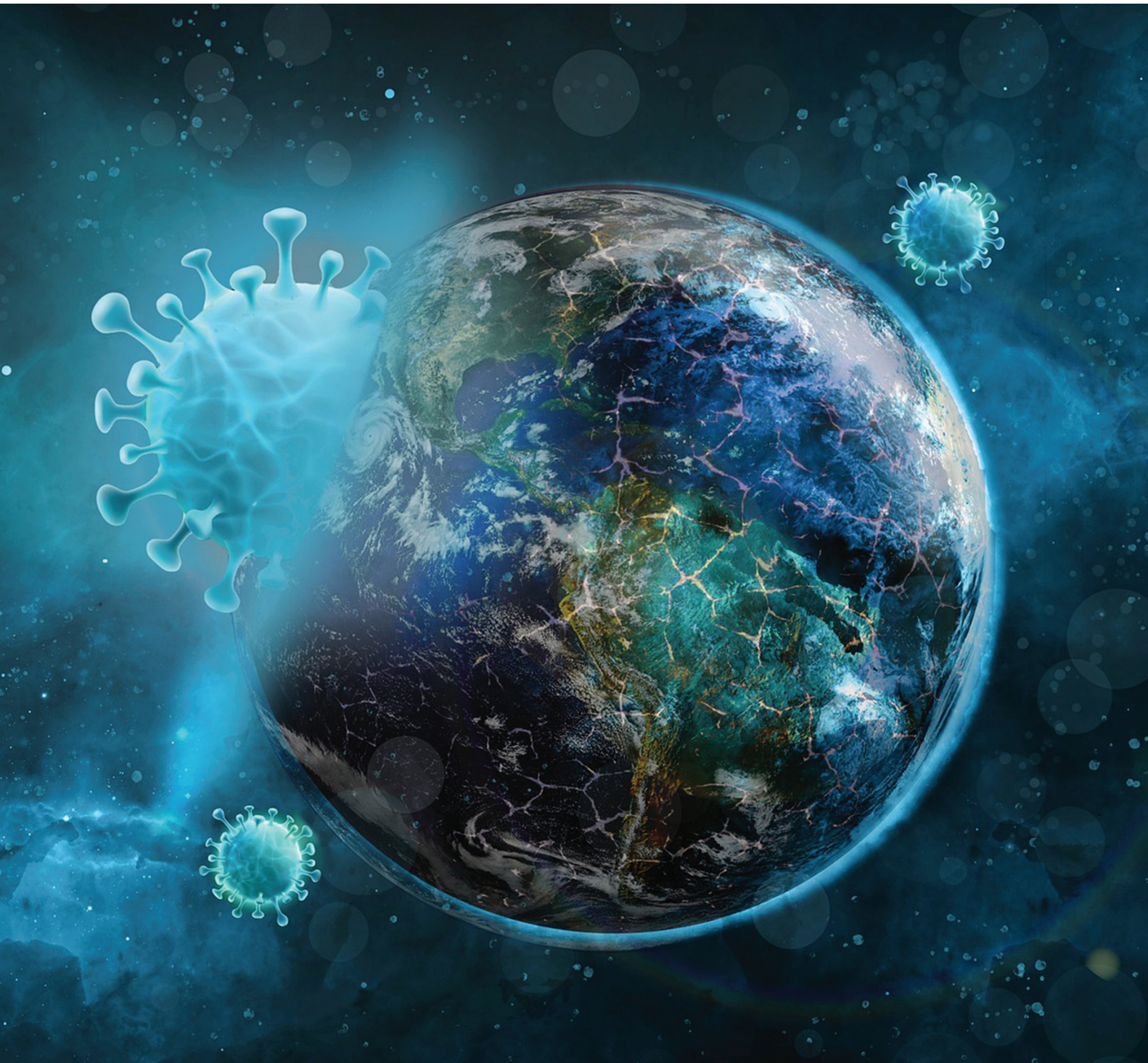

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Original Scientific Article

COMPARATIVE ANALYSIS OF SURGICAL AND ENDOVASCULAR TREATMENT OF INTRACRANIAL ANEURYSMS

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Abstract. *With a prevalence of about 2.3%, intracranial aneurysms represent the most common cause of spontaneous subarachnoid hemorrhage. Many studies compared currently most common therapeutic options-neurosurgical clipping and endovascular embolization, but so far no single solution in which therapeutic method should have an advantage has been found. The aim was to compare the outcome of treatment on discharge, among patients treated with clipping or embolization. The data of 62 patients treated at the Neurosurgery Clinic of the Clinical Center of Serbia, were obtained through discharge lists. The analysis included data on sex, age, Glasgow coma scale, aneurysm rupture, Hunt & Hess scale, localization, number of aneurysms, length of overall and postoperative stay as well as the existence of complications. The condition on discharge was assessed using the Glasgow outcome scale. In our study 37 subjects (59.7%) had a subarachnoid hemorrhage, while 25 (41.9%) patients were without bleeding. 30 patients underwent surgery, while 32 were treated by embolization. No significant relationship between the treatment modality and outcome on discharge was observed (p=0.115). Patients without bleeding had a significantly better outcome on discharge when treated by endovascular method (p<0.001), whereas in the group with a rupture no differences were found (p=0.35). Complications were significantly more common after surgery (p=0.026). No difference among the groups in the length of the total hospital stay was found (p=0.246), while a significantly longer postprocedural period was recorded following neurosurgical treatment (p=0.029). Groups treated with different modalities did not differ in the outcome on discharge. However, the percentage of complications was greater in the group of patients undergoing surgery, as well as the length of postoperative hospital stay. We believe that furthe detailed analyzes offering information on the condition of patients after a long period of follow-up iare required.*

Key words: *aneurysms, clips, embolization.*

Introduction

Intracranial aneurysms are the most common cause of spontaneous subarachnoid hemorrhage. They can be of congenital, arteriosclerotic, traumatic, mycotic, embolic, and neoplastic origin. The prevalence of intracranial aneurysms is thought to be about 2.3%. Aneurysms that lead to spontaneous subarachnoid hemorrhage are most often not of congenital origin and are mostly localized in the anterior segment of the circle of Willis.

Currently, the most common therapeutic options in the treatment of intracranial aneurysms are neurosurgical clipping aneurysms and endovascular embolization. The goal in the treatment of aneurysms is to achieve immediate and permanent occlusion of the aneurysm lumen while preserving the parent blood vessel, surrounding blood vessels, as well as the brain parenchyma. Current recommendations indicate that unruptured symptomatic aneurysms should also be treated, given the possibility of rupture and the development of further complications [1].

Until the development of endovascular techniques, neurosurgery had primacy in the treatment of cerebral aneurysms. Since the beginning of the '80s of the last century, great progress has been made in technology, imaging methods, development of new materials, which enabled rapid progress in the field of interventional radiological methods and led to a reorientation of many health systems to different therapy of these lesions. Since then, studies have been constantly conducted comparing these two treatments, but so far no unique solution has been found as to which method should be preferred [2].

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The Goals

This study aimed to compare the treatment outcome on discharge of patients treated with neurosurgical clipping or endovascular embolization of intracranial aneurysms.

Materials and Methods

The study included 62 patients treated from 1.1.2017 to 31.10.2017. at the Clinic for Neurosurgery of the Clinical Center of Serbia. Data were obtained by reviewing discharge lists and included gender, age, Glasgow Coma Scale (GCS) at admission for all subjects, and aneurysm rupture data. The severity of subarachnoid hemorrhage in patients with a ruptured aneurysm was determined by grading patients on the Hunt and Hess (H&H) scale. The location and number of aneurysms, the method of treatment, the time when the intervention was performed were determined. Also, information was collected on the

length of intrahospital stay and postoperative course and the existence of complications. The condition of patients on discharge was determined by the Glasgow Outcome Scale (GOS) - death outcome (1), persistent vegetative state (2), severe (3) or moderate disability (4), and good recovery - no symptoms or mild deficit (5).

The collected data were analyzed using standard statistical tests in the SPSS software package.

Results

The study included 62 patients, 45 females (72.6%), and 17 males (27.4%) (Fig. 1). The observed number of aneurysms was 70.

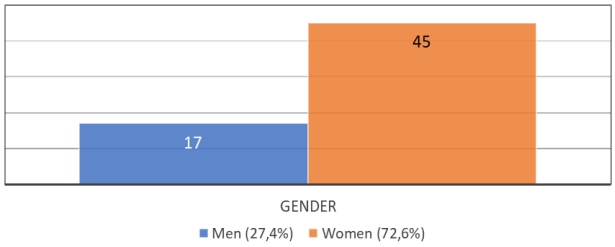

Fig. 1 Distribution of patients by gender.

The mean age was 53.47 years (SD = 11.94), the youngest respondent was 12 years old, and the oldest 78. No significant difference in mean age was observed between patients of different sexes (t = −0.93; p = 0.920) (Table 1).

Table 1 The average age of the patient

Gender	Average age	
Men	Average	53.24
	St. Deviation	10.59
Women	Average	53.56
	St. deviation	12.60

At admission 40 subjects had GCS 15 (64.5), 16 subjects GCS 13 and 14 (25.8), respectively, and 4 GCS between 9 and 12 (6.5%). GCS could not be determined in two subjects at admission because they were sedated (Fig. 2).

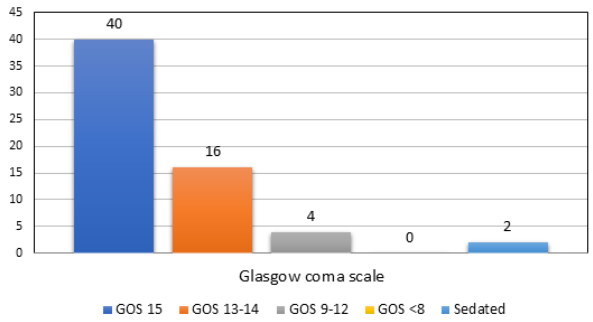

Fig. 2 Glasgow coma scale of patients on admission.

Subarachnoid hemorrhage was noted in 37 subjects (59.7%), while 25 (41.9%) were without bleeding.

Only one respondent (1.6%) had Hunt and Hess grade V, 9 respondents (14.5%) grade III, 24 (38.7%) grade II, 2 (3.2%) grade Ib, and 1 (1.6%) degree Ia. The majority of the patients in this study (25 respondents or 40.3%) were rated with 0 (Fig. 3).

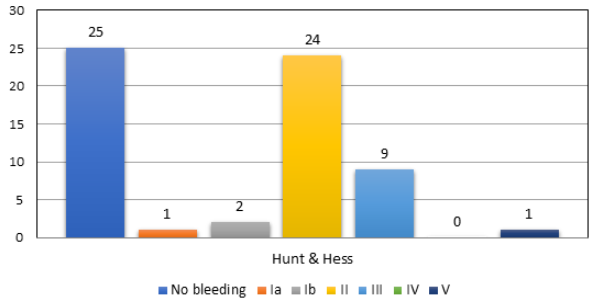

Fig. 3 Patient grades according to the Hunt & Hess scale.

The number of aneurysms ranged from 1 to 3 per subject. The largest number of subjects - 54 had 1 aneurysm (87.1%), 7 subjects (11.3%) had 2, and one subject (1.6%) 3 aneurysms.

Almost all of the aneurysms (95.8%) were localized in the anterior circulation and 3 (4.2%) in the posterior. Internal carotid artery aneurysms were the most common - 32 (45.7%), followed by middle cerebral artery aneurysms (ACM) - 19 (27.1%), followed by anterior communicating artery aneurysms (AcoAnt) - 11 (15.7%), aneurysms a. perisallosae were present in 4 cases (5.7%), a. superior cerebellum, a. anterior choroid, a. basilaris, and a. vertebralis 1 (1.4%) (Fig. 4).

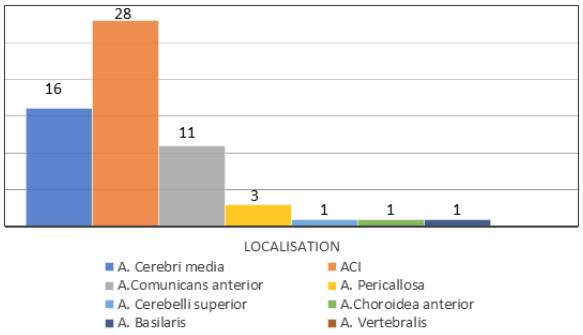

Fig. 4 Prevalence of aneurysms by localization.

Of the 62 patients presenting with subarachnoid haemorrhage included in the study, 30 patients underwent surgical treatment, while 32 were treated with the endovascular method. Statistical analysis did not show a significant association between therapy type and discharge outcomes (Man-Whitney = 380.5; p = 0.115).

Looking only at the group of patients with subarachnoid hemorrhage due to aneurysm rupture, no significant difference was found in the outcome of discharge among patients treated with surgery or endovascular method (Man-Whitney = 134.5; p = 0.35), while in the group of patients without

previous rupture, the outcome at discharge was significantly better after endovascular treatment (Man-Whitney = 15.5; p <0.001) (Fig. 5).

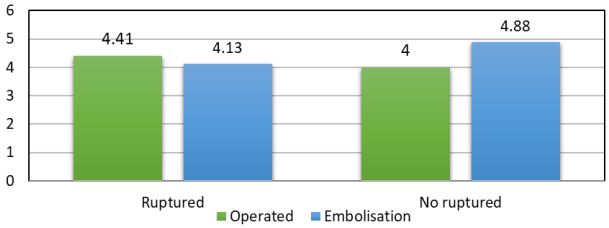

Fig. 5 Condition of patients at discharge expressed by the Glasgow outcome scale.

The percentage of complications was significantly higher in the group of patients treated with microsurgical aneurysm clipping (p = 0.026) (Table 2).

Table 2 Prevalence of complications, length of intrahospital stay, and duration of the postoperative treatment period

	Type of the procedure	
	Clipping	Embolization
Complication	11 (36.7%)	4 (12.5%)
Length of total hospital treatment (in days)	15.07	12.41
Length of postoperative period (in days)	12.2	8.03

Of the complications, ischemia (6.5%) was noted in 4 patients, 2 patients each had septicemia (3.2%), cranial nerve paresis (3.2%), and edema (3.2%), while 1 observed bleeding (1.6%), transient diabetes insipidus (1.6%), meningitis (1.6%) and hydrocephalus (1.6%) and diarrhea (1.6%).

There was no difference in the length of the total intrahospital stay among groups of patients treated endovascularly or surgically endovascular and surgical (t = 1,171; p = 0,246), while the postoperative period was significantly longer in the group of patients undergoing neurosurgical treatment (t = 2,223; p = 0,029) (Table 2).

Statistical analysis did not show a significant correlation between the age of the respondents and the outcome at discharge (ANOVA = 1.753; p = 0.182).

No significant association was found between the time elapsed from admission to the procedure and the discharge outcome (Kruskal-Wallis test; p = 0.975).

In our study, the localization of the aneurysmal dilatation was not statistically significantly associated with the outcome at discharge (Kruskal-Wallis; p = 0.288), nor with the existence of treatment complications (Fisher = 4.188; p = 0.758).

Discussion

The development of medical science and technology in the second half of the 20th and the beginning of the 21st century enabled interventional radiological methods to stand side by side with neurosurgery. Since the first major studies in the 1980s, numerous studies have been conducted on an ongoing basis to determine which treatment for brain aneurysms should be preferred. To date, no consensus has been reached on which method is more effective, and the opinion has been accepted that the decision on the therapy that will be implemented should be made by the teams for the treatment of cerebrovascular diseases, for each patient individually. Some of the proposed guidelines that can help make a decision take into account the location, morphology of the aneurysm, preoperative grade and age of the patient, the possibility of dissecting the aneurysm after previous operations, and the possibility of injury to perforating branches [3].

The largest study comparing surgery and endovascular embolization of ruptured aneurysms was the International Subarachnoid Bleeding Study (ISAT) [4]. ISAT results showed that ruptured aneurysms that could be treated in both ways had a better outcome when treated by endovascular approach, but with a higher degree of rebleeding. ISAT results after 5 years of follow-up showed a lower mortality rate in the group treated by the endovascular approach, but there was no difference in morbidity. Also, higher rate of rebleeding in the group of embolized patients was demonstrated [5]. Numerous studies have shown that the degree of recanalization of blood vessels after endovascular treatment is higher [6]. Other studies showed better results in the early postprocedural period following endovascular obliteration, but demonstrated no significant difference in morbidity and mortality over longer follow-up [7]. The results of our study showed that there was no difference in the outcome at discharge between the groups of patients treated with different methods, while the outcome in the immediate perioperative period was better in the group of embolized patients, while over time mortality and morbidity equalized [8]. Above all, we should not forget other advantages of open surgery, such as the evacuation of blood from the cistern, which contributes to the development of vasospasm and allows normal cerebrospinal fluid flow, for which endovascular methods are not possible. It should be kept in mind that when combining surgical clipping and endovascular coiling, or subsequent surgery after the endovascular intervention, hematoma evacuation carries the risk of hematoma enlargement due to the use of antiplatelet drugs. Also, the observed incidence of vasospasm does not differ between patients treated with clipping or embolization [9]. Various studies demonstrated no significant difference in morbidity depending on treatment method in unruptured aneurysms, but better occlusion if treated by microvascular clipping [10–13]. The results of this study show that patients undergoing endovascular treatment have a better outcome at discharge in the group of unruptured aneurysms. However, the observed higher rate of complications after surgical treatment is consistent with the opinion of most authors [4]. In contrast to other studies, subjects included in our study did not show an association between age and discharge outcomes [3]. Our findings support claims that the

length of postoperative stay in the hospital for longer in patients undergoing surgery [14].

Conclusion

Among the groups treated with neurosurgical clipping or endovascular embolization, cerebral artery aneurysms did not show a difference in the outcome of treatment on discharge, expressed by the Glasgow outcome scale. However, the percentage of complications is higher in the group of operated patients, as well as the length of postoperative intrahospital stay.

Given the increasing prevalence of arterial hypertension, it is possible that non-traumatic subarachnoid hemorrhage, most often caused by rupture of the intracranial aneurysm, will be an even more common problem in the future with more cases, and thus a greater burden on neurosurgeons, interventional radiologists and the health system as a whole. We believe that the final judgment on the choice of therapeutic method to be given priority in treatment requires a further comprehensive analysis that will, in addition to information on the condition of patients on discharge, provide data on the condition of patients after a long follow-up period.

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Case Report

ORIGIN OF THE LEFT VERTEBRAL ARTERY FROM IPSILATERAL COMMON CAROTID ARTERY IN A HUMAN FETUS: CASE REPORT AND BRIEF REVIEW OF THE LITERATURE

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Abstract. *Although the vertebral artery (VA) is a type of artery with a lot of variations, its origin from the common carotid artery (CCA) is one of the rarities that deserves to be presented.Microdissection of injected arteries in the thorax and neck of human fetuses under the operating microscope was used and documented in the workbook and photos. The origin of the left VA from ipsilateral CCA, and its entry into the transverse foramen of the fourth cervical vertebra (C IV), associated with the normal vascular arrangement of branches of the aortic arch were the main features of this case. Simultaneously, the right VA of normal (subclavian) origin entered the C V transverse foramen. A present case of the left VA−CCA variant is the only one discovered in human fetuses, respectively, in total it is the tenth human case in the literature. However, the future findings could show whether the right aortic arch and the carotid stenosis discovered in half of known adult cases can be possible causal and/or consequent abnormalities of the left VA−CCA variant*.

Key words: *human fetus, vertebral artery, variable origin, common carotid artery.*

Introduction

The vertebral artery (VA), a collateral branch of the ipsilateral subclavian artery (SA) may be missing on one or both sides [1,2], or can present a lot of other variations at the origin, and/or course and/or its termination [3−5].

The origin of the (right or left)VA from ipsilateral common carotid artery (CCA) or so-called VA−CCA variant was first described on the right side in 1768 by Murray [6], as cited [7], and on the left side in 1836 by Hyrtl [8], as cited [9].

It is well known that determination of anomalous VA origin is important before the performance of surgical or endovascular interventions and, besides, an appearance of atherosclerotic plaques in close proximity to the VA aberrant origin is also possible [10].

The purpose of this report was to present a unique left VA-CCA variant in the human fetus and point out the morphofunctional importance of this variant in adult life.

Materials and Methods

A very rare case was found by a retrospective analysis of the co-author's workbook and archive of slides. The present case was an incidental finding after the investigation of human fetuses at the Department of Anatomy of Faculty of Medicine in Niš during the preparation of the co-author's doctoral thesis [11]. The gestational ages of fetuses in a 50-year old anatomical collection of our department, which were estimated according to the crown-rump lengths (CRLs), as has presented in Patten's book [12], varied from the third to the eighth lunar month. The arteries were injected with Latex or Micropaque solution through the left cardiac ventricle or through the CCA and kept in 10% solution of formalin until nowadays. The arteries were dissected under the operating microscope (Olympus), while their lengths and outer diameters were measured via an installed micrometer scale. Each case was sketched in the workbook and photographed.

All fetuses were medicolegally provided from the Clinic of Gynecology and Obstetrics in Niš that as a part of our Faculty of Medicine participated as professional cooperation and internal Ethical control; the Council for Postgraduate study of our Faculty issued the main permit to the co-author for an investigation of the fetal material in the period 1983-1990.

The embryology basis of the VA−CCA variant was explained according to Padget [13], and a scheme in Lie's book [3].

Case Report

A unique case was identified in a female fetus of 220-mm CRL or 23 post-menstrual weeks. The left VA originated from the posterior wall of the ipsilateral CCA at the level of C VII vertebra. It coursed upward and out of transverse foramen of the sixth and fifth cervical (C VI and C V) vertebrae and entered the same of the C IV vertebra. Simultaneously, the right VA was of SA origin; it entered the C V transverse foramen. The outer diameter of the left VA was twice as large as on the right one. The pattern of branching of the aortic arch was as usual—the brachiocephalic trunk (BT), left CCA, and left SA (Fig. 1).

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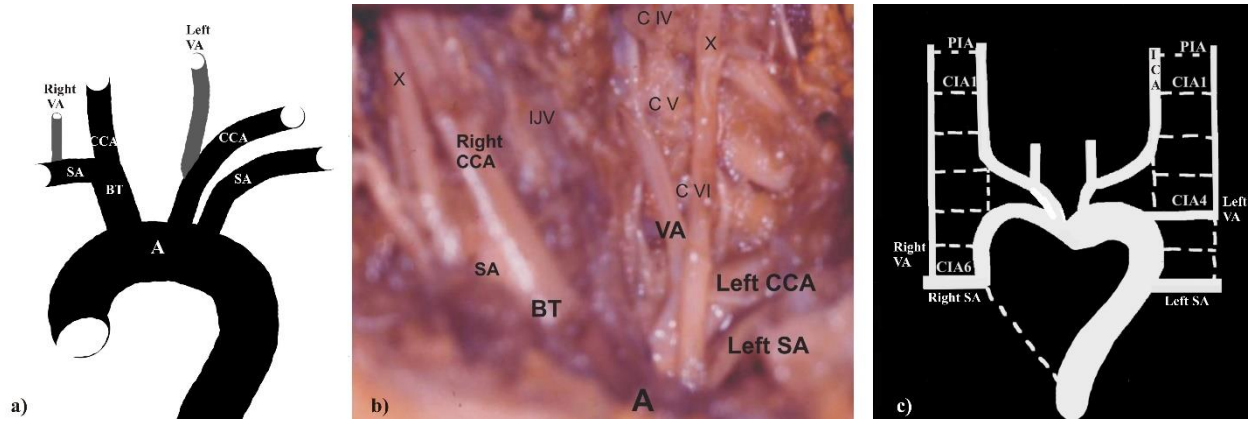

Fig. 1 Left vertebral–common carotid (VA–CCA) variant in a human fetus. **a –** scheme of variant; **b –** original picture of aortic branches and variable origin of the left VA from the ipsilateral CCA; **c –** modified diagram of embryological basis of the left VA–CCA variant according to Lie [3].
Note: We did not measure the outer diameters of arteries due to the poorly injected arteries by Micropaque.
Additional abbreviations: SA, subclavian artery; BT, brachiocephalic trunk; A, arch of the aorta (a); X, vagus nerve; IJV, internal jugular vein; C (IV, V, VI), cervical vertebra (b); PIA, proatlantal intersegmental artery; CIA, cervical intersegmental artery; ICA, internal carotid artery (c).

The recent case has been added to the list of nine published cases [8, 14–21] so far (Table 1).

Discussion

As already described, the variable origin of the left vertebral artery is relatively frequent. Its aortic arch (AA) origin was established in 2.4–5.8% of adult specimens, as cited [22], or in 23/200 fetuses [11]– as a single trunk in 19/200 or 9.5% (one case was associated with a variable origin of the right VA from the thyrocervical trunk) and as a segmentally duplicated in 4/200 or 2% of cases (one VA trunk was of the AA origin, while the other VA trunk was of SA origin); the left VA was usually localized on the AA between the left CCA and left SA and only in one case distally from the left SA [4]. The presented case was not included for consideration in the thesis due to non-injected cerebral arteries [11], but the left VA–CCA variant was noted as a rare variation. Furthermore, as cited [5], the left VA was presented as a simple or common vessel or segmentally duplicated at the level of the origin from the ipsilateral SA, or CCA, or external carotid artery (ECA), or thyrocervical trunk, or so-called left brachiocephalic artery or special left lateral SA. Additionally, there were reports about the bilateral internal carotid (ICA) origin of the VA [22] and the left ICA–VA common origin [23].

A variable left VA origin from the ipsilateral CCA was the object of this paper. The fact that in the period of 170 years, i.e. from 1844 [15] to 2013 [16] there was no published or quoted work about the left VA–CCA variant demonstrates of what arterial rarity it is.

Embryologically, the third, fourth, and sixth primitive aortic arches (PAAs) are important for the human arteries, as cited [4,5], because the first and second PAAs already disappear at day 29 of gestation, while the fifth PAA either never forms or regresses as an incomplete arch.

Padget [13] pointed out that serial cervical parts in the human embryo represent eight nerves, seven vertebrae, and six cervical intersegmental arteries (CIAs), as branches of the corresponding dorsal aorta. At the 7–12 mm (5th to 6th week), the VA is formed by an interconnection of the primitive proatlantal intersegmental artery (PIA), which is located between the occipital and cervical somites, and dorsal branches of proximal six CIAs. Bilaterally, the CIA6 also contributes to the development of the SA and a part of the definitive arch of the aorta on the left side, and a part of the SA on the right side (distal to the internal thoracic artery).

According to Patil et al. [18], the left VA–CCA variant is a result of the persistence of the left PIA continuing as the left VA with obliteration of proximal longitudinal anastomoses. We suppose that the left VA–CCA variant in our case was the consequence of the longitudinal anastomosis between the PIA and only four CIAs, associated with an involution of the anastomosis between CIA4 and CIA6, as Padget [13] and Lie [3] have proposed; simultaneously, the left CIA6 continued as the left SA. However, Inaba et al. [19] noted that it is a consequence of the persistent left CIA5 and involution of the ipsilateral middle dorsal aorta between the persistent CIA5 and CIA7, while Sharma et al. [21] described that it is caused by the persistence of any of the CIA3 to CIA6 and its migration to the level of the left CCA followed also by involution of the ipsilateral middle dorsal aorta.

Although we have found only nine published cases [8, 14–21] so far, Yuan et al. [24] evidenced two cases of the left VA–CCA variant analysing 1,231 cases of aberrant VA origin in the literature, while Sharma et al. [21] noted only 1/1,286 patients in a meta-analysis; this spe-

Table 1 Left vertebral artery (VA) origin from ipsilateral common carotid artery (CCA)

No	Age/ gender	Country	Initial symptoms or a reason of discovery	Level of VA origin	Associated variations	Associated pathology	Authors*
1							Hyrtl (1836)**
2							Hyrtl (1841)**
3							Quain (1844)**
4	76/M	USA	CTA evaluation			70% stenosis of the left CCA. Parkinson disease.	Troutman et al. [16]
5	68/M	Ireland	Headache / chest and neck pain		Right-sided aortic arch. Aberrant left SA arose from Kommerell diverticulum. Hypoplastic left VA.	>80% stenosis of both ICAs	Shaikh et al. [17]
6	64/M	India	Weakness in the right upper and lower limbs / slurred speech	C III vertebra	Trifurcation of the left CCA. Left VA entered C I transverse foramen. Absent right A1 part.	Small acute nonhemorrhagic infarct in the left thalamus. Gliotic area in the head of right caudate nucleus.	Patil et al. [18]
7	55/M	Japan	Diagnostic evaluation		Right-sided aortic arch. Aberrant left SA arose from a large Kommerell diverticulum. Left VA entered C V transverse foramen.		Inaba et al. [19]
8.	22/F	India	CTA evaluation		Right-sided aortic arch. Aberrant left SA. Origin of dilated left pulmonary a. from the ascending aorta.	Tetralogy of Fallot	Pandey et al. [20]
9.	12/U	India	CTA		Double-outlet right ventricle. A subaortic ventricular septal defect. Pulmonary stenosis. Early bifurcation of the left CCA.	Congenital heart disease	Sharma et al. [21]
10.	Fetus/F	Serbia	Incidental finding during anatomy dissection	CVII vertebra	Left and right VAs entered C IV and C V transverse foramina, respectively.		Recent case

*The authors are listed according to age-published data.
**Hyrtl, 1836; 1841 [8,14], and Quain, 1844 [15] were cited by Bernardi and Dettori [9].
M, male; CTA, computed tomography angiography; SA, subclavian artery; C (I), cervical vertebra (atlas); ICA, internal carotid artery; PIA, proatlantal intersegmental artery; A1, pre-communicating part of the anterior cerebral artery; CIA, cervical intersegmental artery; F, female; U, unknown gender.

cial incidence would range from 0.16−0.7%. In comparison to the incidence of this variant on the right side of 0.18% presented by Layton et al. [10], the left VA−CCA variant, paradoxically, could be more frequent than the right one.

We claim that case of a persistence of the left primitive PIA originating from the CCA bifurcation and its course outside of transverse foramina of all cervical vertebrae, presented by Palmer and Philips [25], should be differed from a case of the VA of the same origin and its course through the transverse foramen of the atlas showed by Patil et al. [18]. Although we described this manner of VA−CCA variant as a type 2 of the persistent PIA [1], we now think that an artery passing through the transverse foramen of one or all (seven) cervical vertebrae represents the VA rather than a PIA or its variant.

Initial symptoms or reasons of discovery of 7/10 known cases, as presented in Table 1, were unspecific (headache and chest pain or weakness of the extremities) [17,18], or incidental during diagnostic computed tomography angiography evaluations [1,19−21], or anatomy dissection in the recent case. The age of the known previous cases (4 of unknown gender, 4 of male, and 2 of female) ranged from 12−76 years.

A case of the posterior circulation infarct associated with the left VA−CCA variant induced a hypothesis

about a possible alteration of the cerebral hemodynamic in this variant [18]. It was noted that anomalous VA might be a source of aortopulmonary collaterals that may need preoperative embolization [21], e.g. in associated cyanotic congenital heart diseases [20,21]. Two cases of the left VA−CCA variant associated with stenosis of some carotid arteries could be explained as a consequence of atheromatous plaques in vessels of older patients, independent from the left VA−CCA variant [16,17].

There was an association of the left VA−CCA variant and the right aortic arch (RAA) in 3/6 known cases [17,19,20]. It would be speculative to talk about their common association, especially since the RAAs are revealed in a minor percentage (0.01% to 0.1%) in the general population [26]. Whether the genetic defects, e.g. 22q deletion that characterized the RAA [26] can lead to the discovery of the left VA−CCA variant, the future findings will show.

Conclusion

Although a fetal sample was presented, this is only the tenth case of a left VA−CCA variant discovered in the last 185 years.

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Review Article

SEVEN ARGUMENTS AGAINST STARTING WARFARIN ON THE FIRST DAY OF PULMONARY THROMBOEMBOLISM

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Abstract. *The aim is to analyze how the advances in pulmonary thromboembolism (PTE) may influence its therapeutic protocol, focusing on the anti-vitamin K (AVK) start. Narrative review (analyzing the most important Guidelines) was used for the synthesis of the improved approach regarding the time to start AVK in PTE. For PTE, it is crucial to instantly provide an optimal anticoagulant effect of both unfractionated heparin and AVK, a difficult task indeed. By delaying AVK we may avoid the AVK use (and thus the overlap); instead, following a parenteral anticoagulant, we may proceed with direct oral anticoagulant-DOAC (if the escalation therapy is not needed). There are seven new important arguments to postpone AVK commencement from the first day of PTE treatment (although recommended in contemporary guidelines for PTE patients who are not planned for thrombolysis). A more appropriate time to start the oral anticoagulant (preferably-DOAC) is when PTE comes under control and the need for escalation of fibirinolytic treatment is gone*.

Key words: *oral anticoagulants, warfarin, pulmonary thromboembolism.*

Introduction

We are witnesses to a rapid improvement of pharmacology in the field of pulmonary thromboembolism (PTE) [1]. It imposes a need to optimize utilization of new drugs together with the incorporation of clinical experience and current understandings of the disease into our contemporary approach to PTE patients [2–5]. Treatment of PTE has been complex due to a pronounced variability in the age, the number, and severity of comorbidities, thrombus burden and location, degree of cardiopulmonary reserve, variable response to therapy and treatment-related complications, etc. Nine years ago a number of reasons have been published to postpone anti-vitamin K (AVK) from the admission day [6]:

1. In PTE patients without shock and hypotension and therefore without the need for immediate „primary" fibrinolysis, the *escalation therapy* (*„secondary fibrinolysis"*) may be required on e.g., the fifth day. In such patients, AVK given on the first day will pose an unnecessary and avoidable risk of bleeding [6]. This is an important drawback of the protocol with AVK from the first day of admission because the prevalence of patients with PTE who need the escalation therapy (due to clinical markers of worse prognosis such as new-onset hemodynamic instability, worsening right ventricular dysfunction, or respiratory failure, or substantial myocardial necrosis), has not been low. In one study, cited in the 2014 European Society of Cardiology (ESC) guidelines, an escalation to the emergency treatment varied from 10.2% to 24.6% in PTE patients [7].

2. AVKs act *procoagulantly* for the first few days of being administered [8–10]. The worst time to expose PTE patients to the procoagulant effect of AVK, is exactly during the first days when the thrombus burden and the blood hypercoagulablility are maximal. Moreover, it is now easy to avoid the application of AVK from the first day because we have other, safer options – direct oral anticoagulants (DOACs), that do not act procoagulantly [6].

3. A later start of AVK makes the *vena cava filter* implantation less risky if indicated [2] e.g. when venous thromboembolism (VTE) had occurred despite the ongoing anticoagulation [11].

Materials and Methods

Narrative review (analyzing the important Guidelines) was used for the synthesis of the improved approach regarding the time to start AVK in PTE.

Results

The traditional beginning of the AVK treatment on admission day (as recommended by contemporary guidelines) [2, 7, 11, 12] is not imperative at all, because an AVK neither enhances thrombus degradation during the first critical days by itself nor allows the endogenous fibrinolytic system to do it. Actually, in addition to the three above-mentioned, one can list at least seven other shortcomings of the AVK commencing exactly on the day of admission.

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Additional weaknesses of the current protocol with the early AVK treatment (from the admission day)

1. When we give AVK on the first day, together with a parenteral anticoagulant, in order to avoid complications (bleeding on one hand and thrombus propagation or re-embolization on the other), *it is crucial to instantly provide the optimal anticoagulant effect of both unfractionated heparin (UFH) and AVK, which is a very difficult task.* It is not easy at all to provide a stable adequate anticoagulant effect of an AVK (e.g., warfarin), during the first days and even weeks from the introduction [12]. For example, in one study the median time to reach a therapeutic international normalized ratio (INR) was 21 days in the genotype-guided group as compared to no less than 29 days in the usual care group [12]. Difficulties in the INR targeting (requiring frequent INR measurements and dose adjustments) are the consequences of warfarin's narrow therapeutic index and drug–to-drug interactions, as well as patient's health status, hepatic metabolism, and genotype, and diet [10]. It is also difficult to promptly provide the optimal anticoagulant effect of UFH, as measured by activated partial thromboplastin time (aPTT) or anti-Xa [3, 9]. Over 300 various reagent-coagulometer combinations are currently applied in the practice. As a result, therapeutic heparin concentration (0.3–0.7 anti-Xa u/ml) corresponds to wide aPTT ratios, from 1.6–2.7 to 3.7–6.2 times control [9]. The quality of UFH control also depends in practice on the capability of the local laboratory to measure aPTT or anti-Xa frequently, quickly, and 24 hours a day. According to the recommendations, the aPTT or anti-Xa levels ought to be checked every 6 hours until two consecutive therapeutic results are obtained [9, 10], which is not feasible in all hospitals. It was very important to obtain adequate aPTT during the first days of admission, as it considerably influenced the prognosis [8]. If PTE patients do not receive at least 30,000 UFH units a day, sub-therapeutic aPTTs within the first 24–48 hours will correlate with VTE recurrence [9]. The overactivity of each, and particularly of both UFH and AVK, substantially increases the risk of bleeding, while the insufficient anticoagulant effect can result in thrombosis extension in an already highly thrombogenic situation such as PTE.

2. Starting AVK on the first day, *precludes later in-hospital switching from a parenteral anticoagulant to a DOAC,* because it has been recommended not to change one oral anticoagulant (OAC) for another [2]. Each of the four approved DOACs has evidence of a better benefit /risk ratio in comparison with AVK [1, 3, 12]. DOACs have been shown to be safer than AVK, as they decrease the risk of bleeding [1]. Moreover, DOAC is much easier and quicker to introduce: there is no need to overlap DOAC with parenteral anticoagulant; we can simply administer DOAC 0–2 hours prior to and instead of the next scheduled regular dose of the original parenteral anticoagulant (low molecular weight heparin - LMWH or fondaparinoux in the majority of patients, as suggested by contemporary guidelines) [1, 7, 9, 12]. Thus, by introducing AVK on the first day we omit the possibility to choose in the hospital probably better option for prolonged treatment – DOAC [1]. Rivaroxaban was administered following LMWH/UFH, without introducing AVK on the first day in the important study of Sharifi et al. [13] Therefore, the decision which drug to administer during the coming months or even years (AVK or DOAC), is better not to make too early on the day of admission but once PTE gets under feasible control. Most physicians treating PTE patients have preferences regarding AVK or DOAC; therefore the intention to use one or another is present already at the patient's presentation. When this preference is DOAC, the agreement of a patient for financial participation is needed in many countries. In the absence of the patient's agreement for DOAC, we ought to proceed with LMWH and AVK combination. Admittedly, it is somewhat prematurely during the first day or two of hospital admission to explain to the patient with e.g. intermediate-high risk PTE the possible therapeutic options (and elaborate about their cost-effectiveness, LMWH, and AVK vs. DOAC). It seems more adequate to discuss post-acute treatment a couple of days before discharge, when PTE is under control and when the patient's confidence is obtained.

3. If one or two INR values >2 have been achieved relatively rapidly, e.g., in 5 days (which is the minimum allowed by actual guidelines) [7, 9, 11, 12], the overlap is finished and the *period of the administration of stronger anticoagulant* (*the parenteral one*) [14] *may be too short* to help to obtain successful thrombus dissolving. An insufficiently long administration of more efficient, parenteral anticoagulant is probably one of the important reasons why (with classic PTE protocol) residual thrombosis in pulmonary arteries has been found after 6 or 12 months of follow-up in a disturbing number of patients, e.g., 50% [8, 15] or 70% [16]. This residual thrombosis in VTE may increase the probability of re-thrombosis [3, 7, 8]. Therefore, it is better to cease more efficient parenteral anticoagulant treatment when we judge that it is the right time (once when we have objective evidence of improvement of patients' findings, such as respiration rate, hemoglobin oxygen saturation, electrocardiogram - ECG, echocardiogram, computer tomography pulmonary angiography - CTPA, D-dimer, etc.) than when it happens that the patient achieves INR >2 once [5] or two times, 24 hours apart (indicating the end of the overlap) [2, 3, 7, 11].

4. Starting AVK may turn out to be *a mistake for* approximately 10% of the patients with unprovoked PTE, in whom we find *cancer later* during hospitalization (in the quick examination of all patients aged over 40 years with a first unprovoked PTE or deep venous thrombosis - DVT, searching for the cause of VTE, using e.g., basal clinical investigation, laboratory, and chest X-ray, plus an abdomino-pelvic CT scan - and a mammogram for women) [11]. Since Traussaud described the temporal re-

lation between cancer and VTE in 1865 [17], it is wise to have it in mind. This relation is named "metachronous" because each of the two may come first or they can be present simultaneously. Therefore, cancer screening is recommended [18]. It is particularly important to search for cancer in patients with "unprovoked" PTE, because as many as 10% may have a diagnosis of cancer during the first year of follow-up [19]. The reasons are numerous; cancer increases mortality rates in VTE and the other way round; cancer presence influences to a great deal the estimation of both re-thrombosis and bleeding; there are several specificities in VTE treatment in patients with cancer, etc. The optimal thoroughness of the cancer screening is a matter of long-standing debate. On the one hand, it is not sound to spend a lot of time, effort, hospital capacities, and resources as the probability of positive finding is not high; on the other hand, it is a mistake to miss cancer in a patient with VTE and jeopardize his/her prognosis, particularly because this relationship is known for >150 years. Therefore, a balance is needed. The 2019 ESC and National Institute for Health and Care Excellence (NICE) Guideline recommend careful medical history taking and a physical examination, together with baseline laboratory findings, such as complete blood count (CBC), parameters of hepatic and renal function, as well as PT and aPTT [20, 21]. Other investigations are not necessary and they can be invasive, time-consuming, costly, stressogenic, and represent a radiation risk [20]. Starting AVK on the admission day is a mistake for cancer patients with PTE because LMWH has been recommended (provided patient agrees) for 6 months or as long as the cancer is present / under treatment, without the usage of AVK at al [2, 3, 11]. The more comfortable post-acute anticoagulant treatment in VTE patients with cancer is DOAC [21], except for gastrointestinal tumors [21]. Therefore, AVKs are currently regarded insufficient for the majority of patients with VTE and cancer.

5. Keeping in mind the importance of bleeding in PTE [2], it is particularly *important to avoid or postpone procedures and drugs* (*such as AVKs*), *which increase bleeding risk, until patient's clinical state becomes far better*. For example, an eventual major bleed may further deteriorate the already bad condition and may limit the use of otherwise needed anticoagulants in the next period. Thus, eventual major hemorrhage would cause less harm if it occurs on day nine (when thrombus burden, symptoms, and risk are smaller) than on day three of hospitalization (when thrombus burden is still prominent). Therefore, it makes sense to postpone AVK from the first day in order to delay eventual bleeding in an already dangerous disease. It is important to postpone AVK particularly in numerous patients with recent bleeding or high risk of bleeding, such as listed in *Hestia criteria*, e.g., gastrointestinal (GI) bleed within 14 days, recent stroke (within 4 weeks), recent surgery (within 2 weeks), platelets <75,000/μl, uncontrolled arterial hypertension (systolic blood pressure, BP>180 mmHg, diastolic BP>110 mmHg) [3]. It is wise to postpone AVK commencement in PTE patients with a high risk of bleeding, which can be recognized by *RIETE* (Registro Informatizado de la Enfermedad Thomboembolica venosa) Bleeding Score: recent major bleed (1 month), creatinine >1.2 mg/dl, anemia, cancer, clinical presentation as PTE (vs. DVT) and age >75 years [8]. Moreover, *ACCP* (*American College of Chest Physicians*) *Score for Bleeding Risk* also suggests who is on the elevated bleeding risk: age >65 years / >75 years, previous bleed, cancer / with metastasis, renal insufficiency, liver failure, thrombocytopenia, previous stroke, diabetes mellitus, anemia, anti-platelet drugs, poorly controlled anticoagulation, comorbidity, and reduced functional capacity, recent surgery, frequent falls, and alcohol abuse [8]. According to the ESC 2014 PTE guidelines, in the absence of properly evaluated bleeding risk scores for VTE patients, high risk for hemorrhage represents old age (especially >75 years), previous GI bleeding (particularly if the cause is not reversible), any type of the previous stroke, chronic illness of kidney or liver, parallel antiplatelet treatment, other serious diseases, and poor anticoagulation control [7]. Therefore, it is logical to avoid commencing AVK on the first day(s), particularly in the following categories of PTE patients A) with the high thrombus burden, B) with the more central thrombus location, C) with the worse the patients' cardiopulmonary reserve and symptoms (suggesting a severe form of intermediate-risk PTE, and thus life-threatening situation if bleeding occurs), and D) with the higher risk of bleeding. Moreover, high bleeding risk implies that the proper choice of peroral anticoagulant for post-hospital treatment would be one of the DOACs (due to their safer profile) [1, 3, 12]. Therefore, as far as OACs are concerned, if a PTE patient has a high risk for hemorrhage, it is better to decrease it a) by introducing OAC later during the hospitalization and b) by choosing a DOAC instead of AVK.

6. By starting AVK from the beginning of the hospitalization, there is a risk for *AVK hyper-responders* that they may bleed at the worst time possible – when PTE is not under acceptable control. Postponing the overlap (e.g., UFH and AVK) may allow time for a parenteral anticoagulant to clear an important part of thrombus before eventual bleeding may compromise the effects of therapy. We may search for AVK hyper-responders by pharmacogenomic study because variations in two genes correspond to >30% of the dosing variability of warfarin. One gene determines the activity of cytochrome P2C9 that inactivates warfarin's S-enantiomer, and the other regulates the activity of vitamin K epoxide reductase, which produces the active form of vitamin K [7, 12].

7. Starting AVK on the first day exposes patients too early to bleeding risk, before the main job is mostly finished – getting the disease (PTE) under control. An additional risk is imposed by drugs, capable of inducing hemorrhage directly by themselves or indirectly, by influencing AVK. For example, *dual antiplatelet therapy* (*DAPT*) may be necessary (because of e.g., recent coronary artery stent implantation), and the early AVK

Table 1 Important arguments to postpone AVK commencement from the first day of PTE treatment

Number[a]	Reason[b]	References supporting the case
1	AVK given from the day of admission may increase the risk of bleeding in case that *thrombolysis* becomes needed due to eventual deterioration of the patient's hemodynamics.	6, 7
2	AVKs act *procoagulantly* for the first few days of being administered.	8, 9, 10
3	It also makes the *vena cava filter* implantation more risky (if an indication arises).	6, 11
4	It is *challenging to obtain adequate INR during the first week* of AVK and *under-anticoagulation increases the risk of rethrombosis* following the cease of the parenteral anticoagulant.	10, 12
5	Starting AVK on the first day, *precludes later in-hospital switching from a parenteral anticoagulant to a DOAC.*	2
6	If target INR is obtained too soon (before day 5), it may *preclude the full duration of a parenteral anticoagulant* (recommended in PTE Guidelines)	7, 9, 11, 12
7	Starting AVK may be *a mistake for* approximately 10% of the patients with unprovoked PTE, in whom we find *cancer later* during hospitalization because LMWH (and not AVK) has been recommended for cancer patients with PTE.	2, 11, 13
8	*If INR raises too much hemorrhage may occur*. Such a scenario is real and *it is better to postpone AVK in order to have the patient stabilized before starting AVK.*	2, 8
9	It is particularly true if the patient is a *hyper-responder to AVK* and the worst time for very high INR is during the first days of treatment.	7, 12
10	AVK from the day of admission overlapped with LMWH is also a premature approach for *patients with recent coronary artery stent implantation who have an indication for DAPT*, as the bleeding risk is unacceptably high.	23

Legend: AVK anti-vitamin K; INR international normalized ratio; DOAC direct oral anticoagulant; PTE pulmonary thromboembolism; LMWH low-molecular-weight-heparin; DAPT dual antiplatelet therapy.
[a] Serial number of an argument.
[b] Reason to postpone AVK commencement from the first day of PTE.

commencement (on the first day), overlapped with LMWH is not the best approach - due to safety reasons [22].

To sum up, in addition to three already published, there are seven new important arguments (making a total of ten) to postpone AVK commencement from the first day of PTE diagnosis (as recommended in all the contemporary guidelines for PTE patients who are not planned for thrombolysis) (Table 1). On the other hand, why should we introduce AVK on the day of admission? Both obvious reasons to do it are outdated. Firstly, the risk of heparin-induced thrombocytopenia (HIT) with prolonged UHF or LMWH therapy can be avoided by choosing the parenteral anticoagulant which does not induce HIT (fondaparinoux). Secondly, the potential increased cost of prolonged hospitalization due to the postponement of AVK, can be easily outbalanced by avoiding the time-consuming overlap of parenteral anticoagulant and AVK by introducing DOAC without overlap (instead of AVK with overlap), once PTE patient is stabilized. Such an approach has already been tested [13, 23].

Discussion

PTE is very important due to its high incidence, prevalence, morbidity, and mortality. Considering global mortality, PTE is the second most important cardiologic and the third most important cardiovascular disease (after myocardial infarction and stroke). Even a small improvement in the therapy of PTE may result in thousands of lives saved worldwide annually.

The review attempts to analyze if there is room for improvement of the current protocol for PTE treatment. AVK has been the standard of care in PTE therapy for decades; AVK has been recommended to start with on the day of diagnosis, together with UFH/LMWH, overlapping them for at least five days. Nevertheless, up to half of PTE patients who survived a year following hospitalization, have in pulmonary artery/arteries residual thrombosis; this worsens the symptoms and prognosis and proves that the before-mentioned standard PTE protocol is far from being optimal. On the other hand, all historical reasons to introduce AVK from the first day (e.g., 1. risk of HIT with prolonged UHF or LMWH therapy, or 2. increased cost of prolonged hospitalization) can be easily solved by contemporary evidence-based treatment (e.g., 1. by choosing the parenteral anticoagulant which does not induce HIT and 2. by avoiding the time-consuming overlap of parenteral anticoagulant and AVK in this way that we simply introduce DOAC without overlap, once when a PTE patient is stabilized).

Aiming to obviate the complications (bleeding on one hand and thrombus propagation or re-embolization on the other), it is crucial to instantly provide an optimal anticoagulant effect of both UFH and AVK, which is a very difficult task. For intermediate-risk, PTE patients who obtain INR >2 in e.g., five days, the administration of parenteral anticoagulant (which is evidence-based stronger than AVK) may be too short for the efficient thrombus removal and starting AVK early would not allow the individualization of stronger parenteral anticoagulation. Starting AVK may turn out to be a mistake for approximately 10% of the patients with unprovoked PTE in whom we find cancer later during hospitalization. Furthermore, we identified numerous PTE patients who may benefit from postponing AVK from the first day of treatment, for example, patients at high bleeding risk.

Several benefits may be expected for PTE patients: avoiding the procoagulant effect of AVK during the first critical days of the hospitalization, the decrease of the bleeding risk of concomitant therapy (overlap) using difficult-to-control anticoagulant AVK, particularly with another anticoagulant with low bioavailability and predictability of effect (UFH). Furthermore, eventual escalation of therapy (due to hemodynamic compromise, absent at admission, that appeared later during the course of PTE, and required fibrinolysis) would result in less bleeding if the patient is without AVK.

Thus, a patient with a previously unrecognized cancer may get a chance to avoid unnecessary AVK therapy. Additionally, all PTE patients with high bleeding risk and this is a large group, will benefit from the later introduction of OAC (preferably DOAC) because the overlap, i.e., administration of two anticoagulants (parenteral one and AVK) is risky, particularly due to the difficulties to obtain the target INR range for AVK. Thus, it is better to start OAC when the thrombus burden is diminished and the patient is out of a life- threatening situation. It is because should a major bleed eventually occur, we might be forced to temporarily withdraw the anticoagulant; indeed, to temporarily cease the anticoagulant, would result in less harm when thrombosis is under control and critical days for the patient are gone.

Final Remarks

From the aforementioned, the following recommendation can be stated: *Starting LMWH as the only anticoagulant in intermediate-risk PTE patients* has several advantages. One of them is the possibility to individualize treatment in terms of A) *leaving all therapeutic options open*, i.e. being prepared for an escalation of treatment or to –to the contrary- to decrease the intensity and proceed to peroral anticoagulation; and B) tailoring *duration* of LMWH administration according to the improvements in patient's clinical picture (symptoms and signs), as well as in oxygen saturation (or arterial blood gasses), ECG, echocardiography, D dimer, etc. This watchful waiting and re-examination of a need to thrombolyse can help us avoid unnecessarily thrombolysis (on the one hand, with its imminent bleeding risk) and enable full preparedness to escalate treatment (including rescue thrombolysis – if needed, on the other hand). The second advantage of LMWH (as the only anticoagulant treatment from the hospital admission – without AVK) is an *easy and quick transition to DOAC* as soon as aforementioned numerous PTE parameters indicate that the risk of sudden hemodynamic worsening/compromise is over (it became very low). No overlap is needed for LMWH and DOAC (as it is for LMWH and AVK), which may substantially reduce hospital stay and costs. The most recent guideline on PTE recommends direct oral anticoagulants (DOACs) over AVK; therefore, the smooth transition from LMWH to DOAC is warranted [24]. An argument for DOAC is about 30% lower risk of major bleeding (as compared to AVK) [24]. The third advantage of "LMWH only" approach to intermediate-risk PTE patients is the *avoiding premature AVK inclusion, which may almost preclude later switch to DOAC* in case of newly diagnosed cancer. The fourth advantage of "LMWH only" pathway is the *avoiding of premature DOAC initiation* in case of e.g. triple-positive antiphospholipid syndrome (APS) [25], because it will be necessary to switch to AVK later on (and any switch from one oral anticoagulant to the other has to be avoided); this 4th advantage can be obtained by providing enough time to diagnose APS. Therefore, "LMWH only" in intermediate-risk PTE patients is a valid option, with numerous advantages over the early introduction of AVK (on the first or second day); it enables us to avoid the premature introduction of each of the following drugs: thrombolytic, AVK, and DOAC.

The other subset of PTE patients (low-risk subset) inclines to the home-treatment or toward an early discharge and the majority of such patients are candidates for a DOAC, preferably one that does not require prior parenteral anticoagulation.

Conclusion

The prevalence of patients with PTE and at least one of ten listed reasons to postpone AVK is high – the majority of PTE patients are concerned. A better time to start OAC (either AVK or -preferably- DOAC) is when PTE comes under control, and the need for the escalation fibirnolytic treatment is gone (i.e. when clinical condition, ECG, echocardiogram and eventually CTPA findings become correct). Moreover, we have got nothing to lose, because even the hospital stay will become shorter due to the lack of days needed to overlap if we proceed from UFH or LMWH/fondaparinoux (and eventual escalation treatment – secondary fibrinolysis if necessary) to DOAC.

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Review Article

HEAD AND NECK CARCINOMA STEM CELLS. DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC TARGETS

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Abstract. *Head and neck carcinoma arise from the mucosal lining of the upper aerodigestive tract, affecting more than half a million people worldwide each year. Although cancer tissue has many heterogeneous cells with different phenotypes, only a subset of these cancer cells proliferate extensively and have the potential to give rise to all other tumor cells. They are named carcinoma stem cells (CSC). CSCs can persist in tumors and cause relapse and metastasis by producing a new tumor. Head and neck carcinoma stem cells (HNCSC) share a common CD44+ phenotype. What drives a normal stem cell into a malignant stem cell is insufficiently understood. Many regulating pathways were analyzed, such as:Receptor Tyrosine Kinases, Sonic Hedgehog, Notch, Wnt, and Bmi1. Also, the miRNA ratio or epigenetic alteration pattern is potential subject of further studies. This would enable better therapy and survival rate in HNSCC.*

Key words: *head and neck cancer, cancer stem cell, epigenetics, prognostic factor, therapy target.*

Introduction

Head and neck squamous cell carcinomas (HNSCC) represent biologically similar cancers originating from the mucosal surface of the upper aerodigestive tract, such as lip, oral cavity, nasal cavity, paranasal sinuses, pharynx, and larynx. Major risk factors are smoking and alcohol abuse. More than half a million people worldwide each year are affected [1].

Treatment of HNSCC includes surgery, radiotherapy, chemotherapy, and targeted agents. Advances in treatment strategies have improved quality of life and survival rates. A better understanding of the biology of HNSCC is needed for more effective therapies and better results. The introduction of the term "stem cell model of cancer" fromr recent studies should enable a better understanding of tumor pathogenesis [2].

Until recently, cancer was considered as a group of heterogeneous cells with different phenotypes, and the general possibility of extensive proliferation. The new model of cancer proposes that only a subset of these cancer cells can proliferate extensively. These cells are named cancer stem cells (CSC). Other cancer cells, known as “non - stem” cells, have only a limited proliferative potential. CSCs may form new tumors on transplantation, and may even persist in tumors and cause relapse and metastasis by giving rise to a new tumor [3].

The analogies between CSCs and “normal” stem cells were confirmed. Extensive proliferative potential and the ability to give rise to new (normal or abnormal) tissues are characteristic of both types of cells [3]. Both tumors and normal tissues have heterogeneous combinations of cells, different phenotypic characteristics, and different proliferative potentials. Most tumors have a clonal origin, so phenotypically diverse progeny is formed. Self-renewal and differentiation of normal stem cells are characteristics of CSCs [3].

Current therapy of HNSCC is aimed to decrease the size of the tumor, which is effective for a limited amount of time. However, CSCs are less sensitive to the current chemotherapy agents, and they remain virtually untouched. So, further research is directed to targeting mainly the CSC population within the tumor mass, which prevents tumor growth and eventually cures the patient.

Review of Literature

Evidence for HNCSCs

Extensive proliferation of a small subset of tumor cells was confirmed in multiple myeloma and leukemia. Mouse myeloma cells from mouse ascites formed colonies in only 1 in 10,000 up to 1 in 100 cancer cells [4]. Also, only a small proportion of human AML stem cells can transfer AML from human patients to immunocompromised nonobese diabetic or severe combined immunodeficient (NOD/SCID) mice [5].

Identification and confirmation of proliferation and differentiation abilities of CSC were recently investigated [6]. Implantation of specimens obtained from patients undergoing surgical resection under the skin of NOD/SCID mice produced mouse xenograft models of HNSCC. Also, $CD44^+$ cells could form tumors, contrary to $CD44^-$ cells. Only a small number of $CD44^+Lin^-$ cells from a patient’s tumor produced new tumors that were phenotypically diverse for CD44 expression. Additionally, $CD44^+$ expression was confirmed in the basal layer and not in the well- differentiated cells in moderately to well- differentiated HNSCC. These tumors had cytologi-

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cal and architectural features similar to normal squamous epithelium, with differentiation from a basal layer toward an apical layer, and the formation of keratin.

HNCSC Markers

Antigenic markers helped to isolate CSCs in various solid tumors, as demonstrated in various studies. For example, Human Brain Tumor Stem Cells have been confirmed using expression of the cell surface marker CD133 [8], while Prostate Cancer Stem Cells have a $CD44^+/A2B1^{hi}/CD133^+$ phenotype [9], and Pancreatic Stem Cells a $CD44^+CD24^+ESA^+$ phenotype [10]. Cells with a specific cell-surface antigen profile (CD44-positive and CD24-negative) from patients with advanced metastatic breast cancer could become tumor xenografts [11]. Also, the experiments with immunodeficient mice and transplantation of cells under the skin provided an environment similar to that in HNSCC.

The purified CD44 positive cells could differentiate into cells similar to those found in the bulk tumor population [6]. Aldehyde Dehydrogenase (ALDH) expression as a potential functional marker for stem cells and CSCs was confirmed. The majority of HNSCC cells had low ALDH activity [12]. A majority of highly tumorigenic $ALDH^{high}$ cells overlapped with the CD44+ population of cells, and only a small number of CD44+ cells expressed a high ALDH activity. It has been suggested that CD44+ cells contain a mixture of CSCs and non CSCs because some 5000 cells are needed to produce a tumor. So, probably the best way to identify CSCs in HNSCC is a $CD44^+/ALDH^{high}$ phenotype.

Side Population (SP) cell sorting represents another common method of identifying CSC. SP cells efflux Hoechst dye 33342 and contains differentiation and proliferation abilities of CSCs [13]. SP cells in a human laryngeal cancer cell line are consistent with cancer stem or stem-like cells [14]. Also, SP cells had higher proliferation rates, than those of non-SP cells. However, proliferating SP cells give rise to both SP and non-SP cells. High proliferation rates, differentiation capacity, serum-free growth, and sphere formation in vitro imply that SP cells have CSC characteristics [14].

Genetic and Epigenetic Alterations in HNSCSs

Cancer is a consequence of genetic and epigenetic alterations of normal tissue. Also, normal stem cells renew under the control of several signaling pathways. Mutation could lead to the unlimited and uncontrolled proliferative potential of cells. Wnt, Sonic Hedgehog, Notch, PTEN, Bmi1, and EGFR are identified pathways and genes for the proliferation of HNSCC.

Increased production of growth factors, overexpression of growth factor receptors on the cell membrane, and mutations in the receptor can produce abnormal cell signaling via the Receptor Tyrosine Kinases (RTK) causing proliferation, block of apoptosis, angiogenesis, and metastasis [15, 16].

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane receptor consisting of a family of 4 members: EGFR, HER2, HER3, and HER4. EGFR leads to intracellular phosphorylation and exposure of the catalytic cleft, activating different signaling pathways. EGFR is up-regulated in more than 90% of HNSCCs [17]. It is postulated that over-expression rather than mutation promotes HNSCC [19–21].

The Hedgehog signaling pathway is essential for the regulation of proliferation and differentiation of various types of stem cells during embryogenesis. There is an association between Sonic Hedgehog (SHH) and carcinogenesis [22, 23]. SHH overexpression was found in different malignant tumors, such as small cell lung carcinoma [24], medulloblastoma [25], and basal cell carcinoma [26]. Overexpression of SHH signaling was found in HNSCC after concurrent chemo radiation, just before the rise in tumor proliferation rates [27]. This further supports CSC model, with the remaining chemo radio resistant portion after the bulk of the tumor mass has been destroyed.

The Notch signaling pathway has four Notch receptors (Notch1–Notch4) and five structurally similar Notch ligands (Delta-like1, Delta-like3, Delta-like4, Jagged1, and Jagged2). Activation of the Notch pathway results in self-renewal of stem cells or differentiation along a particular lineage [28, 29].

The canonical Wnt signaling is initiated by engaging of Wnt ligand with its Frizzled receptor along with the LDL receptor family member, Lrp 5/6, causing the accumulation of β-catenin activating target genes. A mutation of the Wnt pathway is considered a cause of colorectal cancer, leukemia, and HNSCC [30, 31].

BMI1 or B-cell-specific Moloney murine leukemia virus insertion site 1 controls the cell cycle and self-renewal of tissue stem cells [32]. BMI1 influences the proliferation of many normal human stem cells, but is overexpressed in different malignancies, including HNSCC [33].

These pathways are related to each other. For example, secreted Shh influences the cell fate switch executed by Notch [34]. Hedgehog and Notch regulate normal development with a feedback loop, with deregulation leading to cancer. It is not clear whether the Hedgehog pathway and the Notch pathway can regulate stem cell self-renewal through downstream targets other than Bmi-1. This model could help understand regulation normal and malignant stem cell self-renewal [35].

Cancerogenesis is nowadays not exclusively connected to the alterations in pathways and genetic material. Indeed, many recent papers indicate that mechanisms other than changes in the DNA sequence can lead to malignant processes. Epigenetic alterations include DNA methylation, histone modifications, and nucleosome positioning. Global hypomethylation, specific CpG hypermethylation, overall miRNA downregulation, a global reduction of monoacetylated H4K16, global loss of active mark H3K4me3, silencing of tumor suppressors BRG1 and BRM by hypermethylation is the most fre-

quent types of epigenetic alterations [36]. In HNSCC it includes promoter hypermethylation of genes $p16^{INK4a}$, DAPK and MGMT [37].

Diagnostic and Prognostic Potential of HNCSCs

The improvement of the survival rate of HNSCC is slower than for other common carcinomas. Also, many patients with HNSCC present with locally advanced, stage III or IV disease that requires different therapy modality is mandatory for improvement of the results.

MicroRNAs (mina) are small, non-coding RNAs that can regulate gene expression and seem very important for the prognosis of HNSCC. They downregulate many of their target transcripts and the amount of protein encoded by these transcripts [38]. Few cancer-specific miRNA fingerprints have been identified in different types of cancer.

An abnormal expression of miRNA was confirmed in cancer cells, and also in premalignant stages. The examples are the reduced expression of miR-143 and miR-145 in colon adenomas [39] and reduced expression of miR-16-1 and miR-15s in pituitary adenomas [40]. Also, miR-221, highly overexpressed in, is also overexpressed in papillary thyroid tumors and in normal thyroid tissue adjacent to tumors, but not in normal thyroid tissue [41]. Clinical implications of such overexpression are significant for disease control. Indeed, the difference of miRNA expression in HNSCC tumor tissue compared with normal head and neck epithelia was found. Also, miRNA-21, miRNA-18a, miRNA-221 and miRNA-375 were differentially expressed in HNSCC. The ratio of miRNA-221:miRNA-375 had the strongest predictive ability to distinguish tumor from normal tissue with both high sensitivity (0, 92) and specificity (0, 93). This expression ratio can be applied to determine the potential of malignant alteration in precancerous lesions, or for screening for HNSCC in saliva or mouthwash [42].

A potential marker for prognosis and also a predictor of treatment response could be the different level of DNA methylation. DNA hypermethylation can be documented in the saliva of patients with HNSCC. Studies confirmed aberrant promoter DNA hypermethylation (p16, MGMT, and DAPK genes) in 56% of head and neck primary tumors, contrary to the control group. So, it seems possible to determine abnormal promoter hypermethylation in saliva DNA in HNSCC with earlier detection and better results of treatment of cancer [43].

Therapeutic targets of HNCSCs

The cancer stem cell theory implies that the failure in the treatment of HNSCC by chemoradiotherapy is a consequence of remaining small groups of cancer cells with stem potential, present after the removal of the bulk of the cancer tissue.

In the future more effective therapy modalities have to be found and implemented into clinical praxis; therapy that target cells with high differential and proliferative potential. Another area of interest is the microenvironment of CSCs. Their surrounding niches, extracellular matrix, and soluble factors are critical for the maintenance of cell steaminess. Also, CSC were found in the proximity of blood vessels [44], as well as in perinecrotic hypoxic microenvironment [45]. This further implies that the therapeutic target could be directed not only to cells, but also to the perivascular hypoxic environment, or the CSC niche.

CSCs resistance to chemo and radiotherapy is of utmost importance. It was confirmed that after irradiation or chemotherapy CSCs showed an up-regulation of specific “markers”. The quiescence of CSCs, high expression of ABC drug pumps, enhanced resistance to oxidative DNA damage, and other factors could lead to the negative response to this treatment [46, 47].

The epithelial-mesenchymal transformation (EMT) could also cause cancer progression. As a consequence, epithelial cells` acquire traits typical for mesenchymal cells; cell-cell junctions dissociate and gain the ability to migrate. This process is essential for the invasion, progression, and metastasis of HNSCC. The interruption of EMT is a potential aim of treatment [48].

Elimination of CSCs through the targeting of specific markers is another important therapeutic modality. Possible targets in studies were: Wnt and Frizzled receptors, Sonic Hedgehog and Notch signaling. Also, the effects of antibodies to the extracellular domains of Wnt-1 and Wnt-10b were studied in HNSCC showed tumor growth, apoptosis, or elimination of tumor cells by complement, or antibody dependent cellular toxicity [49]. Increased chemo radioresistance of CSCs may be solved by targeting SHH pathway. One option is the administration of blocking antibody against SHH or PTCH-1, while application of SMOH inhibitors (cyclopamine), and the knockdown of GLI-1/GLI-2 with specific small interference RNA [50–52].

Further studies are mandatory to document the need to investigate the effectiveness of targeted therapy and clinical efficiency. The applicability of epigenetic therapy to solid tumors is yet to be confirmed [53].

Conclusion

The cancer stem cell model has proposed a new perspective in the process of cancerogenesis in HNSCC. It states that only some tumor cells have the ability to differentiate and proliferate extensively. Such cells can be confirmed the expression of surface molecules, such as CD44 in HNSCC, and by filtration of CSCs is Side Population cell sorting and ALDH activity. The transformation of a normal stem cell into malignant stem cell is a poorly understood mechanism. The potential regulating pathways include Receptor Tyrosine Kinases, Sonic Hedgehog, Notch, Wnt, and Bmi1. Epigenetic alterations, such as promoter hypermethylation of genes $p16^{INK4a}$, DAPK, and MGMT, are also an important factor. Better knowledge of these facts offers clinical implementation, and potential improval of the survival rate of HNSCC.

The microRNA expression ratio miRNA-221:miRNA-375, or the promoter hypermethylation pattern of p16, MGMT, and DAPK genes could enable the early detection from saliva or mouthwash samples. Potential therapeutic targets and blockage of a specific signaling pathway demand further investigation.

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Corrigendum

Editorial
Ljiljana Šaranac
TWO CENTURIES AFTER DISCOVERY OF SELENIUM, STILL BETWEEN MEDICAL USE AND MISUSE IN CLINICAL PRACTICE
FACTA UNIVERSITATIS, Series Medicine and Biology Vol. 22, No 2, 2021, pp. i-iv,
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The Editor-in-Chief has been informed that the Editorial 'Two Centuries after Discovery Of Selenium, Still Between Medical Use and Misuse in Clinical Practice', FACTA UNIVERSITATIS, Series Medicine and Biology Vol. 22, No 1, 2021, pp. i-iv, doi: http://doi.org/10.22190/FUMB210210001S, has been omitted in prinded version of the journal. The Editor-in-Chief has decided to publish a corrigendum for this editorial.

Link to the online version of editorial: http://doi.org/10.22190/FUMB210210001S

Key words: *corrigendum, editorial, selenium.*

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