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ММР

Original article

SUCCESSFUL APPLICATION OF ENDOVENOUS RADIOFREQUENCY ABLATION IN TREATMENT OF VARICOSE VEINS

УСПЕШНА ПРИМЕНА НА ЕНДОВЕНОЗНАТА РАДИОФРЕКФЕНТНА АБЛАЦИЈА ВО ТРЕТМАНОТ НА ВАРИКОЗНИ ВЕНИ

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Abstract

Introduction. Varicose veins in the lower extremities involve the great and small saphenous veins and their tributaries between the fascia and the skin. Varicose veins are characterized by subcutaneous dilated, tortuous veins greater than or equal to three millimeters, involving the saphenous veins with reflux of blood, saphenous tributaries, or non-saphenous superficial leg veins with age and family history considered important risk factors. For several reasons, including cosmetic complaints and complications with thrombosis formation, varicose veins should be treated.
Methods. This study included 1053 patients treated for varicose veins and varicosites of the lower extremities in the period between November 2009 and November 2023. Endovenous minimal-invasive approach were used along with endovenous laser ablation (EVLA) and radiofrequency ablation (RFA).
Results. Of the total number of treated patients recurrence appeared in about 3% (31 cases): in 18 cases caused by presence of accessory VSM, in 7 cases because of insufficiency and treatment of all 4 venous junctions and in 6 cases due to large dimensions of VSM (>2.1 cm).

Keywords: varicose veins, saphenous veins, reflux

Апстракт

Вовед. Варикозните вени на долните екстремитети ги вклучуваат големата и малата сафенска вена како и нивните притоки помеѓу фасцијата и кожата. Варикозните вени се карактеризирани со субкутано дилатирани, тортуозни вени еднакви или поголеми од 3мм, вклучително сафенските вени со рефлукс на крв, нивните притоки или не-сафенски површински вени на нозете како возраст и семејна историја се важни ризик фактори. Поради козметички аспект и компликации со венска тромбоза, варикозните вени треба да се третираат.
Методи. Во студијата се анализирани 1053 пациенти третирани поради варикозни вени и варикозитети на долните екстремитети во пердиодот од ноември 2009г. до ноември 2023г. Се користеше ендовенозен, минимално-инвазивен пристап со користење на ендовенозна ласерска аблација (EVLA) и ендовенозна радиофреквентна аблација (РФА).
Резултати. Од вкупната бројка на пациенти кои беа третирани рецидиви се појавиле кај 3% (31 случаи) и тоа: 18 случаи поради присуство на акцесорна ВСМ, кај 7 лица поради постоење на инсуфуциенција на сите 4 венски устија и можност за постоење недетектирани извори на рефлукс како и 6 лица поради неуспешност на РФА во третманот поради поголеми димензии на вената >2.1cm.

Клучни зборови: варикозни вени, сафенски вени, рефлукс.

Introduction

Varicose veins are abnormal, enlarged and tortuous vessels in the legs and feet. Varicose veins or enlarged veins occur when the blood vessels just under the skin become larger, crooked, bulging, increasingly visible and blue or dark purple in color.
Approximately 40% of the population faces this problem at some point in their lives, and it is much more common among women.

Anatomy

The venous system of the lower extremities consists of superficial, deep, perforating and communicating veins.
The superficial veins of the lower extremity originate in the subcutaneous tissue. There are two main superficial veins-the great saphenous vein and the small saphenous vein.
The great saphenous vein is formed by the dorsal venous arch of the foot and the dorsal veins of the big toe. It travels up the medial side of the leg, passing in

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front of the medial malleolus of the ankle and moving to the medial condyle of the knee. As the vein moves up the leg, it receives tributaries from other small superficial veins. The great saphenous vein ends by draining into the femoral vein below the inguinal ligament. The small saphenous vein is formed by the dorsal venous arch of the foot and the dorsal vein of the little toe. It moves up the back of the leg, passing behind the lateral malleolus, along the lateral border of the calcaneal tendon. At the level of the knee, the lesser saphenous vein passes between the two heads of the gastrocnemius muscle and drains into the popliteal vein in the popliteal fossa.
The anatomy of the superficial venous system in patients with valvular insufficiency shows a number of variations such as a high extrafascial origin, the existence of one or more accessory veins, and perforating insufficiency of one or more perforating communications. They are Cockett's perforator (distal, middle and proximal), Boyd's and Dodd's perforator.

Physiology

The role of the venous system is to collect deoxygenated blood from the tissues and drain it to the right atrium.
Several factors affect the proper function of this system: venous valves, the muscle pump bellow the knee, hydrostatic venous pressure, respiratory modulated intrathoracic pressure, cardiac factors.
Varicose veins of the lower extremities occur as a result of chronic valvular weakness of the superficial venous system. Dysfunction or incompetence of the valvular apparatus results in pathological retrograde flow, the so-called reflux.
When reflux occurs, there is an increase in the pressure gradient and a retention of blood distally, as a result of which the veins expand, they become tortuous, and with the prolonged existence of this condition, inflammatory changes in the venous blood vessels appear.

Risk factors

There are numerous risk factors for the appearance of varicose veins including: genetic factors, gender, age, profession, increased body weight, increased intra-abdominal pressure (pregnancy), physical activity/inactivity, hormonal status, cigarette smoking, infections, pelvic trauma, and others.

Signs and symptoms

Most often, the first signs and symptoms appear in the third and fourth decade of life. These are: heaviness in the legs, fatigue, pain and cramps in the legs, redness, swelling that indicates inflammation-thrombophlebitis, change in the color and quality of the skin, reduced hairiness of the skin over varicose veins, bleeding from varicosities, venous ulcers, wounds at the site of existing varicosities.

Materials and methods

The diagnostic algorithm in this study consisted of the past medical history, physical examination and functional tests.
Physical examination included standing inspection, palpation of changes, and auscultation if needed.
Functional tests served to assess the flow rates and the degree of venous reflux.
There are several tests for examination of the functional condition of the veins of the legs, which today have lost their practical significance and are very rarely performed. The following functional tests are used: plethysmography for routine clinical examination and a basis for recommending adequate treatment. (measurement of the regularity of blood flow in the examined limb by comparing it with the results obtained during the examination of a healthy limb), phlebography (x-ray imaging of varicosities with contrast injection), radioisotope scintigraphy, computerized angiography and magnetic angiography that are rarely indicated.

The gold standard in the diagnosis of varicose veins is color duplex Doppler ultrasonography (CDD)

It is performed in a standing or lying position (reverse Trendelenburg). Both positions enable an increase in hydrostatic pressure even in patients with incompetent valves, and the veins dilate and become more clearly visible.
The examination begins with showing the common femoral vein in the inguinal fossa, then the saphenofemoral junction is assessed; the great saphenous vein is examined distally, i.e., to the foot. The popliteal vein in the popliteal fossa with the opening of the lesser saphenous vein is also visualized.
The examination techniques are: proximal compression and the Valsalva maneuver to determine the existence of reflux which speaks of valvular incompetence. Reflux longer than 0.5 seconds measured in a standing position is considered pathological. The compressibility of venous blood vessels, flow spontaneity, respiretory modulation as well as distal compression are also assessed to determine the degree of flow changes that confirms or excludes the existence of venous blood vessel obstruction due to a thrombotic process. During the examination, the deep veins are also examined in order to determine their condition. The testing is safe for patients, can be repeated several times and has a high percentage of specificity and sensitivity.
Because of the wide variety in the definition of chronic venous disorders, the CEAP (Clinical-Etiology-Ana-

Table 1. Guide to CEAP classification

CEAP Classification System and Reporting Standard Revision 2020

C (Clinical Manifestations), **E** (Etiology), **A** (Anatomic Distribution), **P** (Pathophysiology)

C0	No visible or palpable signs of venous disease
C1	Telangiectasias or reticular veins
C2	Varicose veins
C2r	Recurrent varicose veins
C3	Edema
C4	Changes in skin and subcutaneous tissue secondary to chronic venous disease
C4a	Pigmentation or eczema
C4b	Lipodermatosclerosis or atrophie blanche
C4c	Corona phlebectatica
C5	Healed
C6	Active venous ulcer
C6r	Recurrent active venous ulcer

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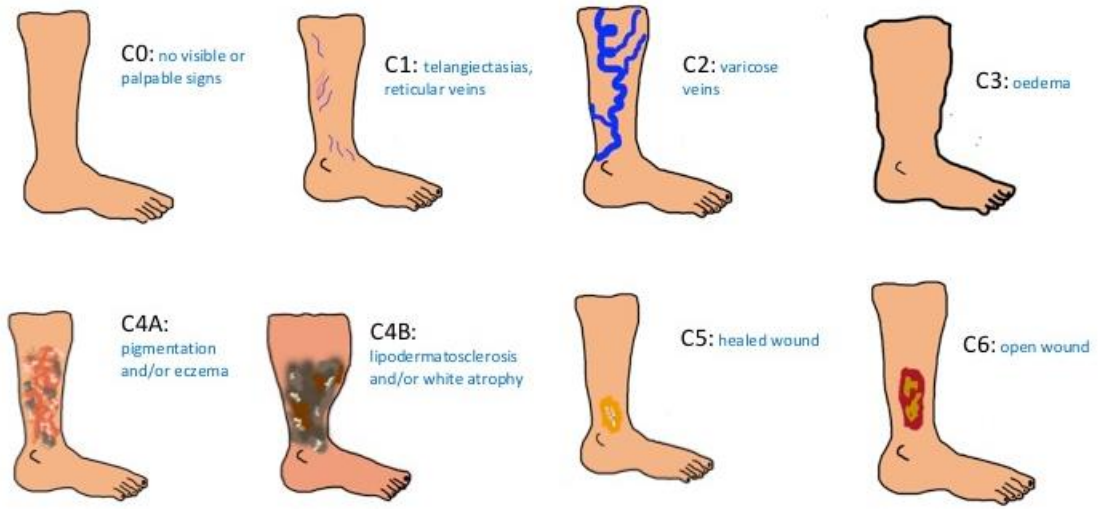

Fig 1. CEAP classification

tomy-Pathophysiology) classification (Figure 1) is widely accepted and serves as a systematic guide (Table 1).

The therapeutic approach to superficial venous insufficiency is conservative and surgical.

Conservative treatment involves treatment with pharmacological therapy and compression therapy with elastic bandages and stockings.

Surgical methods provide a functional solution to varicose veins, and the following surgical techniques are mainly used:

- surgical vein stripping,
- treatments with sclerosing of varicosities,
- endovenous access with laser treatment (EVLT) and radiofrequency ablation (RFA). Both techniques rely on the same principle of working except they a different source of energy.

When there are indications for classical vein stripping (which is rare), the vein above the medial malleolus is prepped and in the area of the saphenous-femoral junction where VSM is ligated, a probe (wire) is inserted, which is pulled down and the vein is extracted i.e., stripped. Those interventions are done under spinal and general anesthesia.

The vein sclerosing procedure can be performed with or without local anesthesia. Color duplex ultrasonographic monitoring is required to perform the sclerosing. Sclerosis is done with aethoxysklerol or by using foam. The foam causes an inflammatory reaction in the vein wall blocking the vein. Then the limb is bandaged. More than one vein can be treated. The goal of ultrasound-guided foam sclerosing to treat varicose veins is to damage the endothelial layer of the vein or varicosity causing scarring and occlusion of the treated vein.

Today, endovenous treatment is the method of choice in the treatment of varicose veins of the lower extremities. Indications are: existence of reflux longer than 0.5 seconds and subjective feeling of fatigue and contraindications are congenital or acquired valvular weakness of the deep venous system.

When performing the radiofrequency ablation, the patient is in a standing position and the site for endovenous access is assessed using an ultrasonographic examination.

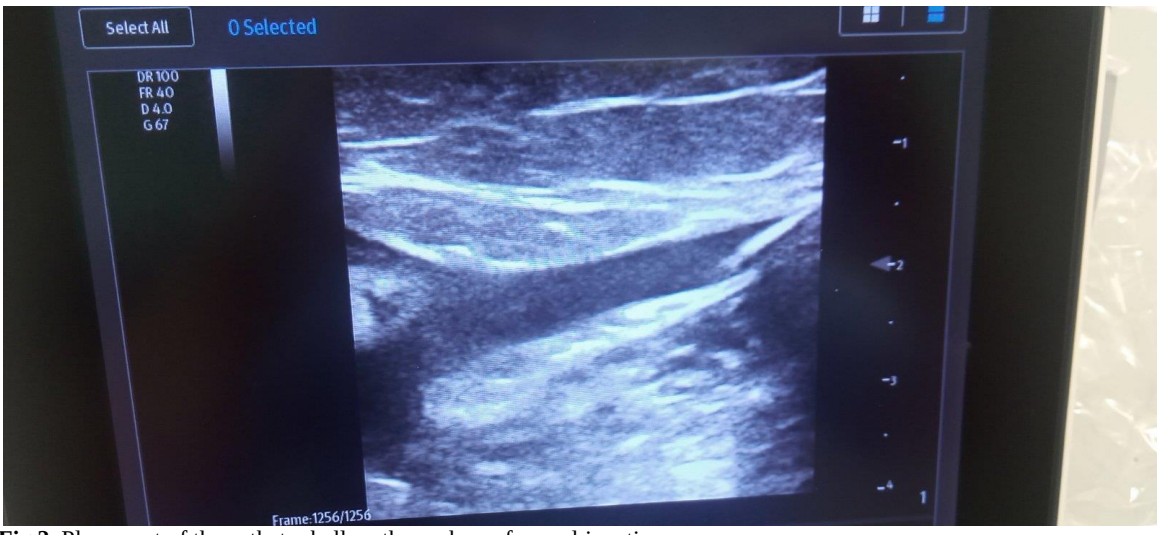

Fig 2. Placement of the catheter bellow the sapheno-femoral junction

Then, the distal varicosities are marked with a permanent marker. The patient is placed in a supine position and a sterile preparation of the extremity is made. Preparation for endovenous percutaneous placement of the catheter is made.

Using the Seldinger technique with a 21G needle and guide, a vein is prepared at a premarked position (predetermined under ultrasound) to provide access for catheterization. After placing the catheter, which is guided by ultrasonography along the vein, 1-2 cm is placed under the proximal valve of the saphenofemoral mouth for *v. saphena magna* (Figure 2) or, the saphenous-popliteal opening for *v. saphena parva*.

Tumescent anesthesia is then applied. 500 ml of Saline 0.9% is used in combination with 2% lidocaine (17.5 ml) + 5 ml bicarbonates + 0.5 ml epinephrine.

The application is under ultrasonographic control along the entire vein

from the SFJ to the insertion of the catheter in the saphenous compartment between the superficial and deep fascia (Figure 3). It acts as a local anesthetic and protects the surrounding tissues from heat damage. After applying the anesthesia, the vein is detached from the surrounding tissue and "floats" in the anesthetic. This phenomenon is known as Egyptian eye (Figure 4).

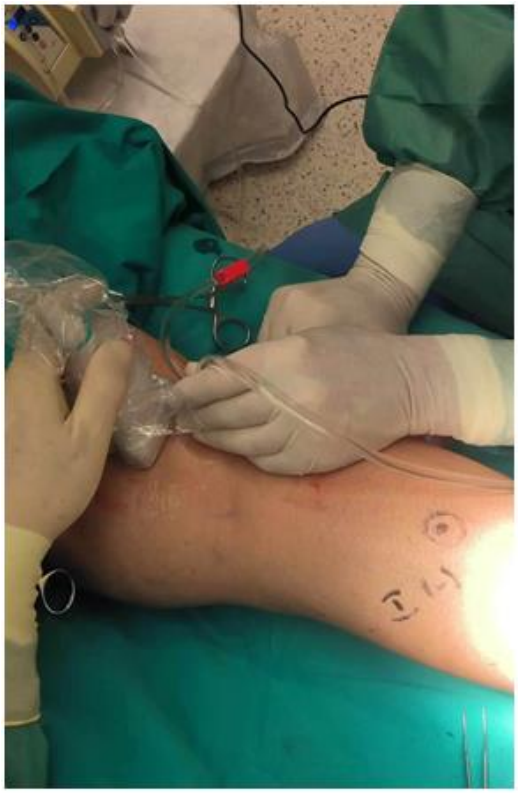

Fig 3. Applying of local tumescent aneshtesia

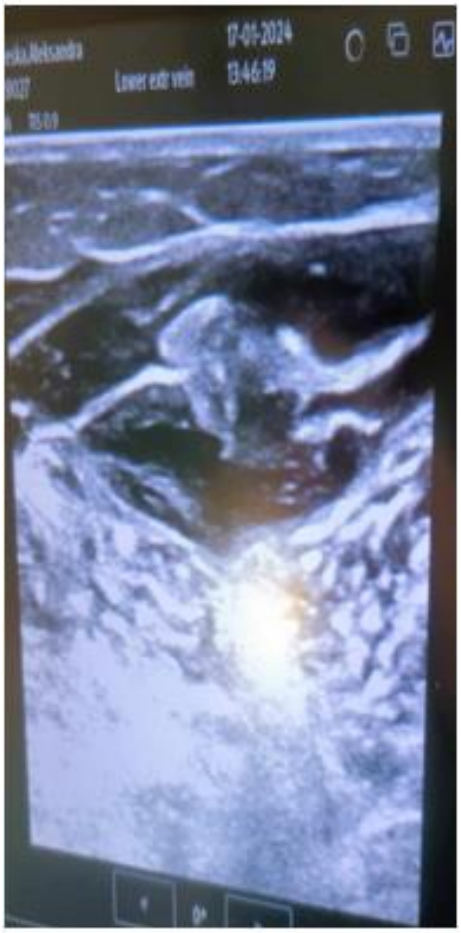

Fig 4. The vein is separated from the surroundings

The patient is placed in Trendelenburg position and exsanguination of the vein is performed with external pressure to ensure better contact between the vein wall and the electrode tip of the catheter. Then, the VNUS closure device is turned on and the vein is obliterated in several stages by pulling the catheter back (Figure 5).

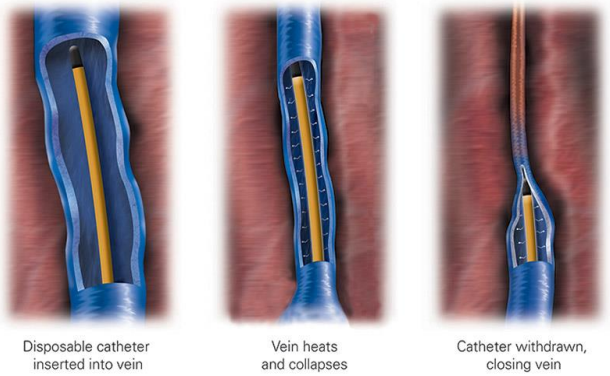

Fig. 5. Obliterating the vein with heating of the inner wall

After the endovenous treatment, the previously marked distal varicosities (Figure 7) are removed with performing a local phlebectomy and avulsion in local anesthesia (Figure 8).

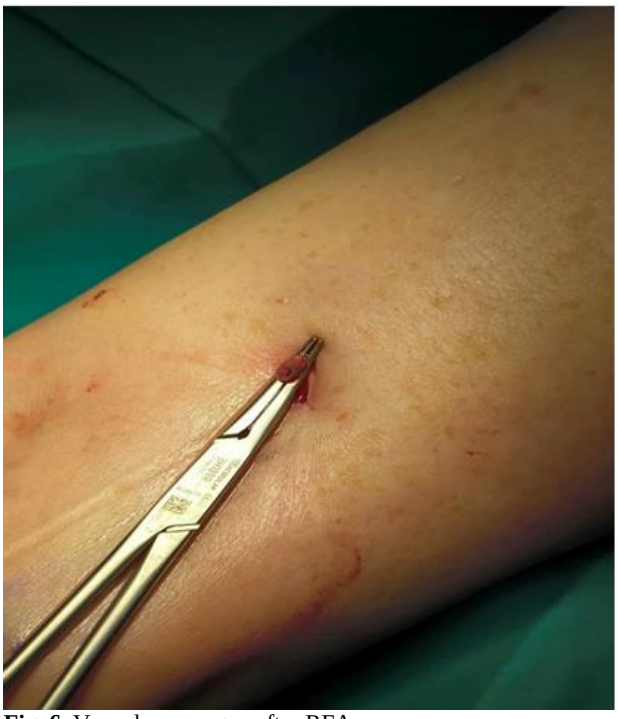

Fig. 6. V. saphena magna after RFA

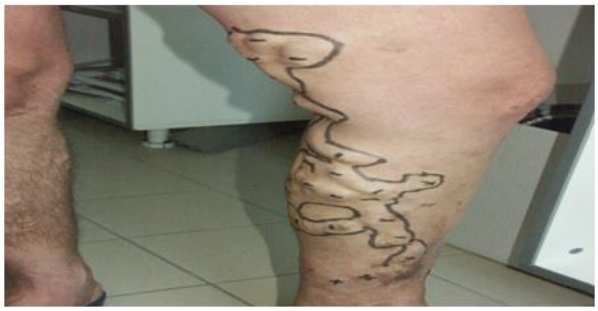

Fig. 7. Pre-operative marking of distal varices

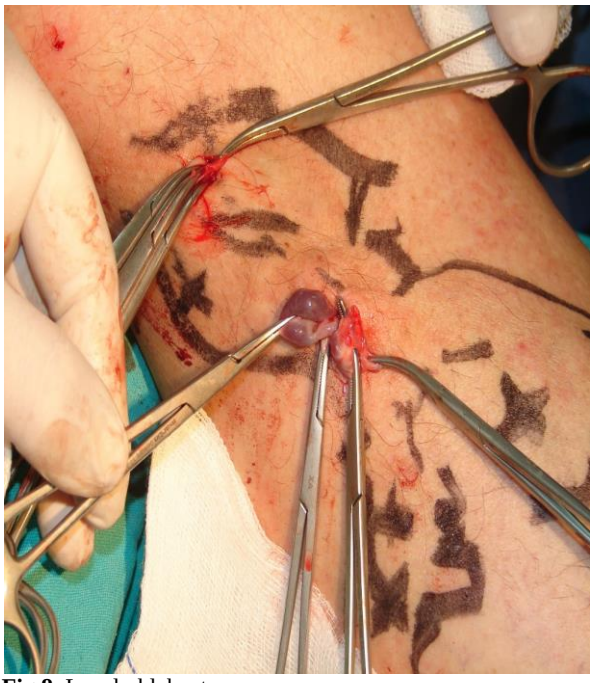

Fig 8. Local phlebectomy

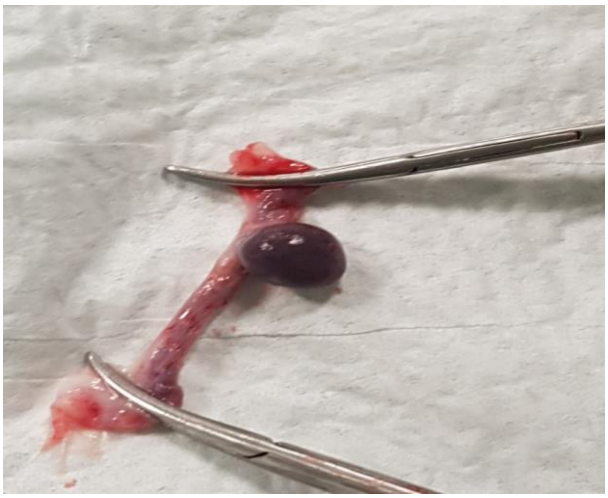

Fig 9. Aneurismatic-like enlargment of a venous blood vessel

After the intervention, a sterile gauze is placed on the phlebotomized parts and along the obliterated vein, which is fixed with an elastic bandage and an elastic stocking for 2 days; then the elastic bandage is removed and only an elastic stocking is worn for 3 weeks. Clinical control of patients is done on the second, seventh day and after 3 weeks; ultrasonographic control after 1 month and after 1 year.

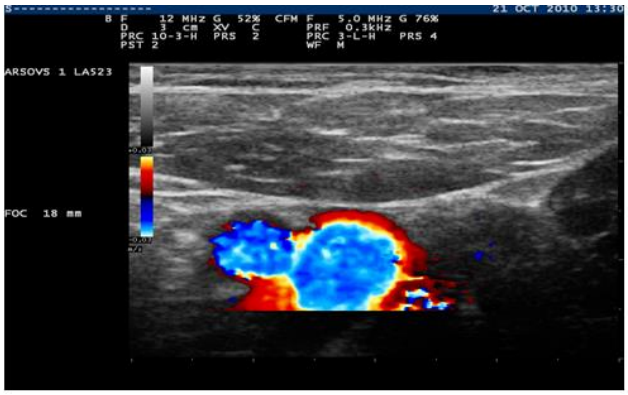

Fig. 10. Ultrasound verification of succesful closing of VSM after the procedure

According to the literature, this method has a success rate of 95% in the first 3 to 5 years, that is, a percentage of relapses of 3-5%, and there is no reliable information for a longer period.
The complications from this method can be divided into early and late. Early complications include: pain, phlebitis, failed vein closure during the intervention, heat-induced venous thrombosis, deep vein thrombosis, skin burns, and lidocaine toxicity. A cause of DVT can be the placement of the catheter in the *v. femoralis* (which is not allowed) and thrombotic tendency such as: antiphospholipid antibody syndrome, activated protein C resistance, increased levels of coagulation factor VIII, malignant disease, protein C deficiency, protein S deficiency, homocysteinemia, dysfibrinolysis (factor XII deficiency).

Fig. 11. Using two probes during during RFA because of an accessory VSM

The following late complications can appear: paresthesia, skin discoloration and local recurrences, ecchymoses (which often occur with laser treatment) and hematoma.

Results

In the period from November 2009 to November 2010, EVLT was used, and after that period RFA, i.e. VNUS technology, was used in both the Private General Hospital „Re-Medika"-Skopje and the Private Clinical Hospital „Acibadem Sistina"-Skopje.
In the period from November 2009 to November 2023, 1053 patients were successfully treated, of which in 818 cases VSM on one leg was treated, of which in 368 cases VSP in addition to VSM was treated; in 395 only VSM was treated; in 55 cases two probes in the ablation process were used due to the existence of accessory VSM, and in 235 cases both legs were treated.
Among the more frequent complications were paresthesias (which were usually transient), ecchymoses (which were more common during laser energy treat-

ment) and hematomas (rarely today). DVT as a thrombosis has not been observed in any case so far. There was a recurrence rate of about 3% after at least one year: 18 cases due to the presence of accessory VSM, in 7 cases due to the existence of insufficiency of all 4 venous junctions and the possibility of the existence of undetected sources of reflux, as well as in 6 cases due to the failure of RFA in treatment due to larger vein dimensions >2.1 cm.

Discussion

The endovenous approach in the management of superficial venous insufficiency is technically the most up-to-date method that has a number of advantages over the classical surgical approach, the so-called stripping. The difference is in the type of anesthesia, i.e., spinal or general anesthesia is used for stripping, while local luminescent anesthesia is used for endovenous access. In the endovenous approach, one incision is made distally, and in the stripping procedure a second incision is made proximally to connect the saphenous-femoral opening. A third major difference is the invasiveness from the minimally invasive approach in endovenous ablation with thermal ablation of the venous vessel to surgical extraction in the classical approach and this has a direct impact on postoperative recovery. In terms of cost *vs*. benefit, the endovenous approach is economically more profitable due to the use of smaller resources in terms of personnel, duration, does not require hospitalization, and it is an outpatient intervention where patients go home the same day after the end of the intervention as opposed to hospitalization after vein stripping.

Conclusion

Endovenous method represents the gold standard in the treatment of venous insufficiency and in combination with local surgical phlebectomy of varicosities show excellent objective results as well as subjective satisfaction among patients. This is a method that is absolutely recommended, sovereign, minimally invasive and with which patients return quickly to their daily activities with very little chance of recurrence if done properly.

Conflict of interest statement. None declared.

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ММР

Original article

RENAL IMPAIRMENT IN NEWLY-DIAGNOSED MYELOMA: FIVE YEAR-ANALYSIS OF CASES IN A UNIVERSITY HOSPITAL

РЕНАЛНО ОШТЕТУВАЊЕ КАЈ НОВО-ДИЈАГНОСТИЦИРАН МУЛТИПЕН МИЕЛОМ: ПЕТ ГОДИШНА АНАЛИЗА НА СЛУЧАИ НА УНИВЕРЗИТЕТСКАТА КЛИНИКА

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Abstract

Introduction. Renal impairment in patients with multiple myeloma may affect 20% of patients at the time of diagnosis, and up to 40-50% during the course of the disease. In newly diagnosed myeloma, renal impairment may be due to various etiology and risk factors, either specific structural renal changes or dehydration, hypercalcemia, infection, or use of NSAID.
Aims. To determine the characteristics and survival of patients with myeloma-related renal impairment in newly diagnosed multiple myeloma (NDMM). Methods: Retrospective data from charts of patients hospitalized in the Department of Nephrology in the period 2015-2020, diagnosed as multiple myeloma, were reviewed. Two groups of patients were compared. Patients with renal impairment and newly diagnosed multiple myeloma (NDMM) at the Department of Nephrology were compared to those previously diagnosed (PDMM). Clinical characteristics and survival were compared between the two groups.
Results. A total of 31 patients were included in the study. Median serum creatinine at start of hemodialysis was 567 umol/l. In 16% of patients, renal biopsy was performed, with findings of myeloma cast nephropathy (MCN), tubulointerstitial changes and amyloidosis. Forty-two percents of patients had improvement in renal function after 3 months. Median time to death was 18 months (range 0-96 months). Patients treated with hemodialysis and chemotherapy had better survival. In 16 patients the diagnosis of multiple myeloma was established at the Department of Nephrology. Newly diagnosed patients with myeloma and renal impairment had shorter survival, 6 *versus* 18 months in patients that were previously diagnosed and treated (P=0.06). Conclusion: Renal impairment in myeloma is consistent with presence of active disease and a high stage of the disease. Hemodialysis and chemotherapy improve survival. NDMM with renal impairment has a significantly shorter survival, thus diagnoses of myeloma should be established in the phase before renal impairment.

Keywords: Multiple myeloma, renal failure, survival, chemotherapy, prognosis

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Апстракт

Вовед. Реналното оштетување кај пациенти со мултипен миелом може да засегне 20% од пациентите во моментот на дијагностицирање и до 40-50% во текот на болеста. Кај новодијагностицираниот миелом, реналното оштетување може да има различна етиологија и фактори на ризик, или специфични структурни ренални промени, дехидрација, хиперкалцемија, инфекција или употреба на нестероидни антиифламаторни лекови (НСАИЛ).
Цели. Да се утврдат карактеристиките и преживувањето на пациентите со бубрежно оштетување поврзано со миелом кај новодијагностициран мултипен миелом (НДММ).
Методи. Вклучени се ретроспективни податоци од медицински картони на пациенти хоспитализирани на Клиниката за нефрологија во периодот 2015-2020 година, дијагностицирани како мултипен миелом. Беа споредени две групи пациенти. Пациентите со ренално оштетување и новодијагностициран мултипен миелом (НДММ) на Клиниката за нефрологија беа споредени со оние претходно дијагностицирани (ПДММ). Клиничките карактеристики и преживувањето беа споредени помеѓу двете групи.
Резултати. Вкупно 31 пациент беа вклучени во студијата. Средниот серумски креатинин при започнување со хемодијализата беше 567 umol/l. Кај 16% од пациентите, направена е ренална биопсија, со наоди за миеломска нефропатија (MCN), тубулоинтерстицијални промени и амилоидоза. Четириесет и два проценти од пациентите имаа подобрување на бубрежната функција по 3 месеци. Просечното време до смртта беше 18 месеци (опсег 0-

96 месеци). Пациентите третирани со хемодијализа и хемотерапија имаа подобро преживување. Кај 16 пациенти, дијагнозата на мултипен миелом е поставена на Клиниката за нефрологија. Новодијагностицираните пациенти со миелом и бубрежно оштетување имале пократко преживување, 6 наспроти 18 месеци кај пациенти кои биле претходно дијагностицирани и третирани (P=0,06).

Заклучок. Реналното оштетување кај миелом е во согласност со присуството на активна болест и висок стадиум на болеста. Хемодијализата и хемотерапијата го подобруваат преживувањето. НДММ со ренално оштетување има значително пократко преживување, така што дијагнозата на миелом треба да се воспостави во фазата пред бубрежното оштетување.

Клучни зборови: мултипен миелом, хронична бубрежна болест, преживување, хемотерапија, прогноза

Introduction

Myeloma is a clonal proliferation of plasma cells and is the second most common hematological malignancy [1], with an annual age-standardized incidence rate (ASIR) worldwide of 2.28 for men, and 1.55 for women per 100,000 persons in 2019 [2]. Estimated ASIR of multiple myeloma for Macedonia in 2020 was 2.6 for men and 1.2 for women per 100,000 population [3]. The number of new cases with multiple myeloma in the Republic of North Macedonia for 2020 was 35, as reported by Global Cancer Observatory [4].

Renal impairment in patients with multiple myeloma may affect 20% of patients at the time of diagnosis to 40-50% during the course of the disease. Symptomatic disease and end-organ damage frequently present as acute renal failure, accompanied with hypercalcemia, anemia and bone lesions (CRAB features) [5]. In newly diagnosed myeloma, renal impairment may be due to various etiology and risk factors, either specific structural renal changes or dehydration, hypercalcemia, infection, or use of NSAID [5,6]. It is associated with poor outcomes. Hemodialysis is needed in only 2-4% of patients with multiple myeloma and renal failure [6].

In the current study, we determined the characteristics and survival of patients with renal impairment in newly *versus* previously diagnosed multiple myeloma patients.

Material and methods

Retrospective data from charts of patients hospitalized in the Department of Nephrology for renal impairment and diagnosed as multiple myeloma, in the period 2015-2020, were reviewed. This study complies with the Declaration of Helsinki. Patients were either diagnosed as multiple myeloma at the Department of Nephrology or at the Department of Hematology. In only one patient on chronic hemodialysis, multiple myeloma was diagnosed after 19 years of hemodialysis and the patient was excluded from the study.

Renal impairment in multiple myeloma was defined, according to IMWG criteria, when serum creatinine was above 177 μmol/l or there was a registered rise in serum creatinine of 20% or more as a result of myeloma [7]. The clinical stage was determined based on Durie-Salmon definition.

GFR was calculated with the use of CKD-EPI. The indications for hemodialysis were: uremia, anuria, metabolic acidosis, hyperkalemia, and excess of extracellular volume.

CRAB was defined as serum calcium above 2.65 mmol/l, renal impairment as creatinine >177 μmol/l, anemia as Hb <100 g/l and bone lesions as osteolytic or osteopenic changes in bones.[7] CRAB is a correlate of end-organ damage and may indicate active disease, while other indicators of active disease are repeated infections, amyloidosis or hyperviscosity.

Statistical evaluation was made by SPSS 20 program (SPSS Inc., Chicago, IL, USA). Data were presented as numbers and percentages or median, where appropriate. Differences in nominal variables were calculated by the use of Student's t test and in continuous variables by the use of chi-square test (or Mann-Whitney test). Survival curves were analyzed by the use of Kaplan-Meier, and Cox proportional hazard regression was used to determine predictors of death.

Results

General characteristics of patients

A total of 31 patients were included in the study. All characteristics of patients are presented in Table 1. The mean age was 64.8 years and 64.5% of patients were females. In 16 patients the diagnosis of multiple myeloma was established at the Department of Nephrology. These patients were admitted for renal impairment. They were considered as newly diagnosed myeloma-related renal impairment (NDMM). At commencement of hemodialysis, 77.4% of patients were already in stage III according to Durie-Salmon. Chemotherapy was started immediately after diagnosis.

Table 1. General characteristics of the group

	Number/ percentage N=31	**Renal impairment in NDMM (Nephrology) N=16**	**Renal impairment in PDMM (Hematology) N=15**	**P**
General characteristics				
Mean age	64.8±10.6 (range 38-82)	62.5±12.2	66.94±8.25	0.46
Sex				
Male (N)	11 (35.48%)	6 (19.35%)	5 (16.1%)	0.61
Female (N)	20 (64.5%)	9 (29%)	11 (35.48%)	
Durie-Salmon stage at commencement of hemodialysis				
Stage I	2 (6.45%)	0	2 (6.45%)	0.31
Stage II	5 (16.1%)	2 (6.45%)	3 (9.68%)	
Stage III	24 (77,4%)	13 (41.9%)	11 (35.5%)	
Active disease and signs at presentation				
Systemic signs of disease				
Hematoma	3 (9.68%)	2 (6.45%)	1 (3.2%)	0.5
Epistaxis	3 (9.68%)	2 (6.45%)	1 (3.2%)	
GIT hemorrhage	2 (6,45%)	2 (6,45%)	0	
Signs of infection	9 (29%)	7 (22.58%)	2 (6.45%)	
Bone pain	13 (41.9%)	9 (29%)	4 (12.9%)	0.04
Bone lesions, X-ray confirmed	18 (58%)	10 (32.25%)	8 (25.8%)	0.34
Bone biopsy at diagnosis	3 (9.68%)	2 (6.45%)	1 (3.2%)	0.78
CRAB syndrome	21 (67,7%)	10 (32.25%)	11 (35.48%)	0.46
Comorbidities				
Hypertension	15 (48.38%)	7 (22.58%)	8 (25.8%)	0.85
Diabetes mellitus	9 (29%)	4 (12.9%)	5 (16.1%)	
Chronic heart disease	4 (12.9%)	2 (6.45%)	2 (6.45%)	
Cerebrovascular accident	1 (3.2%)	0	1 (3.2%)	
Multimorbidity (>=2 comorbidities)	4 (12.9%)	2 (6.45%)	2 (6.45%)	0.9
Renal impairment				
CKD EPI <10 ml/min on admission	20 (64,5%)	8 (25.8%)	12 (38.7%)	0.2
Anuria on admission	8 (25.8%)	6 (19.3%)	2 (6.45%)	0.038
Signs of uremia on admission	18 (58%)	9 (29%)	9 (29%)	0.83
Median serum creatinine at start of hemodialysis (μmol/l)	594.6	871	637	0.036
Median serum creatinine at discharge (μmol/l)	369.8	641	176	0.003
Median serum creatinine after 3 months (μmol/l)	254	180	320	0.003
Treatment with hemodialysis	20 (64,5%)	8 (25.8%)	12 (38.7%)	0.2
Time from diagnosis to commencement of hemodialysis				
0-3 months	12 (60%)	10 (50%)	2 (10%)	0.026
3-6 months	1 (5%)	0	1 (5%)	
6-12 months	1 (5%)	0	1 (5%)	
12-24 months	3 (15%)	0	4 (20%)	
24-36 months	1 (5%)	1 (5%)	1 (5%)	
36-48 months	0	0	0	
48-60 months	0	0	0	
60-72 months	2 (10%)	0	2 (10%)	
Renal biopsy	5 (16.1%)	5 (16.1%)	0	
Pathohistological finding at renal biopsy				
Acute tubulointerstitial nephritis	1 (3.2%)	1 (3.2%)	0	
Myeloma cast nephropathy	3 (9.3%)	3 (9.3%)	0	
Myeloma kidney with amyloidosis	1 (3.2%)	1 (3.2%)	0	

Laboratory results				
Median proteinuria (g/l…g/dU)				
	1.74 g/l (range 0.17-6.9 g/l)	1.6	1.97	0.96
	4.8 g/du (range 0.84-14 g/dU)	4.02	6,0	0.84
Microscopic hematuria	11 (35.48%)	6 (19.35%)	5 (16.1%)	0.61
Hb<100	21 (67,7%)	12 (38.7%)	9 (29%)	0.07
Serum Ca>=2.6 mmol/l	8 (25.8%)	6 (19%)	2 (6.45%)	0.05
Mean total protein g/l	78±21.9	75.5 ±27.2	79.9 ±16.9	0.62
Mean albumin/globulin ratio (AGR)	1.06 ±0.55	1.14±0.62	0.98±0.5	0.48
Number of patients with kappa/lambda ratio >1.65	3 (9.68%)	2 (6.45%)	1 (3.2%)	0.67
Treatment				
Chemotherapy				
CTD	9 (29%)	4 (12.4%)	5 (16.1%)	0.19
Lenalidomid	1 (3.2%)	0	1 (3.2%)	
VTD	3 (9.68%)	2 (6.45%)	1 (3.2%)	
MPT	5 (16.1%)	2 (6.45%)	3 (9.68%)	
Bone marrow transplantation	6 (19.3%)	2 (6.45%)	4 (12.9%)	0.048
Outcome				
Reversible kidney disease (within 3 months)	13 (41.9%)	5 (16.1%)	8 (25.8%)	0.2
Survival longer than 12 months	18 (58%)	6 (19.3%)	12 (38.7%)	0.048
Death	20 (64.5%)	8 (25.8%)	12 (38.7%)	0.2
Median time to death (months)	18 months (range 0-96 months)	5.8	24.7	0.024

Fifteen patients were diagnosed with myeloma at the Department of Hematology and already treated with chemotherapy when renal impairment occurred, for which they were referred to the Department of Nephrology. They were considered as previously diagnosed multiple myeloma with myeloma-related renal impairment (PDMM). One patient had history of monoclonal gammopathy of undetermined significance (MGUS) and one had history of smoldering myeloma. Predominant chemotherapy protocol was CTD (29%), while therapeutic protocols with VTD and Lenalidomid were used in 9.3% and 3.1% of patients, respectively. Transplantation with autologous stem cells was performed in 19.3% of patients. Four patients were treated with radiotherapy for bone lesions. Relapse was registered in 4 patients.

Time of follow-up was 25.5 months (range 0-96 months). Death occurred in 20 patients. Median time to death was 18 months from diagnosis (range 0-96 months).

Clinical features at admission

Majority of patients, 21 patients (67.7%), on admission to the Department of Nephrology had CRAB features (hypercalcemia, renal failure, anemia and bone disease), implying active myeloma. Bone lesions, ranging from osteopenia, osteolysis to fractures were present in 18(58%) patients. Thirteen patients reported bone pain and in 7 patients the pain lasted 30-90 days.

Systemic signs of disease at hospitalization were present in 8 patients and signs of infection in 9 patients.

Multimorbidity (>=2 comorbidities) was present in 13% of patients (mainly diabetes and hypertension). Twelve patients (39%) received chronic antihypertensive therapy with ACEI and ARB.

Renal impairment

On admission to the Department of Nephrology, 8 patients were anuric and 18 had signs of uremia. CKD-EPI <10 ml/min was registered in 20 patients and they were treated with hemodialysis, mainly in the first three months from diagnosis of myeloma. Median serum creatinine at start of hemodialysis was 594 µmol/l. Median serum creatinine at discharge was 369.8 µmol/l. Forty-two percents of patients had improved renal function after 3 months.

Laboratory results revealed microscopic hematuria in 11 patients, mean total serum protein 71 g/l, and median proteinuria 1.74 g/l (4.8 g/dU). Serum and urinary kappa/

lambda ratios were above 1.65 in 3 patients. Hypercalcemia was found in 25.8% and anemia in 67.7% of patients.
Renal biopsy was performed in 5 patients, 3 of whom had myeloma kidney, one had myeloma kidney with amyloidosis and one had tubulointerstitial changes.

Newly diagnosed renal impairment related to multiple myeloma

No difference was found in the group with NDDM *versus* PDMM considering age, sex and Durie-Salmon staging (Table 1). Systemic signs of disease, signs of infection, bone lesions, comorbidities and CRAB did not differ in both groups. The group with renal impairment in NDDM had significantly more anuric patients, significantly higher serum creatinine at start of hemodialysis and at discharge, yet after 3 months, serum creatinine was significantly lower. Time to commencement of dialysis in the NDMM group was less than three months from diagnosis in 60% of patients treated with hemodialysis. Bone marrow transplantation was more frequent in patients with previously diagnosed MM.

Survival

Patients with renal impairment in NDMM (Figure 1) had shorter survival than PDMM (log-rank 0.06). No differences in survival between the group with NDMM *vs.* PDMM were found, when adjusted for reversibility of renal impairment, diuresis, comorbidities, time to dialysis, chemotherapy or combined chemotherapy with hemodialysis. Survival at 12 months was significantly shorter in NIDDM group.

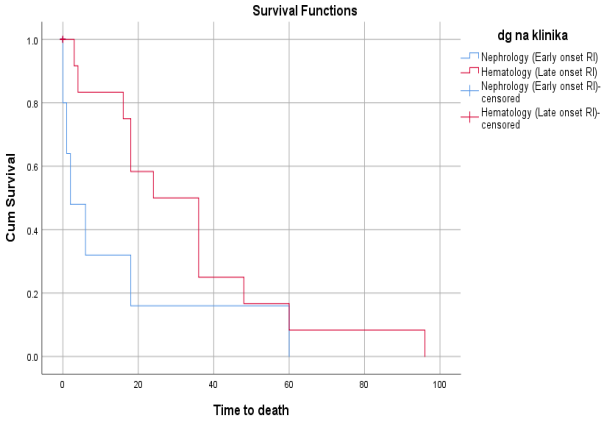

Fig. 1. Overall survival in patients with RI in NDMM *vs.* PDMM (log-rank 0.06)

Survival was significantly better in the whole group of patients (Figure 2) when comparing patients treated with chemotherapy and hemodialysis, favoring both treatments.

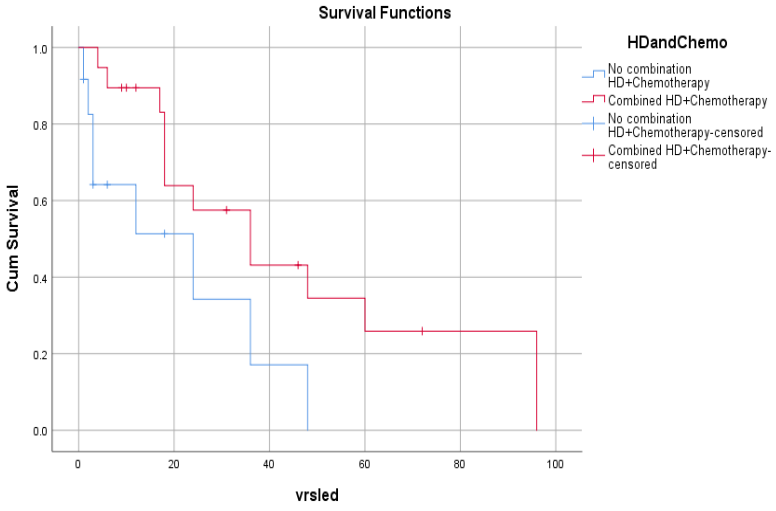

Fig. 2. Survival in patients treated with combination of chemotherapy and hemodialysis (log-rank 0.04)

Treatment with hemodialysis and irreversibility of renal impairment (Table 2) were found as significant predictors: irreversibility as a predictor of death, while hemodialysis as a protective factor in multivariate Cox regression analysis for the whole cohort of patients (P=0.035).

Limitations of the study

This study was conveyed retrospectively and with a small number of patients. Data on free light chains were not accessible for all patients, as some of them were not done on a regular basis.

Table 2. Multivariate Cox-proportional regression analysis of myeloma-specific survival (P=0.035)

						95.0% CI for Exp(B)	
B	**SE**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
-1.393	.615	5.127	1	.024	.248	.074	.829
1.402	.592	5.614	1	.018	4.062	1.274	12.95

Discussion

With the given estimate of 35 new cases per year, patients affected with renal impairment and admitted at the Department of Nephrology for renal impairment constitute about 17% of all myeloma patients per year. Patients who were treated as outpatients were not included in the study, so that overall incidence of renal impairment is probably higher. Reported incidence of renal impairment in the literature is between 15 to 40% of myeloma patients [8]. When IMWG criteria are used, myeloma-related renal impairment is defined as reduced creatinine clearance below 40 ml/min as a result of myeloma [9] and according to this classification, prevalence of acute kidney injury may be as high as 10-25%.

Most patients admitted in the Department of Nephrology had a very low GFR and majority of patients were already in advanced, Durie-Salmon stage III. An active and symptomatic disease, reflected by the high frequency of CRAB syndrome, was present in majority of patients. Therefore, our patients can be profiled as a high-risk group. Although the International Staging System provides better classification of the stage of multiple myeloma, it was not used in our study due to unavailability of parameters needed for the stratifycation. Systemic signs of disease and infection were found in about a quarter of patients.

In 51% of all patients in our study, diagnosis of multiple myeloma was established by a nephrologist. These patients were newly-diagnosed multiple myeloma patients with renal impairment. In only five patients, diagnosis was established by the use of renal biopsy. Predominant biopsy findings were light-chain cast nephropathy (LCCN). In the study of Nasr [10], frequency of LCCN was 37%, while in another study [11], the frequency was 66-87% in all myeloma-related kidney biopsies. Histopathological findings may include also amyloidosis and acute tubular necrosis, monoclonal immunoglobulin deposition disease and less common findings such as fibrillary and immunotactoid glomerulonephritis [12]. Determination of the histopathological findings is important for the prognosis. Further analysis of kidney biopsies with light-chain nephropathy may include two histologic features that are predictive for the outcome: highest number of casts per millimeter square in the cortex and degree of interstitial fibrosis/tubular atrophy [13]. It was presumed that patients with light-chain cast nephropathy may benefit from chemotherapy, combined with intensive hemodialysis using new-generation “high cut-off” dialyzers. Disappointingly, clinical outcomes for patients did not improve in trials MYRE24 and EuLite88 [14,15].

Proteinuria of nephrotic range was found in three patients in our study. This infrequent finding suggests absence of myeloma cast nephropathy, as nephrotic range proteinuria is consistent with amyloidosis or glomerular involvement. Hematuria was found in 35%, as reported by other authors, too [16]. Although anuria on admission was found more frequently in newly diagnosed myeloma, it was not a predictor of death, contrary to the findings of Jung, who reported a risk of 3.628 [17].

Patients with newly diagnosed multiple myeloma had more bone pain, higher serum creatinine, and higher albumin-globulin ratio. Serum creatinine at start of hemodialysis and at discharge were higher in the group with newly diagnosed multiple myeloma. After three months, with the commencement of chemotherapy, serum creatinine in the group with newly diagnosed myeloma, improved significantly. Yet, serum creatinine is a weak evaluation tool in myeloma because it does not define chronic kidney disease or residual renal function [18].

Sixty-four percents of patients admitted at the Department of Nephrology required hemodialysis and median time from diagnosis to staring hemodialysis was 2 months. Initiation of hemodialysis had bimodal distribution: while most of the patients (60%) started hemodialysis in the first 3 months from the diagnosis of the disease, the others started in the period from 3 to 72 months. A significant difference in the time of commencement of hemodialysis in newly *versus* previously diagnosed myeloma indicates different pathogeneses of the disease and different risk factors.

Renal recovery is associated with a good outcome for patients with myeloma, but the mechanism is not clear [19,20]. In 42% of all patients in our study, renal impairment was transitory and reversed to the laboratory values before hospitalization. Reversible renal impairment, with achievement of hematological response and independence from hemodialysis are found to be predictors of improved overall survival in NDMM [21]. Contrary to these findings, in a recent study no advantage in overall survival was found in patients with improvement of renal function, but with newer chemotherapy renal function did not decline significantly after 12 months [22].

In newly diagnosed myeloma, renal impairment is a result of an active disease and use of medications, or

dehydration. Hypercalcemia may contribute by vasoconstriction of renal vessels, and reduced blood flow in the kidney, and exacerbation of natriuresis and volume depletion [12]. These causes may be efficiently solved if adequately recognized.

In our study, hemodialysis was a predictive factor and irreversibility of renal failure was predictive of death in patients with myeloma-related renal impairment. The use of hemodialysis in combination with chemotherapy improved the overall survival in the whole group of patients with myeloma-related renal impairment. Time to death was generally shorter in the group with newly diagnosed myeloma, as well as survival at 12 months, which is consistent with findings from other authors [23].

Early diagnosis of multiple myeloma, risk profiling, accurate determination of pathogenesis and combination of hemodialysis with chemotherapy in this high-risk group of newly diagnosed myeloma patients with renal impairment may be beneficial for the renal and overall outcome in patients with multiple myeloma and renal impairment.

Conflict of interest statement. None declared.

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ММР

Original article

INTERNATIONAL PERSPECTIVES ON RARE GYNECOLOGICAL CANCERS – AN OVERVIEW

МЕЃУНАРОДНИ ПЕРСПЕКТИВИ ЗА РЕТКИТЕ ГИНЕКОЛОШКИ КАРЦИНОМИ

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Abstract

Introduction. Rare cancers are not so rare. Their incidence is higher than any of the single common cancers alone, and this group will become larger since a growing number of rare subtypes of common cancers are being identified based on their molecular aberrations. More than 50% of all gynecological cancers are considered rare.
Aim. This overview examines the unique challenges that rare cancers pose and the advancements in healthcare regarding the understanding, diagnosis, and treatment of rare gynecological cancers.
Methods. Literature search was performed using PubMed. The selection of scientific papers was based on their relevance to rare gynecological cancers, including epidemiology, diagnosis, treatment modalities, research challenges, and potential solutions.
Results. Rare cancers pose unique challenges regarding diagnosis and treatment, due to their low incidence and limited research options. In recent years immunohistochemistry and molecular studies have contributed significantly to diagnosis in the field of gynecological neoplasia. Due to the increasing understanding of the molecular basis of cancers, as well as the discovery of specific molecular targets, targeted agents and modern immunotherapies have been developed. Many of the challenges in rare cancer treatment could be overcome by centralization of care, biobanks, and international collaboration.
Conclusion. Although progress has been made in the understanding and treatment of rare gynecological cancers, significant research gaps remain. Through quality international research and collaboration, centralization of treatment, as well as involvement of pharmaceutical industries, new cost-effective and targeted treatments and prevention strategies will be developed more rapidly, which will ultimately lead to better patient care and improved patient outcomes.

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Keywords: rare, gynecological cancer, international, biobanking, target therapy

Апстракт

Вовед. Ретките карциноми не се толку ретки. Нивната инциденца е повисока во однос на инциденцата на било кој од поединечните чести карциноми. Оваа група ќе стане уште поголема бидејќи се идентификуваат нови ретки подтипови на карциноми, врз основа на нивните молекуларни аберации. Повеќе од 50% од сите гинеколошки карциноми се сметаат за ретки.
Цел. Овој преглед на литературата ги испитува уникатните предизвици поврзани со ретките карциноми, како и напредокот во разбирањето, дијагнозата и третманот на ретките гинеколошки карциноми.
Методи. Пребарувањето на литературата беше извршено користејќи ја датабазата PubMed. Изборот на научни трудови беше заснован на нивната релевантност за ретките гинеколошки карциноми, вклучително нивната епидемиологија, дијагноза, модалитети на третман, истражувачки предизвици и потенцијални решенија.
Резултати. Предизвиците во дијагнозата и третманот на ретките карциноми се должат на нивната ниска инциденца, како и лимитираните можности за спроведување на научни истражувања. Во последните години, имунохистохемиските анализи и молекуларните студии значително придонесоа за дијагностицирање во областа на гинеколошката неоплазија. Напредокот во разбирањето на молекуларната основа на карциномите, како и откривањето на специфични молекуларни цели, водат кон развој на таргетирана терапија и модерна имунотерапија. Многу од предизвиците во третманот на ретките карциноми би можеле да се надминат со централизација на лекувањето, т.е. со воспоставување референтни центри за третман, развој на биобанки и меѓународна соработка.
Заклучок. Спроведувањето квалитетни клинички испитувања и меѓународна соработка, како и вклу-

чување на фармацевтските индустрии, водат кон развој на нови ефективни и таргетирани терапии и стратегии за превенција, што резултира со подобра грижа и подобар исход за пациентите.

Клучни зборови: ретки карциноми, гинеколошки карцином, биобанки, таргетирана терапија

Introduction

Cancer is a growing global burden and therefore its prevention is one of the most important public health challenges of the 21st century. Today we are witnesses of an epidemiological transition, which has begun in the previous century and in which the dominance of infectious diseases is superseded by noncommunicable diseases, making cancer and cardiovascular diseases the leading causes of premature death. According to present ranks and recent trends, cancer may surpass cardiovascular diseases as the leading cause of premature death in most countries in the course of this century (Figure 1) [1]. Therefore, cancer research is of the utmost importance in contemporary medicine. Research has led to accumulation of extensive knowledge about the biological processes involved in cancer onset, growth, and metastases. Those discoveries have resulted in improvement of cancer detection, diagnostics, treatment, follow- up and prevention strategies.

Appendix 1

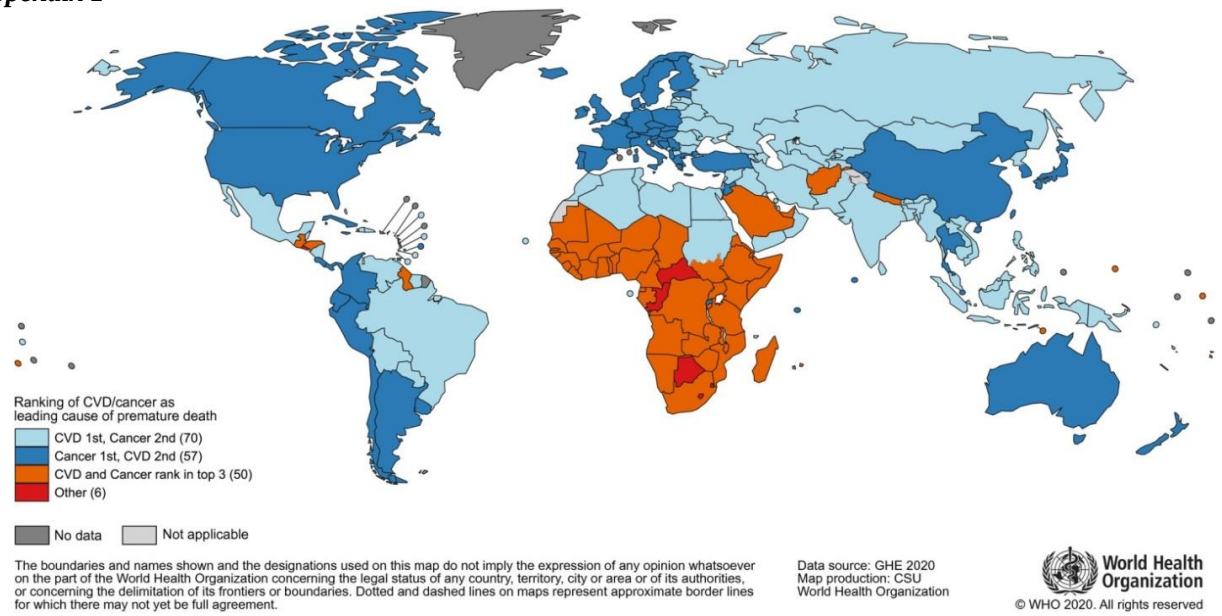

Fig. 1. Global map of the ranking of CVD and cancer as leading causes of premature death (at the ages of 30-70 years) in 183 countries in 2019. Source: World Health Organization

But, the current situation regarding rare cancers is not similar to the common malignancies. There is no universal definition of a rare cancer. The FDA defines the term "rare disease or condition" as any disease or condition which affects less than 200.000 persons in the United States [2]. Rare cancers are defined by the United States National Cancer Institute (NCI) as those which occur in fewer than 15 per 100,000 people per year [3]. The project Surveillance of Rare Cancers in Europe (RARECARE), which provides estimates of the incidence, prevalence and survival of rare cancers in Europe, defines rare cancers as those with an incidence of <6/100,000/year [4].

Rare cancers are not so rare. Rare cancers account for about 22% of all cancers diagnosed in the EU each year [4]. According to the American Cancer Society, one in five cancer diagnosis in the United States is a rare cancer [5]. Therefore, rare cancer incidence is higher than any of the single common cancers alone. Furthermore, the group of rare cancers will become larger since a growing number of rare subtypes of common cancers are being identified based on their molecular aberrations.

Rare cancers pose unique challenges for both clinicians and patients. Research for rare cancers is limited and therefore it is difficult to identify the etiology as well as to develop prevention and early detection strategies. It is challenging to diagnose them as well as to choose the right treatment modality, as treatment options for rare cancers are often limited and less effective than for more common cancers, partly because of less preclinical research and fewer clinical trials for rare cancers. The reasons for the stagnation in research of rare cancers are multifactorial and include logistical difficulties in carrying out clinical trials in a very small cohort of patients, hesitation/motivation of the pharma-

ceutical industry to invest in developing drugs for small markets and complexity in developing cost-effective drugs [6].

Rare gynecological cancers

Gynecological malignancies comprise 19% of new cancer cases worldwide [7]. Rare gynecological cancers account for more than 50% of all gynecological cancers, with approximately 80,000 new cases per year in Europe, involving more than 30 different histologic diagnoses (Table 1), with a very limited number of patients in each diagnostic category (Table 2) [4,8]. The group of rare tumors is becoming larger as molecular classification further subdivides common tumors. The WHO Classification of Female Genital Tumors in the 5th edition of the WHO classification of tumors from 2020, is revised based on histomorphological and molecular pathology data [9].

Appendix 2

Table 1. Rare gynecological tumors

Site	Morphology	Malignancy
Vulva-Vagina	Epithelial	Paget's disease of the vulva
		Adenocarcinoma
		Other carcinomas
	Germ Cell	Skin adnexal carcinoma
		Yolk sac tumor and other types
Uterine cervix	Epithelial	Adenocarcinoma
		Carcinosarcoma
	Mixed	Adenosarcoma
Uterine corpus	Epithelial	Carcinosarcoma
	Mixed	Adenosarcoma
	Germ Cell	Yolk sac tumor and other types
Fallopian tube	Epithelial	Adenocarcinoma
	Mixed	Adenosarcoma
Ovary	Epithelial	Mucinous adenocarcinoma
		Clear cell adenocarcinoma
		Low-grade serous carcinoma
		Other carcinomas

Adapted from WHO classification of tumors. Editoral Board. Female Genital Tumors. Lyon (France) IARC 2020 (WHO classification of tumors series, 5th ed.; Volume 4). https://publications.iarc.fr/592

The female genital tract is characterized by the occurrence of a greater range of tumor types than any other organ system in the body. This is especially so in the ovary where numerous diverse tumors, benign and malignant, occur [10]. The term 'rare tumor' refers mostly to non-epithelial subtypes. However, histologically different epithelial subtypes of ovarian, endometrial and cervical cancers are also to be categorized as rare tumors due to their different pathological behaviors [11]. Some examples of rare gynecologic cancers include, but are not limited to: cervical adenocarcinoma, papillary serous tumors of the endometrium, clear cell cancers of the gynecologic tract, carcinosarcomas, gynecologic sarcomas, tumors of the vulva and vagina, sex cord tumors, small cell tumors of the gynecologic tract, germ cell tumors, gestational trophoblastic tumors.

Challenges in the diagnosis of rare gynecological cancers

Rare gynecological cancers are associated with worse outcomes compared to common cancers. It is challenging to reach the accurate and timely diagnosis since clinical inexperience leads to a delayed diagnosis and the necessity for second opinions extends the time to cancer first treatment. Furthermore, correct pathologic diagnosis of a rare cancer is difficult to reach because of the rarity of the condition, specialist subjectivity, limited experience and because rare cancers tend to have a complex histotypic appearance [6]. Expert consults and a centralized pathological review are ways to overcome these difficulties. In recent years immunohistochemistry has contributed significantly to diagnosis in the field of gynecological neoplasia. Also, molecular studies have shown characteristic genetic abnormalities in different tumor types, which has contributed to establishing the histogenesis of various neoplasms. As an example, high-grade serous carcinomas in the uterus and ovary have been demonstrated to consistently harbor p53 mutations, while endometrioid adenocarcinomas arising in the same organs not uncommonly exhibit microsatellite instability and mutations in beta catenin, k-RAS, PIK3CA and PTEN genes [12]. Molecular studies have also shown that there are two distinct types of ovarian serous carcinoma, termed low-grade and high-grade serous carcinoma and these are two separate neoplastic types rather than high-grade and low-grade variants of the same neoplasm. Much more common high-grade ovarian serous carcinomas are characterized by p53 mutations and BRCA1 and BRCA2

abnormalities, while low-grade serous carcinomas are characterized by k-RAS and BRAF mutations [10].

Appendix 3

Table 2. Estimates of incidence and survival for rare gynecological cancers

Cancer entity		Incidence rate per 100,000 per year	Five-year relative survival (%)
Rare epithelial tumors of corpus uteri		0.70	43.2
	Squamous cell carcinoma with variants of corpus uteri	0.06	54.6
	Adenoid cystic carcinoma of corpus uteri	0.00	31.3
	Clear cell adenocarcinoma, NOS of corpus uteri	0.16	56.2
	Serous (papillary) carcinoma of corpus uteri	0.08	36.5
	Mullerian mixed tumor of corpus uteri	0.40	35.5
Epithelial tumors of cervix uteri		6.28	65.1
	Squamous cell carcinoma with variants of cervix uteri	4.73	66.5
	Adenocarcinoma with variants of cervix uteri	0.91	66.5
	Undifferentiated carcinoma of cervix uteri	0.03	30.9
	Mullerian mixed tumor of cervix uteri	0.02	28.1
Epithelial tumor of ovary and fallopian tube		9.38	37.2
	Adenocarcinoma with variants of ovary	5.95	38.3
	Mucinous adenocarcinoma of ovary	0.77	58.9
	Clear cell adenocarcinoma of ovary	0.30	53.8
	Primary peritoneal serous/papillary carcinoma of ovary	0.08	19.1
	Mullerian mixed tumor of ovary	0.14	19.5
	Adenocarcinoma with variants of fallopian tube	0.17	56.8
Non-epithelial tumors of ovary		0.25	80.6
	Sex cord tumors of ovary	0.13	76.5
	Malignant/Immature teratomas of ovary	0.05	80.6
	Germ cell tumor of ovary	0.07	84.4
Epithelial tumors of vulva and vagina		1.97	57.3
	Squamous cell carcinoma with variants of vulva and vagina	1.69	59.0
	Adenocarcinoma with variants of vulva and vagina	0.07	42.3
	Paget's disease of vulva and vagina	0.05	83.7
	Undifferentiated carcinoma of vulva and vagina	0.01	15.8
Trophoblastic tumor of placenta		0.02	85.3
	Choriocarcinoma of placenta	0.02	86.5

Adapted from: Gatta G, Capocaccia R, Botta L, *et al*. Burden and centralised treatment in Europe of rare tumours: results of RARECAREnet-a population-based study. Lancet Oncol. 2017; 18(8): 1022-1039.

Novelties in treatment modalities

Regarding treatment, surgery is the first choice for most gynecologic cancers, followed or preceded by chemotherapy and radiotherapy. However, treatment is often ineffective, and these traditional therapies do not remove the malignant cells completely. Changes have been made to treatment protocols due to the increasing understanding of the molecular basis of cancers and discovery of specific molecular targets resulting in the development of targeted agents and modern immunotherapy, including PDL1 inhibitors, anti-angiogenic drugs, and PARP inhibitors [11,13].

The care for a patient with a rare gynecological cancer differs fundamentally compared to the care for a patient with a more common cancer. As all these cancers have different clinical and pathological characteristics, as well as different molecular pathways, they also differ in the response to treatment. The clinical behavior of the cancer is more influenced by the histology and tumor biology than the disease site [14]. Different types of cancer have different molecular biological pathways and therefore knowledge of each individual one will be needed in order to develop new diagnostic, prognostic and treatment strategies, such as therapy that interferes with specific molecular targets [15]. Targeted therapy has higher tumor sensitivity and less toxic effects to healthy cells. Unlike chemotherapy, which inhibits DNA replication and mitosis, these agents target cancer signaling pathways, stroma, immune

microenvironment, and angiogenesis in tumor tissues. PARP inhibitors which target defective DNA repair are used in the treatment of advanced ovarian cancer. Also, drugs which target angiogenesis are used in the treatment of advanced or recurrent ovarian and cervical cancers. Immune check-point inhibitors such as anti-PD-1/PD-L1 antibodies have proved successful for mismatch repair-deficient endometrial cancers and HPV-targeted therapies are under development for HPV-related malignancies [16].

Potential non-invasive molecular markers for cancer diagnosis, prognosis and treatment are microRNAs, which are a class of non-coding RNAs involved in regulation of gene expression. Their expression is dysregulated in different cancers, and they may function as tumor suppressor or oncogene under certain circumstances. Restoration or silencing of miRNA function is a promising strategy for cancer treatment. MiRNA expression signatures are associated with tumor type, tumor grade and clinical outcomes [17]. Further research is necessary to identify miRNAs, their biological functions and target genes in order to use them as diagnostic biomarkers, prognostic biomarkers, and therapeutic targets [18].

Rare gynecological cancer research

The lack of knowledge as well as therapeutic options [19] for rare gynecologic cancers is due to the fact that developing effective treatment for rare cancers is with slow progress and, in some cases, non-existent. There are several reasons for the stagnation in rare cancer research. There is limited availability of tumor tissue for characterizing tumor biology and for developing cell lines and animal models to test hypotheses and candidate therapeutics. Also, it is challenging to conduct randomized controlled trials for testing therapies for rare tumors, due to the low incidence of these tumors in the overall population, as well as the fact that these patients may be too geographically dispersed for any clinical center to accrue enough patients to conduct a meaningful study. These issues exacerbate the difficulty in studying the natural history of rare tumors, which has limited our understanding of the biology and clinical course of many of these tumors. Although research on rare tumors has been challenging to conduct, past studies of certain rare tumors have helped clarify fundamental mechanisms of tumorigenesis for both rare and common tumors [20].

Clinical trial design

Since there is a small number of patients with rare gynecological cancers, it is challenging to perform clinical trials and secure funds for research [21]. Also, there is insufficient focus on effective treatment modalities and evidence-based guidelines. As a result, treatment is developed based on retrospective studies, expert opinion or by drawing experience from treatment of more common cancers or from cancers with similar pathologic characteristics [22]. Since it is difficult to perform randomized clinical trials, which provide the highest level of evidence to support diagnostic tools and treatment, and even more challenging to successfully complete them, different approaches have been proposed for rare cancer research. Trial designs can be undertaken using a wide array of possibilities. There is no ‘one size fits all’ solution [23]. For example, clinical trials based on cognate genomic aberrations could be used for molecularly targeted therapies. Also, open-ended single arm trials, or prospective registry studies could be used for multimodality organ site-specific therapies, including surgery, medical therapies, and radiation therapy [21]. Reporting and accessing data on tumor biology and clinical information of patients should be standardized in order to achieve best use of these data. The challenges in conducting clinical trials for rare cancers could be overcome by robust international collaborations [8]. A high-quality management of rare gynecologic cancers should be based on scientific evidence, international consensus guidelines, multidisciplinary approach, and high-quality clinical trials, as well as reference centers and networks sharing multidisciplinary expertise [24].

Advantages of use of biobanks in rare gynecological cancers

One of the ways toward collaborative scientific research is the development of biobanks dedicated to rare gynecological cancers. Biobanking is the collection, processing and storing of biological samples and data for research. Information obtained from high quality biological specimens opens opportunities to learn more about the causes, prevention, and treatment of the disease. International comparisons made possible by the study of sample collections from different parts of the world in combination with epidemiological and clinical data, are invaluable in the pursuit of the evidence base for cancer control [25].

Studies of many rare forms of cancer are limited by the difficulty of recruiting a sufficient number of cases within any single collection center. Networking of biobanks can lead to accumulating higher-quality samples and data from different banks, as well as conducting larger research trials, which will enable the procurement of significant numbers of rare cancers. This will facilitate future research into biomarkers and molecular markers of use in a diagnostic setting as well as in a predictive or prognostic sense [10]. In order to have proper replicability and reliability of the findings obtained from multi-site-based studies, biobanks have to adopt common technical standards for specimen collection, storage, and annotation, and for

data collection and management. Networking of biobanks necessitates the implementation of legislatives in different countries, active participation of many professionals and most importantly has ethical implications. Therefore, appropriate methods need to be developed in order to obtain informed consent from a potential participant, develop research protocols that are fully compatible with the ethical principles of beneficence, non-maleficence, and justice, protect personal data, ensure biological and environmental safety, and make collections accessible and available for reuse for research purposes under defined conditions [26]. The development of biobanks dedicated to rare gynecological cancers which follow the Standard Operating Procedures (SOAPs) plays a crucial role in collecting samples and clinical data for personalized medicine [27,28].

Centralization of treatment

Many of the challenges in rare cancer treatment could be overcome by centralization of care, i.e., by establishment of reference centers at an appropriate geographical level (regional, national, and supranational). Reference centers should focus on a certain rare cancer and have a multidisciplinary approach in order to provide more accurate diagnosis and treatment of rare cancers. This is made possible because of the significantly larger number of cases, the multidisciplinary setting involving expert professionals and access to clinical trials. Some of the disadvantages of centralization are the need for patients to move, the risk of a longer waiting list, with consequent discomfort and possible negative effects on outcome [29,30].

Importance of patients' associations

Finally, it is important to emphasize the role of patient associations in the process of guiding the patient to reference centers and clinical trials. Patient advocacy groups have additional means of communicating with patients and their families, and through these communications they have the potential to speed dissemination of information on emerging cancer therapies. Social media platforms now enable virtual patient aggregation on a larger scale [21].

Conclusion

Although progress has been made in the understanding and treatment of rare gynecological cancers, as treatment has shifted from the 'one size fits all' paradigm to specific target therapies, there is still a need for further research. Investigating and treating rare gynecological cancers is challenging because of the small cohort of patients for clinical trials, obstacles in international cooperation and financial support for research. Since it is difficult to perform randomized clinical trials, and even more challenging to successfully complete them, different clinical trial designs need to be implemented for rare cancer research.

International collaboration is necessary for the conduct of clinical trials with adequate statistical power to provide scientific evidence for development of diagnostic tools, treatment and to evaluate prognostic factors. One of the ways toward collaborative scientific research is the development of biobanks dedicated to rare gynecological cancers. International comparisons made possible by the study of sample collections from different parts of the world are invaluable in the pursuit of the evidence base for cancer control.

Diagnostic accuracy coupled with appropriate treatment policies would ultimately help patients with rare tumors improving survival and quality of life, while simultaneously maintain healthcare expenditures at a reasonable level. The complex care of patients with rare gynecological cancers should be based on scientific evidence, international consensus guidelines, high-quality clinical trials, and a multidisciplinary approach, including gynecologists, pathologists, oncologists, molecular biologists, radiologists, geneticists, etc. Many of the challenges in rare cancer treatment could be overcome by centralization of care, i.e., by establishment of reference centers.

Through quality international research and collaboration, as well as involvement of pharmaceutical industries, new cost-effective and targeted treatments and prevention strategies will be developed more rapidly, which will ultimately lead to better patient care and improved patient outcomes.

Conflict of interest statement. None declared.

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Original article

ROLE OF CYTOLOGY AND CYTOBLOCK IN DIAGNOSIS OF MALIGNANT PLEURAL EFFUSIONS

УЛОГАТА НА ЦИТОЛОГИЈАТА И КЛЕТОЧНИОТ БЛОК ВО ДИЈАГНОЗАТА НА МАЛИГНИТЕ ПЛЕВРАЛЕН ИЗЛИВИ

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Abstract

Introduction. Pleural effusion is a common complication of malignant diseases and its precise diagnosis is an important factor for staging and optimizing the treatment of a patient. The aim of this study was to determine the diagnostic value of cytology and cytoblock in the detection of malignant pleural effusion in patients with pulmonary and extra-pulmonary neoplasms.
Methods. We conducted a cross-sectional study from January 2022 to March 2023 with retrospective approach. We included 92 patients with malignant diseases referred to our Clinic of Pulmonology for diagnosis of their new-onset pleural effusion. We analyzed their medical history, pathohistology of the primary tumor and cytology results from cytospin and cytoblock preparation from their pleural effusions samples
Results. Of the 92 included patients, positive cytology (classification group IV and V) was obtained in 31 (33.69%), negative (group I and II) in 57 (61.95%) and group III in 4 patients (4.34%). Positive cytology was obtained in 17 patients (54.83%) with pulmonary neoplasms, 11(35.48%) with extra-pulmonary neoplasms and 3(9.67%) neoplasms with undefined etiology. Comparation of the results from cytoblock and cytospin preparations showed that the diagnostic yield of cytoblock was greater that cytospin preparation. The results from cytoblock were compatible with the pathohistology result from the primary neoplasm in 88.46% of patients.
Conclusion. Cytology and cytoblock obtained from pleural effusion could be valid diagnostic procedures, especially in patients with advanced malignant disease who are not candidates for more invasive diagnostic procedures.

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Keywords: pleural effusion, sensitivity, malignant diseases, cytopathology

Апстракт

Вовед. Плевралниот излив е честа компликација на малигните болести и неговата прецизна дијагностика е значаен фактор при одредувањето на стадиумот на болеста и донесување на одлука за оптимален третман на болниот. Целта на оваа студија да се одреди дијагностичката вредност на цитологијата и клеточниот блок во детекција на малигните плеврални изливи кај болните со белодробни и вонбелодробни неоплазми..
Методи. Направивме студија на пресек за период Јануари 2022 до Март 2023 година со ретроспективен пристап. Вклучивме 92 пациенти со малигно заболување, пратени на клиниката за Пулмологија за дијагностика наново настанат плеврален излив. Ја анализиравме нивната медицинската историја, патохистологијата од примарниот тумор, и резултатите од цитоспин препаратот и клеточниот блок од примерокот од добиената плеврална течност.
Резултати. Од вклучените 92 болни, позитивна цитологија (4 и 5 класификациона група) е добиена кај 31 болен (33,69%), негативна (1 и 2 група) кај 57(61,95%) болни, а 3 група кај 4 болни (4,34%). Позитивна цитологија е добиена кај 17 болни (54.83%) со белодробен тумор, 11(35,48%) болни со вон белодробен тумор и 3(9.67%) малигноми со нејасна етиологија. Споредбата на клеточниот блок и цитоспин препаратот покажуваат дека дијагностичката вредност на цитоблокот е поголема во однос на цитоспин препаратот. Резултатите од клеточен блок во 88,46% соодветствуваа со патохистолошкиот наод од примарната болест.
Заклучок. Цитологијата и клеточниот блок од плеврален излив би можеле да бидат валидни дијагнос-

тички процедури, пред се кај пациенти со напредната малигна болест, кои не се во состојба да издржат инвазивни дијагностички постапки.

Клучни зборови: плеврален излив, сензитивност, малигни болести, цитопатологија

Introduction

Malignant diseases are imposed as one of the leading and at the same time the most serious causes of pleural effusion and are accounted for approximately 23.7% of all pleural effusions [1]. Most of the cases of malignant pleural effusions (MPE) are the result of a metastatic deposits on the pleura from the primary disease, of which the most common cause is lung cancer in men and breast cancer in women. These two diseases together account for 50-65% of all malignant pleural effusions [2,3]. Mesothelioma is the most common primary tumor of the pleura, associated with exudative pleural effusion in over 90% of cases [4]. The most common malignancies causing malignant pleural effusions in men are lung malignancy (49.1%), lymphoma/leukemia (21.1%), gastrointestinal tract malignancies (7.0%), genitourinary tract malignancies (6%) and malignant melanoma (1.4%). In women, breast cancer is the most prevalent with 37.4%, genital tract malignancies (mostly ovary) 20.3%, lung cancer 15%, lymphoma 8.0% and neoplasms of the gastrointestinal tract with 4.3% [5]. In 5-10% of all malignant pleural effusions, the primary cause remains unknown [6].

Cytological analysis of fluids obtained from serous cavities (mostly pleural and peritoneal) is among the greatest challenges of modern cytopathology. Cytology is established and already represents a routine method in the identification of the causes of pleural and/or peritoneal effusion. However, the distinction between benign and malignant cells in serous effusions with cytological methods (cytospin preparations, cytoblocks) often represents a diagnostic challenge. This primarily refers to the relatively low sensitivity of cytological methods in the evaluation of the liquid, which is sometimes due to unclear visualization of certain morphological details of the cells themselves, overlapping or loss of cells and other changes that occur during the laboratory processing of the material [7]. Other factors that also affect the sensitivity of cytological methods is the presence of blood clots or tissue fragments in the liquid, which is especially important for cytoblocks, which are more successful if the samples are mixed with blood and if there are tissue fragments [8]. Although cytospin preparations are a fast, efficient and inexpensive method to increase the number of visible cells in microscopic preparations with good preservation of cellular morphology, the production of cytoblocks is associated with increased cellularity of preparations, improved morphological details of cells, preservation of architectural structures (papillary, three-dimensional, "cell balls") as well as better nuclear and cytoplasmic preservation. Other advantages are the possibility of multiple sections of the same material in order to make additional special stainings and immunohistochemical analyses [9], and more recently, the possibility of molecular testing [10]. The possibility of molecular and immunological profiling is particularly important for determining therapeutic indications for targeted and/or immunotherapy. One of the biggest advantages of cytoblock preparations is the appearance of the cells in them, which corresponds to that of the histopathological preparations [11]. The complementary use of several cytopathological techniques together: cytospin preparations, cytoblocks and immunohistochemistry greatly increases the diagnostic sensitivity of cytology in the detection of malignant pleural and peritoneal effusions. In a study of 150 samples of pleural and peritoneal fluid [12], pleural effusion of malignant etiology was detected by classical cytology in 19.23% of samples, while cytology combined with cell block and immunohistochemistry confirmed malignant etiology of pleural effusion in 34.61% of the samples. Similar results were obtained from peritoneal fluid samples: 17.65% *versus* 30.88%. The authors conclude that the use of cytoblocks and immunohistochemistry in the cytopathological analysis of pleural and peritoneal fluid greatly increases the diagnostic sensitivity and specificity of cytology in the detection of malignant pleural and peritoneal effusions and that the combined use of cytoblocks and immunohistochemistry is more useful than cytological analysis alone.

In a clinical study of 50 patients comparing cytological methods in the evaluation of pleural effusions with pleural biopsy as the gold standard in the diagnosis of malignant and non-malignant pleural effusions [13], in 8 patients malignant pleural effusion was proven by classic cytology, in 10 patients by the cytoblock technique, and in 11 patients malignant disease was diagnosed by pleural biopsy. The authors conclude that the cytoblock technique of pleural fluid processing is complementary to classical cytology in the diagnosis of pleural effusions, however, due to the preserved archetectonic structures of the cells in the cytoblock preparations, it is superior to classical cytology in the detection of malignant pleural effusions and can represent a bridge between cytopathological and pathohistological methods. Considering the difficulty and invasiveness of the diagnostic methods used to provide an adequate histopathological sample (pleural biopsy, bronchobiopsy, surgical biopsy) as opposed to pleural paracentesis used to provide a sample for pathocytological analysis (cytospin, cytoblock studies) the development and utilization of certain cytopathological me-

thods (the cytoblock technique) in the evaluation of malignant pleural effusions is increasing even more.

Objectives of the study

To determine the distribution of patients with pleural effusion and malignant disease by sex, age and type of primary tumor
To determine the frequency of malignant pleural effusion in patients with confirmed malignant disease and pleural effusion
To determine the frequency of certain pathohistological types of malignant diseases and the occurrence of pleural effusion by gender distribution
Determining the diagnostic value of cytology and cell block from pleural effusion in patients with pulmonary and extrapulmonary malignancies.

Material and methods

Material

The study included patients with a diagnosed malignant disease aged over 18, who were referred to the University Clinic for Pulmonology and Allergy, Skopje for additional diagnostics of a present pleural effusion, in the period from January 2022 to March 2023. The pathocytological analysis of the samples from the pleural effusion was performed at the University Clinic for Radiotherapy and Oncology and the Institute of Pathological Anatomy at the Faculty of Medicine in Skopje.

Methods

A cross-sectional study was done with a retrospective approach. Data from the medical history of patients treated at the University Clinic for Pulmonology and Allergy in the period from January 2022 to March 2023 were analyzed. In all patients, a diagnostic pleural paracentesis was performed and a sample of 60 mL of fluid was collected. Cytopathological analysis of cytospin preparation and cell block was requested for all samples.
The following parameters were analyzed:

- Demographic characteristics of patients (gender, age)
- Results of the pathohistological findings of the primary malignant disease
- Cytopathological findings from cytospin preparations and cell block from pleural punctate
- Standard descriptive statistical analysis, with determination of frequencies, was made with Microsoft Excel, 2016.

Results

The medical history of 92 patients with malignant disease and accompanying pleural effusion was analyzed. Patients were equally represented according to gender distribution, i.e. 48(52.17%) men and 44(47.82%) women with an average age of 61.42±4.79 (CL 95%) years.
In the analyzed material, extra-pulmonary neoplasms were the cause of most of the pleural effusions associated with a confirmed malignant disease in 45 patients (48.91%), while the other part was due to neoplasms of pulmonary origin 40(43.47%). In 7(7.61%) patients, the origin of the primary malignant disease was not clearly identified. The distribution of patients by gender indicated that lung neoplasms were dominant in men (55.77%), while 65.0% of extra-pulmonary neoplasms with pleural effusion were found in women (Table 1).

Table 1. Distribution of patients according to gender and primary localization of the neoplasm

Primary localization of the neoplasm	Total men	%	Total women	%	In total	%
Pulmonary neoplasms	29	55.77%	11	27.5%	40	43.48%
Extrapulmonary neoplasms	19	36.54%	26	65.0%	45	48.91%
Undefined	4	7.69%	3	7.5%	7	7.61%
Total by gender	52	100%	40	100%	92	100

Cytopathological confirmation of malignant cells in pleural effusion, in relation to the total number, was found in 31(33.69%) of patients. Of the samples with positive cytology, 17(54.84%) came from patients with pulmonary neoplasm, 11(35.48%) from patients with extra-pulmonary neoplasm and in 3(9.68%) the primary neoplasm was not defined (Figure 1).
Table 2 presents the results of the cytological analysis of the preparations according to the type of primary neoplasm. In patients with primary lung neoplasm, positive cytology (marked as classification group IV and V) was obtained in 17(42.50%), negative cytology (classification group I and II) was obtained in 21 (52.50%) patients, and classification group III, defined as “a finding suspicious for a malignant pleural effusion”, in 2(5.00%) of the samples. Regarding extrapulmonary primary malignancies, positive cytology (classification group IV and V) was obtained in 11 (24.44%),

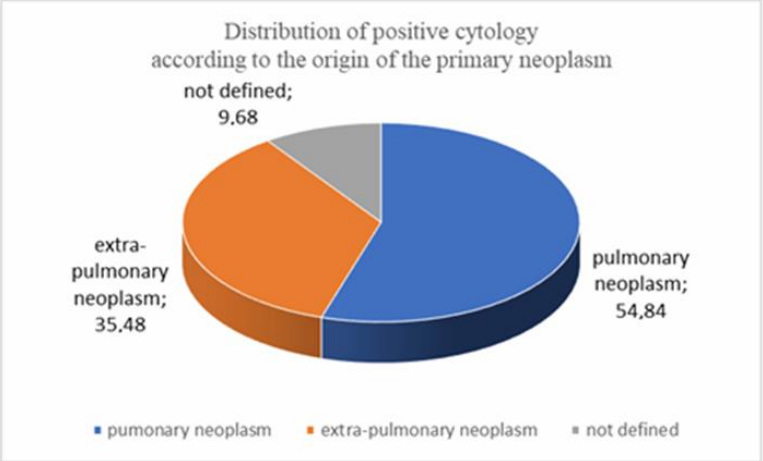

Fig. 1. Distribution of positive cytology according to the origin of the primary neoplasm

negative cytology (classification group I and II) in 32 (71.11%), and classification group III, that is, a finding suspicious for a malignant pleural effusion, in 2 (4.44%) of the samples.

The highest percentage of positive malignant cytopathological findings in the pleural effusion was found in lung adenocarcinoma (45%), followed by breast cancer (16%) and mesothelioma (39%) (Figure 2).

Table 2. Presentation of cytological results of pleural effusion in patients with confirmed malignant disease

Classification group	Pulmonary	Extra-pulmonary	Undefined	Total
I+II	21(52.50%)	32(71.11%)	4(57.14%)	57(61.96%)
III	2(5.00%)	2(4.44%)	0(0%)	4(4.35%)
IV+V	17(42.50%)	11(24.44%)	3(42.85%)	31(33.69%)
Total	40(100%)	45(100%)	7(100%)	92(100%)

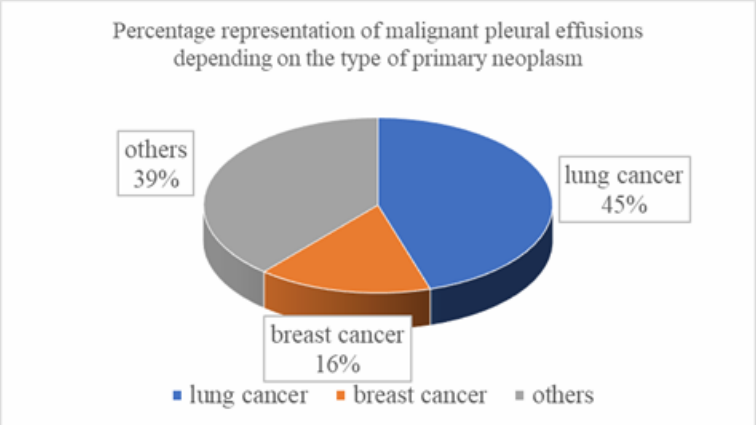

Fig. 2. Percentage representation of malignant pleural effusions depending on the type of primary neoplasm

Lung cancer and breast cancer together accounted for 61% of confirmed malignant pleural effusions in this study. Among patients with lung cancer, lung adenocarcinoma was prevalent with 71.43%.

The results form the cell blocks of 26 of the patients with malignant disease and associated pleural effusion were analyzed, of which 10(38.46%) were men and 16(61.54%) were women.

According to the pathohistology of the primary tumor, 13(50.0%) samples were from patients with lung neoplasm, 4(15.38%) patients with breast cancer, 2(7.69%) with lymphoma, 2(7.69%) with ovarian cancer, 1(3.85 %) with bladder cancer, 1(3.85%) with melanoma and 3(11.54%) neoplasms of undefined etiology.

The results of the pathocytological analysis of the cell block were compared with the results of the cytospin preparation from the same patients.

The results obtained with cell block showed that in 24 of a total of 26(92.31%) preparations a clear confirmation of malignant pleural effusion was obtained, that is, the result was positive and conclusive. In 2(7.69%)

cell block preparations, a result interpreted as a suspicion of malignant disease was obtained.
The results obtained with the cytospin technique clearly confirmed the existence of a malignant etiology of the pleural effusion (classification group IV and V) in 22 of 26 patients (84.62%), malignant etiology was not detected in the effusion (classification group I and II) in 1 patient (3.85%), while in 3 (11.54%) patients, a classification group III result was obtained, where, the finding was inconclusive or the malignant etiology or malignant effusion was not clearly confirmed (Figure 3).

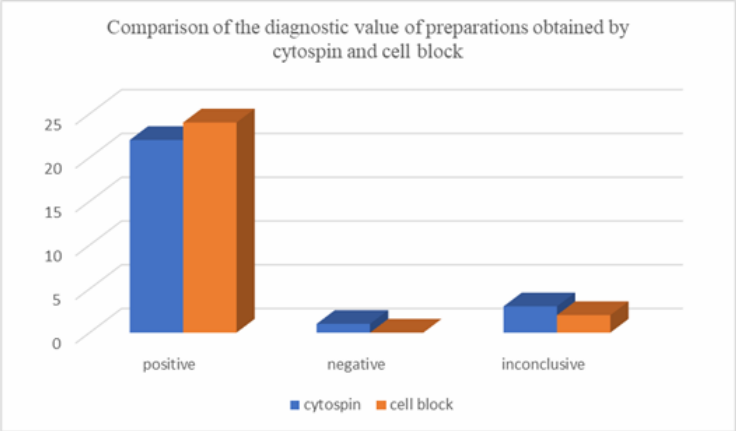

Fig. 3. Comparison of the diagnostic value of preparations obtained by cytospin and cell block

The results of cell block in 88.46% (23 of 26 patients) corresponded with the pathohistological finding of the primary disease.

Discussion

Malignant pleural effusion is the second most common cause of pleural exudate [14] and occurs in 15% of cancer patients [15]. Determining the exact etiology of the pleural effusion in patients with concomitant malignant disease is necessary when making a decision on the optimal treatment for a patient. Confirmation of a malignant etiology of the pleural effusion always means an increase ("up-grade") of the stage of the disease and limits the possibility of surgical treatment of the primary disease. Occurrence or worsening of pleural effusion, during or after completion of oncological treatment, may necessitate a change in therapy. On the other hand, due to the severe general condition of oncology patients, often associated with impaired heart, kidney or liver function, as well as malnutrition and possible hypoproteinemia, the pleural effusion will not always be of malignant etiology. In such a case, timely recognition and appropriate treatment is crucial.
In this study, extrapulmonary tumors were most commonly associated with pleural effusion (48.91%), followed by lung neoplasms (43.47%) and neoplasms of unknown origin (7.61%). However, cytospin and cytoblock confirmed malignant pleural effusions in this study to be most commonly of pulmonary origin (42%), followed by breast cancer (16%) and all other neoplasms (39%). This is in accordance with some other studies, such as that by Shahini *et al.* [16] where the cytoblock technique was used for cytological analysis of suspected malignant pleural effusions. In the same study of 152 positive malignant effusions, confirmed by cytoblocks, in 82 of them the cause of the malignant pleural effusion was lung cancer, followed by 15 cases of breast cancer, 8 tumors of the gastrointestinal tract, 6 of the female genital tract and 24 of unknown origin.
A large number of studies have confirmed the relatively low diagnostic sensitivity of cytological preparations in the confirmation of malignant pleural effusions (58.2%) with significant differences between studies [17]. Although the repetition of diagnostic paracentesis increases the diagnostic value of cytological preparations by about 27%, the greater number of paracenteses (over two) increases the diagnostic value of cytology by only 5%, which is why it is not recommended in most guidelines [18]. Also, the role of a larger volume of fluid sent for cytology may have a certain role in increasing the sensitivity of cytological preparations, especially if the first finding is negative, but more studies are needed to define exactly the amount of fluid that should be sent [19].
In the study by Porcel *et al.* [20], 632 cytological smears and 554 cytoblocks from 414 patients with pleural effusion were evaluated. The diagnostic value of the cytological analysis from the first sample of the fluid was 44% regardless of whether smears or cytoblocks were performed. Evaluation of the additional fluid sample increased the diagnostic value of both smears and cytoblocks to 56%. About 11% of the samples that were negative for malignancy in the cytological smears, showed malignant cells in cytoblocks;

however, and conversely 15% of the negative cytological smears were reported as positive in the cytoblocks. The authors conclude that the simultaneous use of cytological smears and cytoblocks is necessary to increase the sensitivity of the pathocytological evaluation of pleural effusions. The results of this study showed a greater diagnostic value of cytoblock preparations. In 24 out of a total of 26(92.31%) of them, a clear confirmation of malignant pleural effusion was obtained in relation to classic cytospin preparations, in which malignant pleural effusion was confirmed in 22 of 26 patients (84.62%). It is in accordance with the study of Shivakumarswamy *et al.* [21] who referred to an increased diagnostic sensitivity of cytoblocks by about 15% compared to the classic cytospin preparation in distinguishing malignant pleural effusions.

Although cytoblock preparations show an increased diagnostic value compared to cytospin preparations, the simultaneous use of both increases the sensitivity of the overall pathocytological analysis in the detection of malignant pleural effusions. This is especially significant in cases where an adequate sample for pathohistological diagnosis cannot be provided and the use of the cytoblock represents a bridge between classical cytology and pathohistology.

Conflict of interests: None declared.

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ММР

Original article

FT3/FT4 RATIO PREDICT SURVIVAL IN SURGICALLY TREATED PATIENTS WITH RENAL CELL CARCINOMA

ОДНОСОТ FT3/FT4 ПРЕДВИДУВА ПРЕЖИВУВАЊЕ КАЈ ХИРУРШКИ ТРЕТИРАНИ ПАЦИЕНТИ СО КАРЦИНОМ НА БУБРЕГ

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Abstract

Aim. The primary objective of this study was to examine the connection between hormone levels, specifically the fT3/fT4 ratio, and surgical outcomes in renal cell carcinoma patients. The study aimed to assess the prognostic value of hormone status in predicting progression-free survival and overall survival following surgery.

Methods. This prospective study included patients scheduled for surgical treatment of renal cell carcinoma. Patients aged over 18 years with ASA status I-III were enrolled in the study. Thyroid status was evaluated by pre- and post-surgery hormone values and ultrasonography findings preoperatively. The fT3/fT4 ratio was calculated for every patient.

Results. Analysis was conducted on 40 patients with complete data. The median age of the cohort was 64.3 years, with 1/4 of patients aged over 70. Clear cell carcinoma predominated (92.5%), with 55.1% falling into the intermediate international metastatic renal cell carcinoma database consortium, risk group. Surgery. In 82.5% of patients laparoscopic intervention was performed. The fT3/fT4 ratio significantly correlated with the median progression free survival (5, 14, and 20 months for low, intermediate, and high ratio groups, respectively) and median overall survival (7, 26, and 40 months for low, intermediate, and high ratio groups, respectively) ($p<0.005$).

Conclusion. Our findings underscore the importance of the fT3/fT4 ratio as a valuable prognostic indicator for renal cell carcinoma patients undergoing surgery. The fT3/fT4 ratio was related to worse progression free survival and overall survival, emphasizing the potential role of hormone status in predicting outcomes. potential role of hormone status in predicting outcomes.

Keywords: carcinoma, renal cell, thyroid function test, thyroid hormones

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Апстракт

Вовед. Примарната цел на оваа студија беше да се истражи поврзаноста помеѓу нивоата на хормоните, конкретно односот fT3/fT4 и хируршкиот исход за пациенти со карцином на бубрег. Студијата имаше за цел да ја процени прогностичката вредност на хормонскиот статус во предвидувањето на преживувањето без прогресија и целокупното преживување по операцијата.

Методи. Оваа проспективна студија опфати пациенти закажани за оперативен третман на бубрежен карцином. Вклучува пациенти на возраст над 18 години со АСА статус I-III. Статусот на тироидната жлезда беше евалуиран преку вредностите на хормоните пред и по операцијата и наодот од ултрасонографија на тироидната жлезда предоперативно и хормонскиот статус постоперативно. Односот fT3/fT4 беше пресметан за секој пациент.

Резултати. Спроведена е анализа на 40 пациенти со целосни податоци. Просечната возраст на групата беше 64,3 години, со 1/4 од пациентите на возраст над 70 години. Преовладуваше чисто клеточен карцином (92,5%), при што 55,1% спаѓаат во средно ризичната група од интернационалната база за податоци на бубрежни карциноми. Кај 82.5% од ациентите интервенцијата беше спроведена лапароскопски. Односот fT3/fT4 значајно корелираше со просечното преживување без прогресија (5, 14 и 20 месеци за групи со низок, среден и висок однос, соодветно) и просечно целосно преживување (7, 26

и 40 месеци за низок, среден и висок сооднос групи, соодветно) (p<0,005).
Заклучок. Нашите наоди ја нагласуваат важноста на односот fT3/fT4 како значаен прогностички показател за пациентите со карцином на бубрежни клетки кои се подложени на опертивен третман. Сооднос на fT3/fT4 беше поврзан со полошо преживување без прогресија и целокупно преживување, нагласувајќи ја потенцијалната улога на статусот на хормонот во предвидувањето на исходот.

Клучни зборови: тироидни хормони, тироидна жлезда, бубрежен карцином

Introduction

Recently, hormone levels have been linked to superior outcomes in elderly or vulnerable patients admitted to hospitals for acute illnesses [1,2]. It has been demonstrated that the presence of low-free triiodothyronine (fT3) in the absence of thyroid function abnormalities -referred to as "euthyroid sick syndrome"-is an independent predictor of prognosis in patients admitted to hospitals for coronary failure, acute coronary syndrome, end-stage renal disorder, and others [2-4]. Low fT3 levels in cancer patients are linked to a worse prognosis in individuals with various solid malignancies [5-7]. Rather than watching the averages of the two hormones (fT3, fT4), the fT3/fT4 ratio could be a far better predictor to show what proportion of peripheral deiodination activity there's. It'd even help sort patients whose fT3 levels are normal. Thyrotropin (TSH) is not widely utilized since it is less consistent and tends to remain within the traditional range for an array of reasons, including pituitary dysfunction, decreased synthesis of hypothalamic hormone resistance (THR), and decreased pulsatility of TSH. The fT3/fT4 ratio has also been shown to be predictive of a shorter overall survival (OS) and progression-free survival (PFS) among individuals with metastatic colorectal cancer.
The emergence of hypothyroidism after anti-VEGF tyrosine kinase inhibitor therapy may be a well-known good prognostic factor for renal cell carcinoma (RCC) therapy [8]. However, there is currently insufficient data on the importance of baseline thyroid measurements, specifically the fT3/fT4 ratio.
Therefore, we designed a prospective study to determine the correlation between the initial fT3/fT4 ratio and the outcome of surgery for RCC.

Material and methods

This prospective observational study was conducted at the University Clinic for Urology and University Clinic for Anesthesia, Reanimation, and Intensive Care within the tertiary University Hospital in Skopje after receiving an internal ethical review board approval and consent of patients included in the evaluation. All consecutive patients scheduled for surgical treatment of RCC, aged above 18 years with ASA status I-III, were enrolled in this evaluation. Thyroid status was assessed by the hormone values and ultrasonography findings of the thyroid before surgery, and hormone values after surgery. All biopsies were made in the same accredited hospital laboratory. The ratio of fT3/fT4 was calculated for every subject. In a 40-month period, the Kaplan-Meier approach was employed to gauge OS and PFS from the beginning of surgery to eventual death from any cause or progression of the disease. Every patient was assessed during the follow-up period. Primary outcomes of this analysis were the extent of T3, T4 and T3/T4 ratio. Secondary outcomes were postoperative complications, length of hospital stay, disease free survival and overall survival.

Results

This study included 60 patients. Only 40 patients' data were examined (Figure 1). Consequently, only individuals with complete data and therefore the ability to calculate fT3/fT4 ratio were included in the study. Demographic data and important clinical characteristics of patients are listed in Table 1.

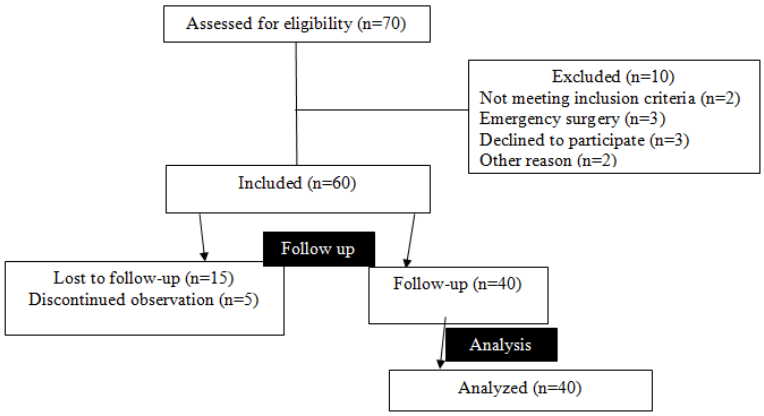

Fig. 1. Consolidated standards of reporting trials flow diagram

Table 1. Patients' characteristics and demographic data

Parameter	Number of patients (%)
Gender	
Male	28(70%)
Female	12(30%)
Age	
Median (range)	64.3
>70 years (%)	12(30%)
Histology	
Clear Cell	37(92.5%)
Other	3(7.5%)
Metastatic Sites	
Lung	10(25%)
Bone	4(10%)
Liver	1(2.5%)
CNS	/
Lymph nodes	20 (50%)
Others	5 (12.5%)
Baseline thyroid hormone levels	
fT3 (median ± SD) pmol/L	4.74±4.2
fT4 (median ± SD) pmol/L	14.5±12.4
IMDC risk classification	
Good	5(12.5%)
Intermediate	32(80%)
Poor	3(7.5%)
N/A	/
Time from diagnosis till treatment	
>12 month	4(10%)
<12 month	36(90%)

CNS, central nervous system; IMDC, international metastatic renal cell carcinoma database consortium, NA, not available

The cohort's median age was 64.3 years, and around one-quarter of the patients were older than 70. Clear cell carcinoma affected the majority of patients, with a predominance of intermediate identification of international metastatic renal cell carcinoma database consortium (IMDC) risk group (55.1%).

All patients underwent surgery. Laparoscopic intervention was performed in 33 patients (82.5%). In seven patients (17.5%) open classical surgery was performed.

Thyroid hormone levels were also assessed before and after surgery. Ultrasonography of the thyroid showed normal findings in 38 patients, two patients had single nodule of the gland. Following fT3/fT4 ratio, we divided patients into three separate groups; the baseline value between the low and intermediate groups was 0.25, and the baseline value between the intermediate and high groups was 0.35.

Table 2. Thyroid hormone levels

Parameter	Baseline	After Surgery
T3 pmol/L	3.81±1.64	4.1±1.9
T4 pmol/L	17.59±4.6	19.9±7.1
TSH uIU/ml	1.5±1.62	2.1±1.1
fT3/fT4 ratio		
low	0.21±0.1	0.20±0.12
intermediate	0.26±0.12	0.25±0.13
high	0.35±0.16	0.35±0.38

There was a significant difference ($p<0.05$) within the median PFS between the low, intermediate, and high fT3/fT4 ratio groups (5, 14, and 20 months, respectively). The median anticipation was 7, 26, and 40 months within the low, middle, and high fT3/fT4 ratio groups, respectively ($p<0.05$). The low fT3/fT4 ratio was related to worst PFS and OS. Hormone levels are presented in Table 2.

Discussion

The results obtained in this study shed a new light on an intriguing connection between hormone metabolism and renal cell carcinoma patients' prognosis and surgical outcomes.

Our analysis reasoning was motivated by clinical circumstances that resembled cancer but were not an equivalent illness. Additionally, new data on the association between hormone and oncological RCC treatment in patients with colorectal cancer was released on the same subject [9-11].

Our analysis on the importance of hormone status as a risk factor assessment that follows RCC surgery validates published data on various neoplasms by showing that hormone insufficiency, represented by a decreased fT3/fT4 ratio, is an important prognostic risk in patients with RCC who undergo a systemic treatment [7,9].

Thyroid hormone levels changing during acute or chronic illness (also referred to as non-thyroidal illness syndrome (NTIS)), may be a common occurrence linked to a poorer prognosis in patients with active spread of disease [1-4].

Nowadays, it is unclear if these alterations need to be considered a real tissue hypothyroidism requiring hormone replacement therapy, or if they represent an adaptive response to sickness [4]. Many variables are under consideration while analyzing the pathophysiology of non-thyroxine syndrome. These include aberrant activity of the hypothalamic-pituitary axis (which is vital within the early stages of acute illness), alterations in hormone metabolism, and abnormalities within the expression of hormone-binding protein and thyroid hormone receptor [4].

The family of enzymes mentioned as iodothyronine deiodinases is really responsible for determining the amounts of the active level of thyroid hormones. These enzymes can convert the biological precursor T4 (produced by the thyroid) into the "active" form T3 (by deiodinases D1 and or D1 and D2) or the inactive forms rT3 (from T4) and T2 (from T3) (by deiodinase 3, D3) [2-4]. The three deiodinases involved in the metabolic pathway vary within the tissues. D2 is expressed in striated muscle, where it is present within the cells and creates most of T3; D3 is an inactivating enzyme that is essential for placental and fetal tissues. is expressed in the liver and kidneys [8].

Chronic systemic inflammation, liver or renal dysfunction, cachexia, and chronic sickness might cause D1 and D2 to be less active and D3 to be more active, which lowers fT3 levels [12-13]. These clinical scenarios are typical in cancer patients, especially when the disease is nearing its end, and patients are usually linked to a dismal prognosis. Negative prognostic variables include neutrophil and platelet count, which are indirect measures of systemic inflammation, are widely known [14-15]. Thus, the impairment of thyronine deiodinases and NTIS may function as indirect indicator of organ dysfunction, cachexia, sarcopenia, or chronic systemic inflammation-all of which are linked to a more advanced disease, a poorer prognosis, and a poorer response to systemic therapy. Nonetheless, the pathophysiological process in cancer patients is still up for debate, as is the impact of hormone and deiodinase activity on the differentiation and proliferation of cancer cells [16]. Furthermore, while substitutive hormone therapy with triiodothyronine does not seem to achieve success in non-cancer disorders, it is uncertain if it can enhance the oncological result or, at least, clinical symptoms [4,13]. Numerous researchers in the field of oncology discovered a correlation between the low fT3 levels and a poorer prognosis in patients with lymphomas and other advanced solid malignancies [5-7]. It is interesting to notice that a worse outcome was related to higher fT4 levels in hepatocarcinoma [6]. In an examination of senior hospital patients, authors initially suggested using the fT3/fT4 ratio as an indirect indicator of deiodination impairment [10]. Even in individuals with normal T3 readings, a fT3/fT4 ratio was related to fragility and a worse outcome [6]. Our results are similar. In patients with colorectal cancer, the fT3/fT4 ratio was found to be an important predictor of outcome in those that had received extensive pretreatment. Consistent with their study, the T3/T4 ratio's prognostic significance stood alone from those of other well-known, reliable prognostic markers [9-10]. Our investigation's findings supported there findings.

Conclusion

The fT3/fT4 ratio is a valuable prognostic indicator for RCC patients undergoing surgery. Our findings contribute to the growing body of evidence suggesting that hormone status, particularly the fT3/fT4 ratio, plays an important role in predicting outcomes in cancer patients, emphasizing the necessity for further research on the topic.

Conflict of interests: None declared.

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ММР

Original article

EVALUATION OF SALIVARY HYPOFUNCTION AND ORAL COMPLICATION AFTER RADIOTHERAPY IN PATIENTS WITH MALIGNANT NEOPLASMS OF HEAD AND NECK

ЕВАЛУАЦИЈА НА САЛИВАРНА ХИПОФУНКЦИЈА И ОРАЛНИ КОМПЛИКАЦИИ ПОСЛЕ РАДИОТЕРАПИЈА КАЈ ПАЦИЕНТИ СО МАЛИГНИ НЕОПЛАЗМИ НА ГЛАВА И ВРАТ

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Abstract

Introduction. In patients with head and neck malignant neoplasms salivary glands are affected by the radiation therapy because they are located close to the place that is exposed to the total dose of radiation. Therefore, xerostomia and dysphagia are the most frequent post-radiation complications that affect the life of these patients. The volumetric modulated radiation therapy enables higher doses of radiation to be focused on the targeted place, and the surrounding healthy tissues to be exposed to the harmful effect of radiation as less as possible.

Method. This study was conducted by cooperation of the University Clinic for Radiotherapy and Oncology and University Clinic of Maxillofacial Surgery-Skopje. The study sample consisted of 30 patients treated with one of the radiation techniques: 3DCRT and VMAT. The stimulated salivary flow from parotid glands was defined using modified Lashley cups. Post-radiation xerostomia and mucositis were noted in grades based on the clinical examination, and the rest of the post-radiation complications during the first control after the radiation therapy were registered if they appeared. The moisture of oral cavity was evaluated by the modified Schirmer method.

Results. The comparison of patients treated with one of the radiation techniques showed significantly larger amount of produced stimulated saliva (ml/min) in patients treated with VMAT compared to those treated with 3DCRT (p=0,0054). An insignificant linear negative correlation was found between the volume of stimulated salivary flow (ml/min) and the localization of the malignant neoplasm-salivary flow was insignificantly decreased in patients with malignant neoplasm of the nasopharynx, oropharynx, hypopharynx and larynx ($R_{(30)}$=-0,151; p=0,4602). The mucositis appearance was significantly associated with the use of 3DCRT method. The low grade of xerostomia was significantly associated with the use of VMAT method.

Conclusion. The use of the new technique, volumetric modulated radiation therapy, enables to spare the salivary function that is proved by the increased salivary flow, the decreased level of xerostomia and the decreased representation of all other post-radiation complications.

Keywords: hyposalivation, xerostomia, malignant neoplasm of head and neck, volumetric modulated radiation therapy, irradiation consequences

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Апстракт

Вовед. Кај пациенти со малигни неоплазми на глава и врат, терапијата со радијација несомнено ги афектира саливарните жлезди од едноставна причина што тие се наоѓаат во близина на местото каде што се дистрибуира тоталната доза на радијација. Последователно на тоа ксеростомијата и дисфагијата се најчестите пострадијациони компликации кои го нарушуваат квалитетот на живот на овие пациенти. Волуметриски модулираната радиотерапија овозможува повисоките дози на радијација да бидат фокусирани на целното место, а околните здрави ткива да бидат колку е можно помалку изложени на штетното дејство од радијацијата.

Методи. Истражувањето се заснова на соработка помеѓу Универзитетската клиника за радиотерапија и онкологија и Универзитетската клиника за максилофацијална хирургија-Скопје. Истажувачкиот примерок вклучува 30 пациенти третирани со една од техниките на радиотерапија: 3D CRT и VMAT. Стимулираниот саливарен проток од паротидни жлезди се одредуваше со помош на модифицирани Lashley cups. Пострадијационата ксеростомија и мукозитис се евидентираше во степени врз

основа на клиничкиот преглед, а сите други пострадијациони компликации за време на првата контрола после завршена радиотерапија, се евидентираа со присуство и отсуство на истите. Мерењето на влажноста на оралната празнина се изведуваше со помош на модифицирана Schirmer метода.

Резултати. Споредбата на пациентите третирани со една од двете техники на радиотерапија укажува на сигнификантно поголема количина на излачена стимулирана салива (ml/min) кај пациентите третирани со VMAT споредено со 3D CRT (p=0,0054). Помеѓу количина на стимулиран саливарен проток (ml/min) и локализацијата на малигна неоплазма утврдена беше несигнификантна линеарна негативна корелација-саливарниот проток несигнификантно се намалуваше кај пациентите со малигном на Nasopharynx, Oropharynx, Hypopharynx, Larynx ($R_{(30)}$= - 0,151; p=0,4602). Од двете методи, појавата на оралниот мукозитис сигнификантно асоцираше со методот 3D CRT. Слабиот степен на ксеростомија сигнификантно асоцираше со методот VMAT.

Заклучок. Примената на новата техника на волуметриски модулирана радиотерапија овозможува да се зачува саливарната функција кое се докажува преку зголемениот саливарен проток, а намален степен на ксеростомија и намалена застапеност на сите пострадијациони компликации.

Клучни зборови: хипосаливација, ксеростомија, малигни неоплазми на глава и врат, волуметриски модулирана зрачна терапија, ирадијациони последици

Introduction

The therapy in patients with malignant head and neck neoplasm that consists of radiation inevitably affects salivary glands and their surroundings; therefore patients face negative consequences such as progressive malfunction of salivary glands, followed by a significant level of xerostomia (Figure 1). Despite all of the efforts to protect the surrounding tissues from damaging during the radiation treatment, salivary glands are still exposed to the radiation due to their close location to the exposed area [1]. Having this in mind, xerostomia in most of the cases is the main cause of the symptoms: oral mucositis, changes in oral microflora, dysphagia, throat inflammations, changes or loss of the taste, caries, changes in the voice quality, halitosis, discomfort, problems with chewing and swallowing, which lead to nutritive complications and loss of weight in the future [2,3].

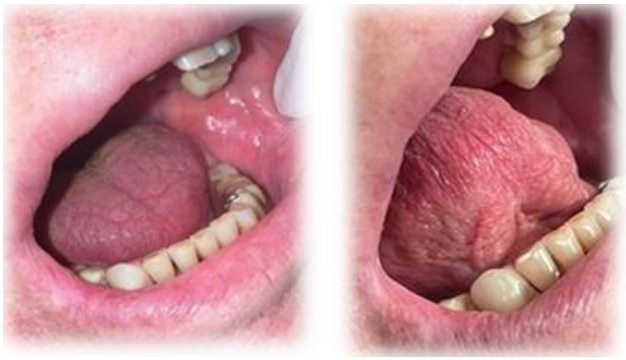
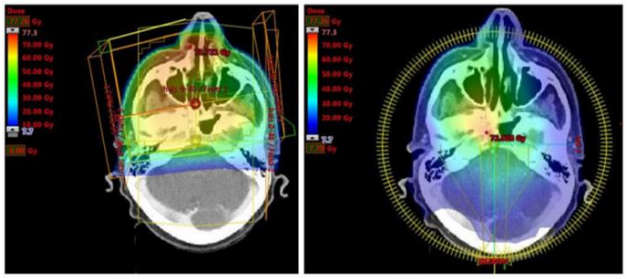


Fig. 1. Dry mouth – xerostomia

Salivary gland impairment due to radiation therapy happens in 4 different phases: the first phase happens from the 1st to the 10th day, when the water component in the tissue is being eliminated, but the acinar cells and amylase secretion are not being affected; the second phase takes place from the 10^{th} to the 120^{th} day, acinar cells are exposed to membrane degeneration and they also lose their ability to produce amylase; the third phase is from the 120^{th} to the 240^{th} day, known as a phase of belated toxicity characterized by losing the function of acinar cells as a result of stem cells loss; the fourth phase is known as a regenerative phase, however the deterioration of the salivary gland function is still continuing in this phase as a result of the nerves damaging used for glands ducts and blood vessels [4,5].

3DCRT-Three-Dimensional Conformal Radiation Therapy (3DCRT) and Volumetric Intensity Modulated Arc Therapy (VMAT) are two different techniques that are aimed at providing a precise and efficient treatment for patients with malignant neoplasms (Figure 2). Three-dimensional conformal radiation therapy is a standard technique that enables a good view of the anatomic structures and radiation beams in three dimensions. The segments for radiation treatment can be modified individually according to the shape of the tumor. On the other hand, the volumetric modulated radiation therapy is considered to be innovative and advanced technique that offers modulation of the radiation intensity in small multiple parts; therefore, this method enables higher doses of radiation to be focused on the targeted place, while the surrounding healthy tissues are exposed to the harmful effects of radiation as less as possible. Recent studies have been focused to prove that the new approach of modified radiation therapy (volumetric modulated radiation therapy-VMAT) is expected to reduce the xerostomia incidence, and subsequently to improve the quality of patients' life that have been treated with this method of radiation therapy [6,7].



(a) (b)

Fig. 2. Comparison between three-dimensional conformal radiation therapy (a) and volumetric modulated radiation therapy (b)

Aim

The main aim of this study was to evaluate the salivary hypofunction by determining the parotid stimulated salivary flow and assessment of the oral complications in patients with malignant neoplasms of the head and neck, treated with three-dimensional conformal radiation therapy and volumetric modulated radiation therapy.

Material and methods

The study sample consisted of 30 patients treated with one or both techniques of radiation therapy: 3DCRT or VMAT. Taking into consideration in advance defined exclusion and inclusion criteria, patients treated with one of the techniques of radiation therapy at the University Clinic for Radiotherapy and Oncology in Skopje were included in the study. The clinical examination involving the measurement of stimulated salivary flow and moisture of oral cavity was performed at the University Clinic of Maxillofacial Surgery with previously obtained patients' written consent. Salivary flow measurement and post-radiation complications were done immediately after the first assessment of the finished radiation therapy. Xerostomia and oral mucositis were noted in grades; however, the rest of the post-radiation complications were noted only if they appeared. Stimulated saliva from the parotid glands was collected using special devices made in line with the modified Lashley cups principle, and they were made by dental laboratory technicians, using silicone mass for duplication and connecting it with a tube system that allows collection of saliva from both parotid glands at the same time. The device is being placed in the upper vestibule where the salivary duct of parotid gland is located, and is fixed by using vacuum. Saliva from the duct flows in the oval part of the device from where it is being moved through the tube system saliva and collected in microliters graduated syringe. The obtained values are being divided by the minutes spent in saliva collection to define the salivary flow in one minute. In order to gather stimulated saliva, stimulation was done by using citric acid applied on a cotton applicator and placed on the dorsum part of the tongue, specifically on the dorso-lateral edges of the tongue, five times in duration of 2 minutes (Figure 3).

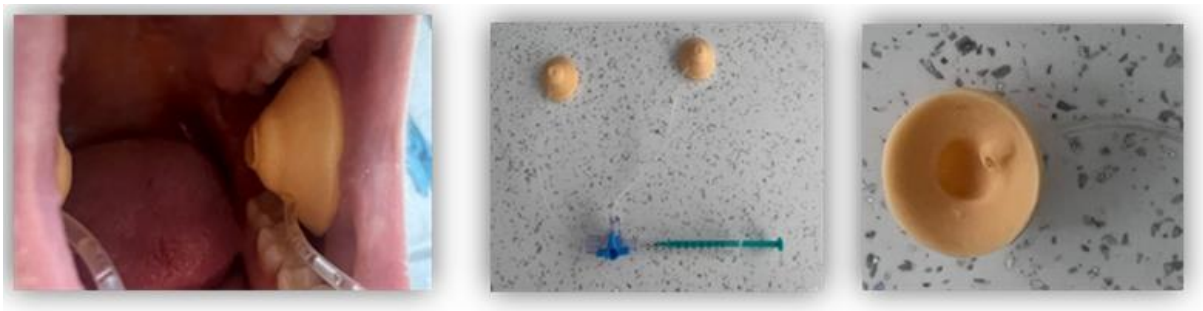


Fig. 3. Modified Lashley cups

By using the modified Schirmer method for measurement of the moisture in the oral cavity, Schirmer stripes are applied at the bottom of the oral cavity of a patient, and the results are available 3 minutes later (Figure 4). The obtained results are noted immediately; the stripes are divided in millimeters starting with 5 mm to 35 mm. If the observed result was lower than 5 mm, we considered it as value 5. The results were registered from the size of wet zone on the stripes in milimeters, that can be easily seen as a darker part.

According to Primary site of malignant neoplasm classification, patients were divided in two large groups. The first group was consisted of patients with malignant neoplasm located on the: lip, oral cavity, tongue, floor of the mouth, buccal mucosa, retromolar fossa, hard palate, maxilla and mandible. The second group was consisted of patients with malignant neoplasm located on the: nasopharynx, oropharynx, hypopharynx and larynx.

Fig. 4. Modified method for measuring mouth moisture

Results

The evaluation of patients with malignant neoplasm located on the lip and oral cavity treated with different methods showed a significantly higher volume of stimulated saliva (ml/min) in patients treated with the VMAT compared to those treated with the 3DCRT technique (p=0.0054).

Patients with malignant neoplasm of the nasopharynx, oropharynx, hypopharynx and larynx, treated with the VMAT method showed insignificantly bigger volume of produced saliva (ml/min) compared to those treated with the 3DCRT method (p=0.0588) (Table 1).

Table 1. Comparison of saliva (ml/min) between 2 groups of participants

Parameters	N	Mean± SD	Min / Max	Median (IQR)	p
Saliva (ml/min) - Lip, Oral cavity (tongue, floor of the mouth, buccal mucosa, retromolar fossa, hard palate, maxilla, mandible)					
VMAT	8	0.21±0.09	0.08/0.30	0.23(0.14-0.30)	Z=2.781
3DCRT	7	0.06±0.01	0.05/0.08	0.06(0.06-0.07)	p=0.0054*
Saliva (ml /min) - Nasopharynx, Oropharynx, Hypopharynx, Larynx					
VMAT	7	0.19±0.11	0.06/0.30	0.21(0.10-0.29)	Z=-1.889
3DCRT	8	0.07±0.02	0.05/0.11	0.08(0.05-0.09)	p=0.0588

SD-standard deviation, IQR-Interquartile range, *significant for p<0.05

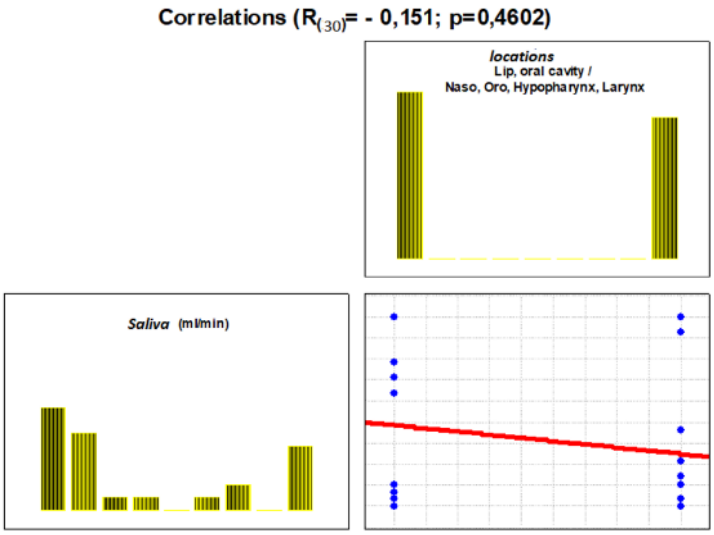


Fig. 5. Correlation between stimulated salivary flow and malignant neoplasm localization

There was an insignificantly linear negative correlation between stimulated salivary flow (ml/min) and malignant neoplasm localization-salivary flow insignificantly decreased in patients with malignant neoplasm of the nasopharynx, oropharynx, hypopharynx, and larynx

($R_{(30)}$= - 0,151; p=0,4602) (Fig.1a)

Table 2. Distribution of post-radiation complications

Post-radiation complications	3DCRT (n=15)	VMAT (n=15)	Total (n=30)	p
grade 1- mild	2(16.66%)	9(75%)	11(45.83%)	p=0.0123*
grade 2 - moderate	8(66.67%)	3(25%)	11(45.83%)	p=0.0996
grade 3 - severe	2(16.66%)	0(0%)	2(8.34%)	-
grade 4- disabling; life threatening	0(0%)	0(0%)	0(0%)	-

Fisher Exact test, *significant for, p<0.05

Mucositis as a complication after treatment was significantly associated with using the 3DCRT method for radiation therapy. The emerge of the other post-radiation complications (difficulties in mastication, dysphagia, dysgeusia and dysphonia) was not associated with any of the methods used for radiation treatment (3DCRT/VMAT) (Table 2).

Table 3. Distribution of xerostomia grades

Xerostomia grading scale - NCI CTCAE	3DCRT (n=15)	VMAT (n=15)	Total (n=30)	p
Oral mucositis	10(83.3%)	4(33.3%)	14(58.3%)	p=0.0384*
Difficult mastication	11(91.6%)	7(58.3%)	18(75%)	p=0.1573
Dysphagia	11(91.6%)	7(58.3%)	18(75%)	p=0.1573
Dysgeusia	11(91.6%)	6(50%)	17(70.8%)	p=0.0724
Dysphonia	9(75%)	5(41.6%)	14(58.3%)	P=0.2149

Fisher Exact test, *significant for, p<0,05

The low level of xerostomia was associated signify-cantly with the usage of the VMAT method. The moderate level of xerostomia was found in borderline association with the 3DCRT method. The severe level of xerostomia was found only in 2 patients treated with the 3DCRT method, and in none of patients treated with the VMAT method. Disabling xerostomia was not registered in any of the examined patients in the study (Table 3).

The results obtained by using the modified Schirmer test were as follows: the average value of produced saliva volume in patients treated with the three-dimensional conformal radiation therapy was 4.2 mm; modus 1 and 0; median 3.5; maximal value was 9 mm and minimal was 0 mm; standard deviation 1.0. In the second group of patients treated with the volumetric modulated radiation therapy, the mean value was 5.5 mm; modus 2; median 6; maximal 12 mm and minimal value was 0.2 mm; standard deviation was 0.4 (Table 4).

Table 4. Average values of collected saliva using the modified Schirmer test measured in mm

Type of radiation therapy	Mean	Modus	Median	Max	Min	SD
3DCRT	4.2	1; 0	3.5	9	0	1.0
VMAT	5.5	2	6	12	0.2	0.4

Discussion

During the radiation process it is necessary to protect the salivary gland function from the damaging effects of the radiation. Protection of the submandibular Salivary glands during radiation is more complicated compared to that of the parotid glands due to their location that is in the same place as the affected lymphatic nodes. The use of volumetric modulated radiation therapy is based on computer controlled linear accelerators that apply the radiation doses precisely on the place that is specified for radiation or more precisely where the tissue is affected by the tumor [7,8].

By introducing the new method of volumetric modulated radiation therapy (VMAT), xerostomia that appears after radiation treatment in patients is significantly reduced, which also contributes to improving the quality of life in patients treated with radiation therapy [9,10].

The study by Taoran C. *et al.* evaluated a total of 222 patients treated with IMRT and VMAT radiation therapy, and were followed in a period of 23 months and 7 months. The results obtained by using the VMAT showed significant improvement that resulted in significantly lower grades of second-degree dysphagia and xerostomia in patients after the received radiation therapy [11,12].

Nutting C M, Morden JP. found that 4% of their study participants suffered of a post-radiation xerostomia after treatment with the VMAT. The main difference in the approach with sparing the parotid glands during treatment had been in the following period of regeneration of the parotid glands.. The damaged parotid glands showed the ability of regeneration to some degree in a 2-year period after the VMAT treatment, which compared to xerostomia in patients treated with the conventional radiation therapy proved to be permanent. After 12 months following the treated patients, xerostomia was registered in 73% of the total number of participants (82). Severe xerostomia was proved to be significantly rare in the group of participants treated with the intensity modulated radiation therapy (38%), compared to the group of patients treated with the conventional radiation therapy where the result was 74% [13,14]. According to Chris Nutting, the modified radiation therapy VMAT needs to be taken and applied as a golden standard in patients with high risk of radiation-related xerostomia [13]. Chottetanaprasith in his evaluation of 33 patients treated with VMAT presented results showing that survival rate at 3 years after treatment was 81%; acute symptoms as a result of the therapy were mucositis in 36% of participants, first grade xerostomia in 57.6%, and second grade xerostomia in 24.2% of participants. A conclusion was derived that using the VMAT approach in treating patients with nasopharyngeal carcinoma was acceptable and it offered good results with high survival rate and acceptable negative consequences [15].

Conclusion

In conclusion, the usage of the new technique-volumetric modulated radiation therapy-offers sparing of the salivary glands function, which can be proved with the increased salivary flow, decreased grade of xerostomia and decreased representation of all post-radiation complications compared to patients treated with the three-dimensional conformal radiation therapy.

Conflict of interest statement. None declared.

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ММР

Original article

DIFFERENTIATING SECONDARY PROGRESSIVE AND RELAPSING-REMITTING MULTIPLE SCLEROSIS: CEREBROSPINAL FLUID BIOMARKERS

ДИФЕРЕНЦИРАНСКА СЕКУНДАРНА ПРОГРЕСИВНА И ПОВТОРНА-РЕМИТИРАЧНА МУЛТИПЛА СКЛЕРОЗА: БИОМАРКЕРИ НА ЦЕРЕБРОСПИНАЛНА ТЕЧНОСТ

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Abstract

Introduction. Multiple sclerosis (MS) is a prevalent autoimmune disorder affecting 2.1 million individuals globally, with the disease mechanisms and effective treatments still not fully understood. Research into cerebrospinal fluid (CSF) biomarkers has provided insights into the inflammatory process's indicative of MS progression, aiding in differential diagnosis and potentially predicting clinically definite MS.
Aims. It was our aim to make a comprehensive evaluation of the admission CSF biomarkers, as well as their association with the CSF immunological patterns for their role as early predictors for MS disease progression in a cohort group of 83 MS patients.
Methods. A 2-year longitudinal cohort study on Interferon beta-1a-treated MS patients employed qualitative (OCGB detection), semiqualitative (DISC-PAGE and densitometry tracing), and quantitative (laser nephelometry) methods. These approaches were aligned to evaluate sensitivity and select the optimal method for distinguishing multiple sclerosis types. The proposed morphological characterization of CSF electrophoretic signatures, associated with CSF immunological types, includes assessed sensitivity and specificity for estimated cut-off values.
Results. The 2-year Interferon beta-1a treatment resulted in a median EDSS score of 2.5 in the cohort group. DISC-PAGE electrophoretic signatures exhibited higher sensitivity than quantitative laser nephelometry for detecting CSF types (INF, TU, and INF+TU). New lower cut-off values for CSF biomarkers discriminating between INF, TU, and combined INF+TU types were proposed with high sensitivity and specificity. A developped CDA model offers a more accurate assessment of immunological CSF types in MS. For OCGBs, the IgG synthesis rate, specifically the TOURT formula, showed the highest association, with a value of ≥ 0.75 mg/24 h considered optimal for detecting intrathecal IgG synthesis (96% sensitivity, 75.8% specificity). In prognostication, routine markers failed, but CSF lactate levels (cut-off ≥ 2.01 mmol/L) emerged as the sole significant early predictor for SPMS development. Additionally, admission CSF lactate levels and CSF IgG were identified as significant early predictors for functional disability. The final model incorporated non-significant predictors, including the presence of OCGBs, combined INF+TU CSF type, CSF IgA levels, and age, contributing significantly to the overall predictive capacity through their direct and indirect effects.
Conclusions. DISC-PAGE electrophoresis with densitometry tracing is recommended for sensitive detection of CSF immunological patterns. Proposed cut-off values for albumin index, IgG index, CSF IgG, and CSF lactates could enhance the accuracy, sensitivity, and comprehensiveness in assessing multiple sclerosis types.

Keywords: cerebrospinal fluid biomarkers, multiple sclerosis, DISC-PAGE, cerebrospinal fluid immunological patterns, cerebrospinal fluid lactate levels, CDA

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Апстракт

Вовед. Мултиплекс склерозата (МС) е распространето автоимуно пореметување кое погодува 2.1 милиони лица на глобално ниво со сеуште нецелосно разбрани механизми на болеста и ефективните третмани за лекување. Истражувањето на биомаркерите на цереброспиналната течност (CSF) дава увид во воспалителните процеси кои укажуваат на прогресијата на МС, помагајќи во диференцијалната дијагноза и потенцијално предвидувајќи клинички дефинитивна МС.
Цели. Наша цел беше сеопфатна евалуација на приемните биомаркери, како и нивната поврзаност со имунолошките обрасци на цереброспиналната течност и нивната улога како рани предвидувачи за прогресијата на МС во група од 83 пациенти.
Методи. Двогодишна лонгитудинална кохортна студија на пациенти со мултиплекс ссклероза третирани со интерферон бета-1а, притоа користевме

квалитативни (OCGB детекција), полуквалитативни (DISC-PAGE и трасирање со дензитометрија) и квантитативни (ласерска нефелометрија) методи. Овие пристапи беа усогласени за да се процени чувствителноста и да се избере оптималниот метод за разликување на типовите мултиплекс склероза. Предложената морфолошка карактеризација на електрофоретските потписи на цереброспиналната течност, поврзани со имунолошките типови на цереброспиналната течност, вклучува проценка на чувствителност и специфичност за определување на гранични вредности.

Резултати. Двогодишниот третман со интерферон бета-1а резултираше со медиана на EDSS од 2,5 во кохортната група. DISC-PAGE електрофоретските потписи на покажаа поголема чувствителност од квантитативната ласерска нефелометрија за откривање на типови CSF (INF, TU и INF+TU). Беа предложени нови пониски гранични вредности на биомаркерите за CSF кои ги разликуваат INF, TU и комбинираните типови INF+TU со висока чувствителност и специфичност. Развиениот CDA модел нуди попрецизна проценка на имунолошките типови на CSF кај МС. За OCGB, стапката на синтеза на IgG, конкретно формулата TOURT, покажа највисока поврзаност, со вредност од ≥0.75 mg/24 ч која се смета за оптимална за откривање на интратекална IgG синтеза (96% чувствителност, 75,8% специфичност). Во прогнозата, рутинските маркери не успеаја, но нивоата на лактат на цереброспиналната течност (cut-off ≥2.01 mmol/L) се појавија како единствен значаен ран предиктор за развој на SPMS. Дополнително, нивоата на лактатво во цереброспиналната течност и нивото на IgG во цереброспиналната течност беа идентификувани како значајни рани предвидувачи за функционална попреченост. Конечниот модел инкорпорираше незначајни предвидувачи, вклучително и присуството на OCGB, комбиниран тип INF+TU CSF, нивоа на CSF IgA и возраст, што значително придонесува за севкупниот капацитет на предвидување преку нивните директни и индиректни ефекти.

Заклучок. DISC-PAGE електрофореза со дензитометриско трасирање се препорачува за чувствителна детекција на имунолошки шеми на CSF. Предложените гранични вредности за индекс на албумин, IgG индекс, CSF IgG и CSF лактати би можеле да ја подобрат точноста, чувствителноста и сеопфатноста во проценката на типовите на мултиплекс склероза.

Клучни зборови: биомаркери на цереброспиналната течност, мултиплекс склероза, DISC-PAGE, имунолошки обрасци на цереброспиналната течност, нивоа на лактат во цереброспиналната течност, CDA

Introduction

Multiple sclerosis (MS) is a persistent, inflammatory autoimmune disorder affecting the brain and spinal cord, characterized by focal lymphocytic infiltration leading to demyelination and neurodegeneration across the central nervous system (CNS). Globally, it constitutes a significant health challenge, impacting approximately 2.1 million individuals and stands as a primary cause of nontraumatic disability among young adults [1,2]. Despite its prevalence, there is a lack of effecttive treatments, and the precise mechanisms driving disease progression remain incompletely understood [3]. Given the inherently unpredictable nature of the disease and the substantial risk of developing severe disability, there is an urgent need for prognostic and predictive biomarkers to facilitate risk stratification, enabling early identification of vulnerable individuals and informed management for secondary prevention of adverse outcomes [4-6].

Cerebrospinal fluid (CSF) biomarkers have been a long-standing focal point in multiple sclerosis (MS) research, owing to their ability to unveil the inflammatory origins of cerebral abnormalities associated with the disease. A comprehensive CSF analysis, encompassing fundamental biochemical assessments, specific CSF protein determinations, and isoelectric focusing (IEF), yields highly sensitive diagnostic insights into concurrent inflammatory processes within the central nervous system (CNS), indicative of MS progression [7]. Previous CSF investigations have substantiated heightened inflammation, axonal damage, demyelination, and oxidative stress in progressive MS [8-11]. The 2010 Revision to the McDonald Criteria underscores the significance of positive CSF findings, such as an elevated immunoglobulin G (IgG) index or the presence of two or more specific oligoclonal IgG bands (OCGBs) solely detectable in the CSF, not the appropriately diluted serum with matched IgG concentration [12]. These findings not only support the inflammatory demyelinating nature of the underlying condition but also aid in differential diagnosis and predicting clinically definite MS.

Despite the typically benign initial clinical course of multiple sclerosis (MS), early pathological studies have revealed considerable axonal injury and tissue loss, suggesting potential utility of admission cerebrospinal fluid (CSF) biochemical markers as early indicators of neurological damage in MS [2]. Given the lack of consensus on the prognostic value of routine CSF biomarkers, quotients, or indices, this study aimed to comprehensively evaluate admission CSF biomarkers and their correlation with CSF immunological patterns to serve as early predictors of disease progression and functional disability in an MS patient cohort. Employing an integrated approach that combines qualitative (oligoclonal band detection), semiqualitative (CSF exami-

nation via DISC-PAGE and densitometry tracing), and quantitative (CSF biomarker levels) methods, we aimed to assess the sensitivity of these approaches and identify the most appropriate method for prognostic-cating the disease course in MS. Additionally, we propose morphological characterization of CSF electrophoretic signatures and their association with defined CSF immunological patterns, providing assessed sensitivity and specificity for the proposed cut-off values.

The investigation of cerebrospinal fluid (CSF) biomarkers and immunological patterns holds immense significance in unraveling the complexities of multiple sclerosis (MS), a neuroinflammatory disorder characterized by diverse clinical courses. Among the analytical tools employed, Canonical Discriminant Analysis (CDA) has emerged as a powerful statistical approach for discerning distinctions among various CSF immunological patterns-specifically those characterized as inflammatory (INF), transudative (TU), and a combination of both (combined INF+TU CSF) [13]. Additionally, CDA serves as a valuable tool for stratifying different MS types based on disease progression, notably distinguishing between Secondary Progressive MS (SPMS) and Relapsing-Remitting MS (RRMS). This research endeavors to harness the potential of CDA in enhancing our understanding of the intricate interplay between CSF immunological patterns and the diverse trajectories of MS, shedding light on crucial diagnostic and prognostic implications.

Material and methods

Study subject

Between January 2011 and December 2017, we conducted an extensive prospective longitudinal cohort study, focusing on individuals receiving Interferon beta-1a treatment for multiple sclerosis (MS). During this period, patients suspected of MS, typically presenting with monofocal or multifocal clinically isolated syndrome (CIS), were admitted to the University Clinic for Neurology as inpatients for initial screening. This comprehensive screening involved a thorough diagnostic work-up, encompassing medical history examination, clinical and paraclinical assessments, MRI scans of the entire neural axis, CSF analyses, and sensory and motor-evoked potentials. The diagnostic lumbar puncture and blood collection were performed on the day of admission. Following completion of the initial screening and inpatient care (typically lasting 2 to 3 weeks), patients received a final diagnosis based on the McDonald criteria [12].

Despite the diagnosis of MS, the study incorporated specific inclusion criteria: participants without concurrent neurological, psychiatric, or neurodegenerative conditions, as well as individuals without immunological disorders or severe comorbidities such as cardiovascular, respiratory, renal, or hepatic diseases, tumors, or cancer. Furthermore, patients should not have received methylprednisolone, other immune-modulating drugs or supplements (such as cyclophosphamide, azathioprine, cyclosporine A, or corticosteroids for a minimum of 2 months prior to admission). Key exclusion criteria during this phase included the detection of a normal or inflammatory transudate type of CSF immunological pattern through DISC-PAGE densitometry and contraindications for lumbar puncture. All participants provided informed consent, and the study received approval from the Human Research Ethics Committee at Ss. Cyril and Methodius University, Faculty of Medicine in Skopje.

Patients underwent a careful two-year follow-up period, after which they underwent re-evaluation. The re-evaluation included a thorough clinical and neurological examination, assessing disease progression, potential medical complications, and rescanning with MRI. Functional disability over the two years was quantified using the Expanded Disability Status Scale (EDSS) [14]. Disease progression, categorized as primary progressive MS (PPMS), secondary progressive (SPMS), or relapsing-remitting MS (RRMS), was determined during re-evaluation. PPMS was diagnosed based on revised McDonald criteria [12], RRMS in patients with two or more relapses in the investigated period, and SPMS in patients with a relapsing-remitting course followed by at least six months of continuous progression during the study, with or without relapses. Exclusion criteria during re-evaluation included PPMS patients (due to their low representation) and those with severe disability (essentially confined to a wheelchair, i.e., EDSS scores >6.5).

Out of 340 patients suspected of MS, 293 met the initial screening criteria and were diagnosed with clinically definite MS. After applying inclusion/exclusion criteria, 163 patients were considered eligible for the cohort study. Over the 2-year follow-up, 80 patients were excluded due to reasons like incomplete data (n=16), therapy changes (n=32), confirmed PPMS diagnosis (n=11), and severe disability on re-evaluation (n=21). The final dataset comprised Interferon beta-1a-treated SPMS and RRMS patients with observable CSF immunological pattern changes on admission, who did not develop severe disability within the 2-year treatment period post-diagnosis.

Biochemical analysis

Upon admission, morning blood samples were collected after overnight fasting, left to clot at room temperature for 30 min, and then centrifuged at 3,000×g for 15 min at +4°C. The obtained serum was stored at –80°C. Concurrently, diagnostic lumbar puncture and CSF sampling were performed; CSF was cooled on an ice bath during sampling, centrifuged at 200×g for 15

min (at 4°C), and the cell-free CSF was aliquoted and stored at –80°C until further analysis.

Quantitative methods

The quantitative detection of total CSF proteins, total serum proteins, CSF lactate levels, CSF glucose, CSF urea levels and CSF creatinine were performed on Mindray BS-200 Chemistry Analyzer, with the following kits from BioSystems S.A. (Costa Brava, Spain): Protein (urine-CSF) Pyrogallol red kit (Cat. No. 21512), Protein (total) biuret kit (Cat. No. 11500), Lactate oxidase/peroxidase kit (Cat. No. 23736), Glucose-hexokinase kit (Cat. No. 11656), Urea-UV urease/glutamate dehydrogenase kit (Cat. No. 11517) and Creatinine-ENZ (Cat. No. 11734).
The quantitative detection of CSF IgG, serum IgG, CSF IgA, CSF IgM, serum albumins and CSF albumins was performed on BN ProSpec System, by means of laser immunonephelometry and particle-enhanced immunonephelometric. A parallel sample of serum and CSF were run for the albumin and IgG levels in order to calculate the corresponding quotients.

Semiquantitative methods

The CSF Discontinuous Polyacrylamide Gel Electrophoresis (DISC-PAGE) was performed followed the Ornstein (1964) and Davis (1964) procedure using the Canalco model 1200 R research disc electrophoresis apparatus. Separation employed 5% stacking (0.125 mol/L Tris-HCL, pH=6.8) and 7.5% separating (0.375 mol/L Tris-HCL, pH=8.8) gels in TRIS-glycine running buffer with ionizable tracking dye (0.005% bromophenol blue) at 5 mA per sample. Gels were stained with Amido black 10B, and densitometric tracing utilized a SHARP JX 330 scanner and Image Master 1D Elite software. CSF protein fraction interpretation was done in line with the standard literature (Epstein *et al*., 1976), and densitometric analyses, detailed in reference [15], quantified each protein zone by peak area, comparing it to a standard.

Morphological characterization of the electrophoretic patterns

Following densitometric analyses, electrophoretic patterns were categorized into distinct cerebrospinal fluid (CSF) immunological types based on the neuroimmunology classification by Schuller and Delasnerie [16], later endorsed by the European Charcot Foundation consensus [17], with minor terminological adjustments. The patterns included:

a) Inflammatory CSF pattern (INF): Indicated local synthesis, featuring significantly elevated G zone (25-30% increase in peak density), dominant G band (around 3 times larger band percentage than normal CSF, exceeding 28%), and a slightly decreased albumin zone (30-38% of the albumin band).
b) Non-inflammatory transudate CSF pattern: Indicated abnormal barrier function, marked by notably reduced prealbumin zone, increased albumin zone (band percentage >48%, peak area >5000), elevated A zone, and decreased B2 zone. Detection of α2-macroglobulin and haptoglobin 1-1 bands between B2 and G zone is typical, along with the presence of haptoglobin 2-1 and 2-2 polymers.
c) Inflammatory plus transudate CSF pattern (INF+ TU): Demonstrated characteristics of both INF and TU patterns, resembling TU with a markedly increased G zone as seen in INF types.

Qualitative methods (detection of oligoclonal IgG bands - OCGBs)

Oligoclonal bands (OCGBs) in CSF were identified by isoelectric focusing (IEF) on polyacrylamide gels (pH 3-9) using silver staining and immunofixation. Conducted on a PhastSystem TM instrument, the IEF followed the manufacturer's guidelines, utilizing the CSF analysis kit for PhastSystem (GE Healthcare, 18-1039-14). To confirm IgG origin, immunofixation employed anti-human IgG (Dako A424). After immunofixation, silver staining was executed with the PlusOne Silver staining kit, Protein (17115001), using the IEF staining program. Only patients exhibiting oligoclonal CSF patterns with polyclonal serum IgG were considered positive for OCGBs (requiring a minimum of three additional OCGBs in CSF detected by silver staining and one by immunofixation).

Statistical analysis

Data were entered into SPSS software (version 20.0, Chicago, IL, USA) and assessed for normal distribution using the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) was employed to compare serum levels of sCD163, sCD4, sCD8, Ig-G, IgM, and Ig-A among cases (CIS, RRMS, SPMS, PPMS, NMO) and healthy controls. Discriminant analysis was conducted on six levels (CIS, RRMS, SPMS, PPMS, NMO, healthy controls) and eight variables (six serum concentrations). Two-tailed tests were utilized, and significance was set at $p \leq 0.05$. Statistical analyses, including $\chi 2$ tests for categorical variables, were performed in IBM SPSS Statistics® 21 and JMP® 14 for modeling. As most quantitative variables deviated from normality, results are presented as median and interquartile range (IQR) with boxplots. The Kruskal-Wallis H test, followed by Mann-Whitney U tests for multiple comparisons, assessed central tendencies among groups.
Canonical Discriminant Analysis (CDA) was conducted to identify discriminative variables distinguishing

various cerebrospinal fluid (CSF) immunological patterns (INF *vs.* TU *vs.* Combined INF+TU CSF) and different multiple sclerosis (MS) types based on disease progression (SPMS *vs.* RRMS). Receiver Operating Characteristic (ROC) curve analyses were employed to evaluate the discriminatory ability of CDA, measured by the Area Under the Curve (AUC). Additionally, ROC analyses were utilized to determine optimal cut-off points, facilitating discrimination between diverse CSF immunological patterns and MS types.

Results

Correlations between CSF biochemical markers on admission and the identification of specific CSF immunological types based on their DISC-PAGE patterns

The neuroimmunology categorization of CSF immunological patterns, based on proposed cut-off values (CSF IgG > 40 mg/L and CSF albumin > 334 mg/L by Schuller and Sagar [18]), yielded suboptimal outcomes when compared to obtained CSF electrophoretic patterns. Despite 16.9% of patients being initially classified with normal CSF by quantitative analysis, detailed electrophoretogram densitometry revealed that 6 cases labeled as "normal" exhibited significantly increased G zone and typical INF signature, while 8 had elevated albumin content with alterations characteristic of the TU signature. Electrophoretic patterns subsequently identified these types as INF CSF and TU CSF, respectively.

The proposed threshold values had low sensitivity (6.3%) and high specificity (94.1%) for detecting the TU type. Using these cut-off values resulted in numerous false negatives (8 cases misclassified as normal, 7 as inflammatory), suggesting that lower CSF albumin levels may offer better sensitivity in detecting the TU type.

Nearly all quantitatively measured variables (excluding CSF glucose, urea, and creatinine levels) displayed some correlations with the defined CSF types based on their electrophoretic signatures (Figure 1, b-c). The most substantial effect size among these associations was observed for CSF IgG levels (H = 37.50, ***p=7.20·10−9, ε^2=0.457) and the total levels of CSF proteins (H=35.56, ***p=1.90·10−8, ε^2=0.434). Strong positive relationships were also noted for CSF albumin levels (H=29.316, ***p=4.31·10−7, ε^2=0.358), albumin index (H=29.19, ***p=4.59·10−7, ε^2=0.356), 24-hour IgG synthesis rate (H=28.31, ***p=7.13·10−7, ε^2=0.345), IgG index (H=26.46, ***p=0.000002, ε^2=0.323), and CSF lactate levels (H=24.72, ***p=0.000004, ε^2= 0.301). Additionally, CSF IgA (H=22.96, ***p= 0.00001, ε^2=0.274) and IgM (H=21.30, ***p=0.00002, ε^2=0.260) levels exhibited associations with specific electrophoretic patterns, albeit with moderately strong effects.

Mann-Whitney tests revealed significantly higher levels of most CSF biomarkers in the combined INF+TU type compared to the INF pattern, with the largest effect size observed for general protein markers (Fig. 1, b): total CSF proteins (r=0.688, ***p=1.77·10−8), CSF albumins (r=0.675, ***p=3.36·10−8), and albumin index (r=0.669, ***p=4.34·10−8). Optimal cut-off points for detecting the combined INF+TU type were determined with ROC analyses (N = 67, Figure 1, d): ≥0.505 g/L for total CSF proteins (92.9% sensitivity, 98.1% specificity, ROC AUC=0.992, ***p=1.8·10−8), ≥251.5 mg/L for CSF albumins (92.9% sensitivity, 94.3% specificity, ROC AUC=0.982, ***p=3.4·10−8), and ≥5.55 for albumin index (92.9% sensitivity, 94.3% specificity, ROC AUC=0.978, ***p=4.4·10−8). Moderate effect size relations were also noted for immunological markers (CSF IgM (r=0.433, ***p=0.0004), CSF IgA (r=0.388, *p=0.0015), CSF IgG (r=0.376, *p =0.002), and 24-hour IgG synthesis rate (r=0.242, *p= 0.048)). The IgG index was the sole parameter with higher values in the INF pattern compared to the combined INF+TU pattern, with a moderate effect size (r= 0.335, *p=0.006). CSF lactate levels showed no significant difference between the combined pattern and the INF+TU pattern (p=0.405).

Additionally, a comparison between the INF CSF type and the TU CSF type was conducted. In contrast to the TU CSF type, the INF CSF type exhibited signifycantly higher levels for most CSF biomarkers. The most substantial effect size was observed for specific immunological IgG-related markers: CSF IgG levels (r= 0.639, ***p=1.13·10−7), 24-hour IgG synthesis rate (r =0.589, ***p=9.93·10−7), IgG index (r=0.562, ***p = 0.000003), and the metabolic marker CSF lactate levels (r=0.563, ***p=0.000003; Figure 1, c). ROC analyses determined optimal values for discrimination between the TU type and INF type (N=69; Figure 1, e): ≥28.4 mg/L for CSF IgG (90.6% sensitivity, 81.2% specificity; ROC AUC=0.940, ***p=1.1·10−7), ≥0.63 for IgG synthesis rate mg/24 h (83% sensitivity, 93.7% specificity, ROC AUC=0.899, ***p=0.000001), ≥0.675 for IgG index (90.6% sensitivity, 87.5% specificity, ROC AUC=0.887, ***p=0.000003), and ≥1.165 mmol/L for CSF lactate levels (88.7% sensitivity, 75% specificity; ROC AUC=0.888, ***p=0.000003). All these variables strongly and significantly associated with the INF type, suggesting their potential as robust predicttors for identifying pathologically increased intrathecal IgG production. CSF IgA (r=0.362, *p=0.003) and CSF IgM (r=0.334; *p=0.006) exhibited significantly higher levels (with a moderate effect size), while total CSF proteins showed the smallest effect size (r=0.272, *p=0.024). CSF albumin levels and albumin index did not differ significantly between the TU and INF patterns.

Lastly, the TU pattern exhibited significantly lower values for most markers compared to the combined (INF+TU) type. Strikingly large effect sizes were observed for CSF IgG (r=0.829, ***p=7.12·10−6) and CSF IgA (r=0.812, ***p=8.63·10−6), indicating that

67.2% of IgG variability and 65.9% of IgA variability can be attributed to the electrophoretic pattern (TU *vs.* INF+TU). Other CSF biomarkers also showed relatively large effect sizes (total CSF proteins: r=0.775, ***p=0.00002; 24-hour IgG synthesis rate: r=0.774, ***p=0.00002, CSF lactate levels: r=0.732, p=0.00006; CSF IgM: r=0.680, ***p=0.0002). The electrophoretic pattern had a moderate effect on the IgG index (moderate to large, r = 0.482, *p =0.008; Figure 1, a, c).

Estimating diacritical cerebrospinal fluid (CSF) biomarkers to distinguish between different CSF immunological types using DISC-PAGE electrophoretic patterns

To validate the predictive capability of the seven proposed admission CSF biomarkers for determining CSF immunological types identified by DISC-PAGE, a canonical discriminant analysis (CDA) was conducted. The model, employing linear discrimination, reduced

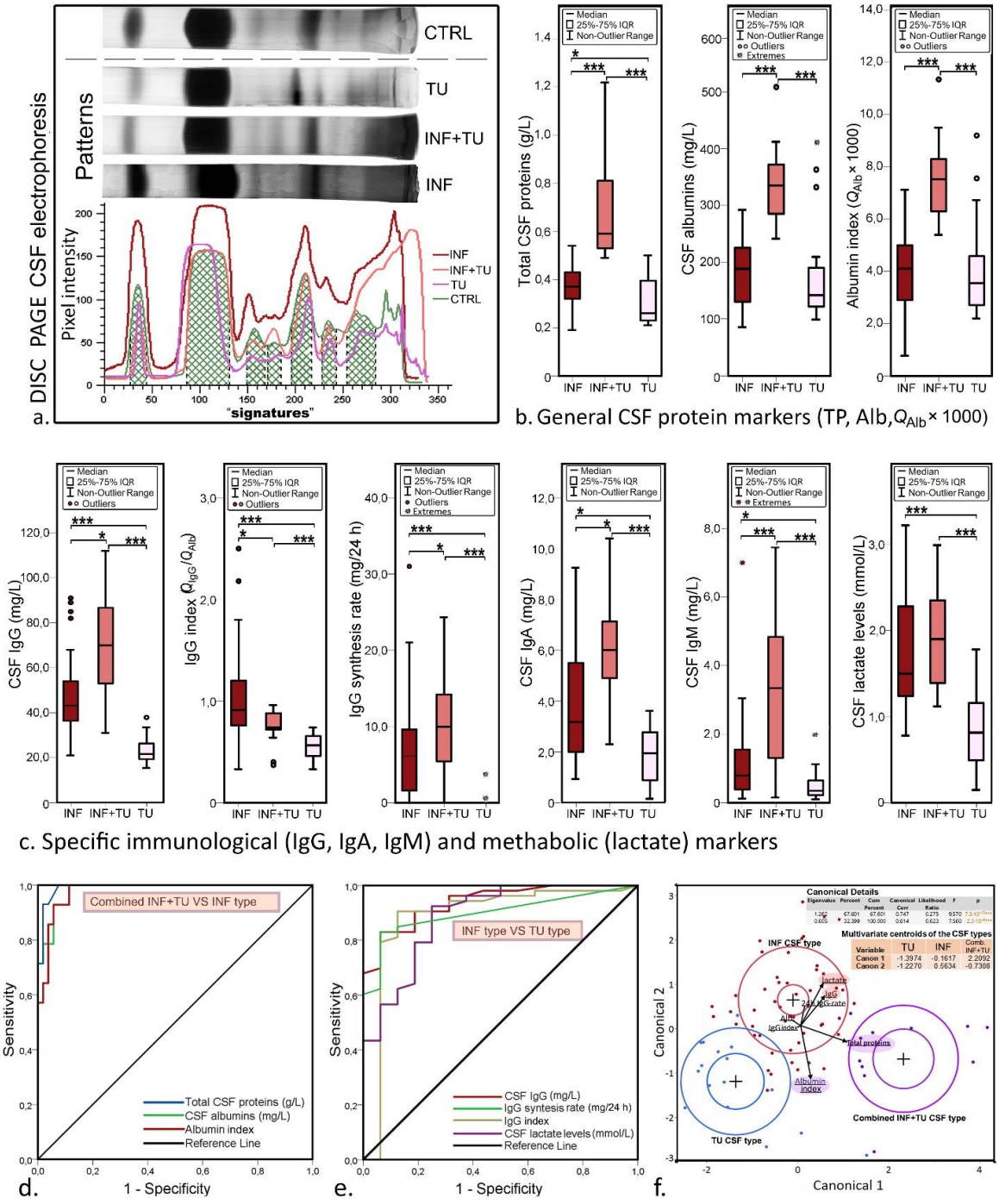

Fig. 1. Associations of the admission CSF biochemical markers (measured by quantitative methods) with the specific CSF immunological types, defined on the basis of their electrophoretic patterns ("signatures").

the seven variables into two major components known as canonical variables (ROC curves-Figure 1, d, e). the seven variables into two major components known as canonical variables (ROC curves-Figure 1, d, e).

As depicted in Figure 1-f, the model revealed distinguishable differences among the three CSF immunological types, with only slight intersection of confidence ellipses between the TU and INF types. The canonical details of the model and precise coordinates of the multivariate means (centroids) for the three MS electrophoretic types are illustrated in Figure 1-f. Statistical tests (Wilk's Lambda, Pillai's Trace, Hotelling-Lawley, and Roy's Max Root) consistently indicated significant differences in centroids among the three patterns (***p<0.0001 for all four tests), affirming their distinguishability based on the proposed seven CSF biomarkers.

The total levels of CSF proteins and the CSF IgG levels were characterized with the highest canonical weight and the highest loading, showing a significant degree of positive association with the first canonical variable (Table 1). Contrarily, the CSF lactate levels and the albumin index had the highest canonical weight and the highest loading with the second canonical variable (Table 1).

In the generated CDA model, only 16.9% of patients were misclassified, demonstrating high sensitivity and specificity for detecting different CSF patterns (81.1% sensitivity, 90.0% specificity for INF; 85.7% sensitivity, 95.6% specificity for INF+TU; 87.5% sensitivity, 85.1% specificity for TU; entropy R2=0.5966; –2log Likelihood=60.5421). Total CSF proteins and CSF IgG levels exhibited the highest canonical weight and loading, indicating a significant positive association with the first canonical variable. Conversely, CSF lactate levels and the albumin index had the highest weight and loading with the second canonical variable. The model identified high total protein levels as indicative of INF+TU CSF type, while low lactate and IgG levels were indicative of TU CSF type. Common cut-off values were proposed for detecting TU and INF+TU patterns, with CSF IgG and CSF lactates aiding further discrimination.

Table 1. CDA model summary (covariates are sorted according to their weight)

Covariates	Scoring Coefficients		Standardized coefficients (*weights*)		Structure coefficients (*loadings*)	
	Canon1	Canon2	Canon1	Canon2	Canon1	Canon2
Total CSF proteins (g/L)	5.429	-1.943	0.669	-0.239	0.918	-0.346
CSF IgG (mg/L)	0.021	0.026	0.351	0.437	0.812	0.295
CSF lactate levels (mmol/L)	0.538	1.042	0.320	0.620	0.537	0.587
Albumin index (Q_{Alb} × 1000)	0.095	-0.500	0.146	-0.764	0.786	-0.501
IgG syntesis rate (mg/24 h)	0.009	0.044	0.058	0.291	0.534	0.323
IgG index	-0.112	-0.055	-0.045	-0.022	-0.037	0.590
CSF albumins (mg/L)	-0.002	0.001	-0.130	0.085	0.798	-0.467

a. Morphological classification and characterization of the MS CSF DISC page electrophoretic patterns. INF-inflammatory CSF type; INF+TU-combined inflammatory plus transudate type; TU-non-inflammatory transudate type. Given comparison with control (CTRL)-"normal" pattern. Panels b-c: evaluation of the levels of admission CSF biomarkers across the three inflammatory CSF types based on electrophoretic patterns. Results summarized as median, IQR and non-outlier range. b. Total CSF protein levels, CSF albumin levels and albumin index (Q_{Alb}×1000) among the pattern types. c. Specific immunological (CSF IgG, IgG index, CSF IgG synthesis rate, CSF IgA, CSF IgM) and metabolic (CSF lactate) biomarkers among the pattern types. d. ROC analyses for discrimination between the combined INF+TU and INF CSF type. e. ROC analyses for discrimination between the TU and INF CSF type. f. CDA analyses for estimation of the diacritical CSF biomarkers in discrimination among the CSF immunological patterns. The multivariate's centroid for each of the three types is denoted by the symbol plus (+), and the Mahalanobis distance from each observation to each group's centroid can be observed. The small ellipses correspond to the region in the space that contains approximately 50% of the observations and the larger ellipses depict the 95% confidence intervals. The set of rays (delivered as multiple of the canonical weights) shows the direction of the covariates in the canonical space and indicates their degree of association with the specific patterns. The four estimated diacritical CSF biomarkers strongly associated with the specific electrophoretic types are depicted with shaded ellipses.

TP-total CSF protein levels; Alb-CSF albumin levels; Q_{Alb}×1000-Albumin index; IQR-interquartile range. *p<0.05; ***p<0.001.

Assessment of the disease course: discrimination between SPMS and RRMS on the basis of admission CSF biochemical markers and CSF electrophoretic signatures

The majority of CSF biochemical markers exhibited comparable levels between patients with SPMS and those with RRMS. Only three variables were signifycantly higher in SPMS patients: CSF lactate levels (U

=226.0; *p=0.001, Figure 2-a), CSF IgG (U=341.0; *p =0.045, Figure 2-d), and albumin index (U=338.5; *p =0.042, Figure 2-e). The effect size of CSF lactate on the disease form was moderate (r=0.369). However, it was noted that the distribution of CSF lactate levels exhibited a binomial pattern with two clear peaks (Figure 2-b), indicating two subpopulations based on CSF lactates. Visual analysis of the histogram in relation to SPMS *vs*. RRMS confirmed a distinct separation of CSF lactate levels between the two MS types

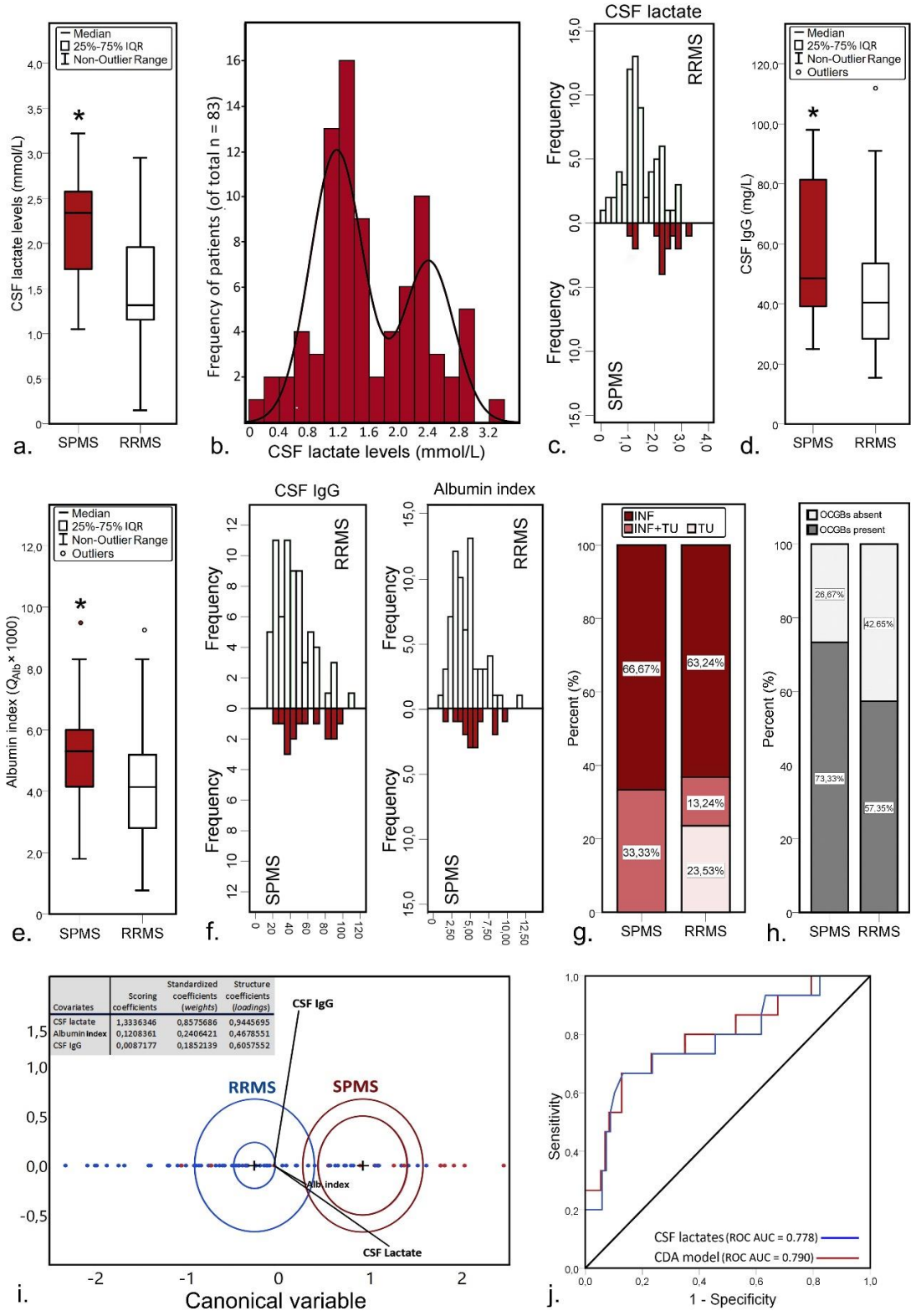

Fig. 2. Discrimination between SPMS and RRMS on the basis of CSF biochemical markers and electrophoretic patterns

(Figure 2-c), suggesting that the two peaks in the binomial distribution corresponded to different MS forms. This implies that the SPMS form might be predicted based on elevated admission lactate levels. In contrast, the other two markers, CSF IgG and the albumin index, demonstrated small effects on the disease form (r=0.220 and r=0.223, respectively). Visual analysis of their histograms in relation to the disease form revealed substantial overlap between values for SPMS and RRMS (Figure 2-f), indicating a limited capacity for CSF IgG and albumin index as prognostic markers for differentiation.

To assess the relevance of CSF IgG and albumin index in predicting disease outcomes, a CDA analysis was conducted (Figure 2-i). The three continuous variables were consolidated into one major component. The model incorporated covariance shrinkage, reducing prediction variance (overall shrinkage=0.8847; overall Lambda=0.1152).

As illustrated in Figure 2-i, the CDA model, utilizing CSF lactates, IgG, and the albumin index, effectively distinguished between the two types of multiple sclerosis (MS). The multivariate centroids for RRMS and SPMS were distinctly separated (assessed as −0.2179 for RRMS and 0.9880 for SPMS), and four statistical tests (Wilk's Lambda, Pillai's Trace, Hotelling-Lawley, and Roy's Max Root) verified significant differences between the two centroids (Likelihood Ratio=0.8192, F=5.8104, *p=0.0012). Only 27.7% of patients were misclassified in this model (73.3% sensitivity, 72.1% specificity for detecting patients with SPMS; entropy R2=-0.1479; -2logLikelihood=90.0359). CSF lactate levels exhibited the highest scoring coefficient (1.3336), canonical weight (0.8576), and loading (0.94457), indicating a substantial positive association with the SPMS form (Figure 2-i).

ROC analyses revealed that CSF lactate levels alone were equally powerful predicting the distinction between SPMS and RRMS as the comprehensive CDA model encompassing all three variables (ROC AUC= 0.778 compared to ROC AUC=0.790 for the CDA model, Figure 2-j). Given the superior predictive capability of CSF lactate levels over IgG levels and the albumin index, we suggest that elevated admission CSF lactate levels can serve as a significant predictive risk factor for SPMS development. A threshold value of ≥2.01 mmol/L CSF lactate is proposed as the optimal cut-off point for discriminating between the two MS types (73.3% sensitivity, 76.5% specificity, ROC AUC=0.778, ***p=0.0007).

Significantly, the two forms of multiple sclerosis (MS) exhibited significant differences in CSF immunological patterns, despite a small effect size. The CSF TU type was exclusively found in RRMS patients, while the combined INF+TU CSF type was more prevalent in SPMS patients ($\chi 2$=6.495, *p=0.039, V=0.209; Figure 2-g). The presence of oligoclonal bands showed relatively equal frequency in both SPMS and RRMS patients ($\chi 2$=1.310, p the seven variables into two major components known as canonical variables (ROC curves-Figure 1, d, e).=0.252; Figure 2-h). disease form (r=0.220 and r=0.223, respectively). Visual analysis of their histograms in relation to the disease form revealed substantial overlap between values for SPMS and RRMS (Figure 2-f), indicating a limited capacity for CSF IgG and albumin index as prognostic markers for differentiation.

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a. Comparison of the CSF lactate levels between SPMS and RRMS patients. b. Binomial distribution of the CSF lactate levels among patients. c. Histogram of the CSF lactates in relation to the two outcomes. A satisfactory separation of the values can be observed, with the highest levels of lactates associated with SPMS form. d. Comparison of the CSF IgG levels between SPMS and RRMS patients. e. Comparison of the albumin index between SPMS and RRMS patients. f. Histograms of CSF IgG levels and albumin index in relation to the two outcomes. A clear overlapping of the values can be observed, suggesting low capacity of the two biomarkers for separation between SPMS and RRMS. g. Frequencies of the three CSF inflammatory types between the two outcomes. It can be noted that the TU pattern was detected only in RRMS patients. h. Approximately equal frequencies of the presence of OCGBs between the two outcomes. i. CDA model for prediction of the outcomes, based on CSF lactates, IgG and albumin index. j. ROC analyses of the capacity of the CDA model and the CSF lactate levels alone to accurately predict the development of SPMS.

Discussion

In this ongoing research, we conducted a prospective 2-year longitudinal cohort study focusing on individuals with MS undergoing Interferon beta-1a treatment. Employing a comprehensive methodology, we integrated various quantitative and qualitative approaches to enhance the sensitivity of our findings. In the initial results section, our focus was on aligning outcomes obtained by diverse methodologies to determine optimal levels of CSF biomarkers associated with specific pathological processes (transudation and local synthesis) and CSF immunological patterns (TU, INF, or combined INF+TU).
In the present study, a thorough morphological examination of cerebrospinal fluid (CSF) electrophoretograms was undertaken, and the observed electrophoretic patterns were compared with the immunological classification of CSF as proposed by Schuller and Delasnerie [16]. Unlike laser nephelometry, which relies solely on the intensity of scattered light proportional to the absolute concentration of the CSF analyte, DISC-PAGE offers multiple variables for assessing CSF content. Importantly, DISC-PAGE captures changes in the TU pattern not only related to albumin content but also discerns detectable alterations in almost all other fractions. Additionally, the identification of protein fractions such as haptoglobin 2-1 and 2-2 polymers, absent in normal CSF, serves as a distinctive abnormality in the TU pattern, strongly indicating transudetion during MS. Therefore, DISC-PAGE electrophoretic patterns can be considered as highly specific signatures, reflecting pathological changes in specific protein fractions within the CSF composition.
Utilizing the acquired electrophoretic patterns and their correlation with quantitatively assessed CSF biomarker levels, we have introduced lower cut-off values for distinguishing between INF, TU, and combined INF-TU types. These values, marked by elevated sensitivity and specificity, were further substantiated by Canonical Discriminant Analysis, affirming their credibility and accuracy. Consequently, we suggest that these revised lower cut-off values for CSF biomarkers offer a more precise and comprehensive means of evaluating immunological CSF types in the context of multiple sclerosis.
The primary objective of this investigation was the comprehensive assessment of CSF immunological patterns and admission CSF biomarkers, with a specific emphasis on their efficacy in providing early prognostic indications of disease outcome and functional disability in individuals with multiple sclerosis. Prognostic and predictive markers hold significant value in MS, contributing to the quality evaluation of the disease trajectory. Their impact on clinical decision-making and treatment recommendations is pivotal, playing a crucial role in averting further neurological impairments. Nevertheless, the existing literature has limitedly addressed the prognostic potential of routinely analyzed CSF biomarkers in the context of MS.
Despite the challenges posed by the concentration of total CSF protein exceeding 1 g/L in diagnosing MS, and the commonly employed albumin index along with the IgG index and 24 h IgG synthesis rate being recognized as key criteria for blood-CSF barrier function assessment and local synthesis reflection, none of these routine markers emerged as promising candidates for early detection of disease progression or functional disability. Our statistical analysis revealed that total CSF proteins, albumin, and IgG indices, along with most other CSF biomarkers, lack accuracy in predicting disease progression and the development of secondary progressive MS (SPMS). Furthermore, CSF immunological patterns and the presence of oligoclonal bands on admission failed to distinguish between relapsing-remitting MS (RRMS) and SPMS patients. Despite the marginal impact of CSF IgG and the albumin index on disease form, canonical discriminant analysis demonstrated their limited relevance in prognosticating the disease course. In contrast, CSF lactate levels emerged as the sole significant early predictor for SPMS. Notably, CSF lactate levels alone exhibited comparable efficacy to the entire canonical discriminant analysis model, which included additional factors such as CSF IgG levels and the albumin index. Given the superior predictive capacity of CSF lactate levels, we propose a threshold of ≥2.01 mmol/L CSF lactate as an optimal cut-off point for early prognostication of SPMS development. These findings align with prior research by Jongen *et al*. [19], which

observed elevated lactate and albumin index in SPMS patients compared to RRMS. Our proposed cut-off value closely corresponds to the suggested threshold of 2.1 mmol/L indicative of central nervous system tissue destruction (Genton and Berger [20], Leib *et al.* [21]).

Conclusion

In summary, we advocate the utilization of DISC-PAGE electrophoresis with densitometry tracing as a superior and dependable method for detecting CSF immunological patterns. Additionally, we posit that our morphological characterization of electrophoretograms, along with the suggested cut-off values for albumin index, IgG index, CSF IgG, and CSF lactates presented in this study, could offer a more precise, sensitive, and comprehensive assessment of the presence of local synthesis and abnormal barrier in the context of multiple sclerosis.

Conflict of interest statement. None declared.

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Case report

СЛУЧАЈ СО ПЕРИВЕНТРИКУЛАРНА НОДУЛАРНА ХЕТЕРОТОПИЈА И NEDD4L ГЕНСКА ВАРИЈАНТА СО НЕПОЗНАТО ЗНАЧЕЊЕ

A CASE OF PERIVENTRICULAR NODULAR HETEROTOPIA AND A NEDD4L GENE VARIANT OF UNCERTAIN SIGNIFICANCE

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Abstract

Introduction. Periventricular nodular heterotopia (PVNH) is characterized by presence of gray matter nodules lining the ventricular walls. Most of the patients present with epilepsy and with or without intellectual impairment. PVNH 7 is associated with mutation in the NEDD4L gene on the chromosome 18. A variant of uncertain significance (VUS) is a variant that cannot be associated with some medical condition because there is not enough evidence for that.

Case presentation. A 17-year-old boy with a history of three seizures in the last two years was referred for reevaluation and treatment initiation. His sleep-deprived EEG study revealed bilateral independent epileptic discharges and his neuropsychological examination revealed borderline intellectual functioning. The MRI studies of the brain showed bilateral asymmetric PVNH but the genetic testing discovered a VUS in the NEDD4L gene.

Discussion. There are five categories of gene variants: pathogenic, likely pathogenic, VUS, likely benign and benign. The interpretation of a variant is influenced by the frequency at which that variant is found in the population, the previous observations of it in other patients with the same clinical features and the predicted impact of the variant. Categorization of a VUS may change as more people get tested.

Conclusion. Reporting patients with PVNH and VUS is important because it can help us in understanding the connection between the malformation and the variant. Genetic testing of the parents may prove that this is a de novo variant, which may be categorized as a likely pathogenic variant.

Keywords: periventricular nodular heterotopia, variant of uncertain significance, NEDD4L, epilepsy, genetics

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Апстракт

Вовед. Перивентрикуларната нодуларна хетеротопија (ПВНХ) се карактеризира со присуство на нодули од сива маса околу вентрикуларните ѕидови. Најголемиот број од пациентите манифестираат епилепсија со или без интелектуално нарушување. ПВНХ 7 е асоцирана со мутација во генот NEDD4L на хромозомот 18. Генските варијанти со непознато значење (ВНЗ) се варијанти кои не можат да се поврзат со некоја болест затоа што не постојат доволно докази за таквата асоцијација.

Презентација на случај. 17 годишно момче со анамнеза за три напади во последните две години беше реферирано за реевалуација и започнување со терапија. Неговиот ЕЕГ наод по депривација на сон прикажа билатерални независни епилептични избивања, а неговото невропсихолошко тестирање прикажа гранично интелектуално функционирање. Наодите од НМР на мозокот покажаа билатерална асиметрична ПВНХ, но генетските анализи откриј ВНЗ во генот NEDD4L.

Дискусија. Постојат пет категории на генски варијанти: патолошки, најверојатно патолошки, ВНЗ, веројатно бенигни и бенигни. Интерпретацијата на една варијанта е под влијание на фрекфенцијата со која таа варијанта е застапена во популацијата, претходните наоди кај пациенти со исти клинички карактеристики и предвидениот импакт на варијантата. Категоризацијата на една ВНЗ може да се промени во колку повеќе луѓе се тестираат.

Заклучок. Прикажувањето на пациенти со ПВНХ и ВНЗ е важно затоа што тоа може да ни помогне во разбирањето на врската помеѓу малформацијата и варијантата. Генетското тестирање на родителите може да докаже дека овде станува збор за de novo варијанта, а тоа може да ја категоризира истата како веројанто патогена варијанта.

Клучни зборови: перивентрикуларна нодуларна хетеротопија, варијанта со непознато значење, NEDD4L, епилепсија, генетика

Introduction

The development of the cerebral cortex is a complex but well-controlled process that is consisted of three major stages: cell proliferation and apoptosis; cell migration; and post-migrational development. Many internal and external factors can disrupt the normal cortical formation at any stage and can cause a large group of disorders called malformations of cortical development [1].

Neuronal migration starts between 5-6 gestational week, peaks between 5-6 gestational month and ends between 30-35 gestational week. At this stage, neurons migrate from the ventricular and subventricular zone to the cortical plate in a radial or tangential way [1-2]. Disruptions of this process can lead to migrational malformations, such as gray matter heterotopia [1,3]. Gray matter heterotopias are defined as masses of neurons localized in the white matter between the lateral ventricles and the normal cortex [1]. Two of several types of gray matter heterotopia are periventricular nodular heterotopia caused by disruption of the early migration and subcortical band heterotopia caused by disruption of the late migration [1,3].

Periventricular nodular heterotopia (PVNH) is characterized by unilateral or bilateral presence of gray matter nodules lining the ventricular walls that vary in number, size and form and with variable association with other brain or systemic malformations [1].

The clinical presentation of PVNH depends on the extent of the malformation and whether it is unilateral or bilateral or associated with other malformations [2]. Most of the patients (up to 90%) present with epilepsy that is frequently drug-resistant and with or without intellectual impairment [2,4,5]. The seizures are focal onset seizures and focal to bilateral tonic-clonic seizures with features referable to the location of the heterotopia, and the intellectual impairment may vary from mild to severe [4]. The diagnosis of PVNH is usually based on the clinical, neurophysiological and neuroimaging findings but genetic analyses may reveal a possible genetic etiology of the heterotopia [4,6]. The treatment of PVNH includes anti-seizure medications, neuromodulation and epilepsy surgery [3,6].

Periventricular nodular heterotopia (PVNH) is a congenital malformation and its etiology may be genetic or associated with an intrauterine insult such as infection, trauma or a vascular accident [7]. The genetic PVNH may be caused by gene abnormalities or chromosomal abnormalities [4]. Gene abnormalities are more frequent and they represent acquired or inherited gene mutations with autosomal recessive, autosomal dominant or X-linked pattern of inheritance. PVNH is most commonly caused by mutations in the FLNA gene and the other responsible genes include ARFGEF2, ERMARD, NEDD4L, ARF1 and MAP1B. Chromosomal abnormalities are less frequent and they usually involve anomalies of the chromosome 5 [8].

PVNH is a genetically heterogeneous condition which means that different genetic defects can cause the same cortical malformation. There are 9 types of PVNH which represent nine different genotype-phenotype correlations. Periventricular nodular heterotopia 7 is associated with mutation in the NEDD4L gene on the chromosome 18 [8]. This gene encodes an ubiquitin ligase that regulates ion channels and it appears to have roles in various neuronal, cardiovascular, respiratory and renal functions [9].

A variant of uncertain significance is a gene variant that cannot be associated with a certain medical condition because there is not enough evidence that can support such association. Variants of uncertain significance are considered neither pathogenic nor non-pathogenic gene variants but their classification can change over time [10].

We present a patient with epilepsy caused by periventricular nodular heterotopia whose genetic testing revealed NEDD4L gene variant of uncertain significance. The aims of this case report were to describe the clinical features of our patient, to discuss the known genetic defects associated with this cortical malformation and to discuss the meaning of the gene variants that are of uncertain significance.

Case presentation

A 17-year-old male patient with a history of three seizures in the last two years was referred to the University Clinic for Neurology in Skopje for reevaluation and treatment initiation. Medical records including two routine electroencephalography (EEG) studies and two magnetic resonance imaging (MRI) studies of the brain were brought along. During the hospitalization at the Clinic, a thorough medical history was taken and all the medical records were carefully evaluated. Additionally, routine blood tests, neurological examination, neuropsychological examination, sleep-deprived EEG study and genetic testing were made.

The patient is a left-handed high school student, unmarried and childless. He lives with his parents and one older brother. No family history for epilepsy or any hereditary diseases were reported. His prenatal, natal and postnatal history were normal. No comorbidities, chronic therapy or food and drug allergies were reported.

He doesn't take any drugs, he doesn't smoke cigarettes and he doesn't drink alcohol. According to his family, the patient 'has never been fond of studying' and has always had low grades in school. He is well socialized

but quiet, likes to play video games and often stays up at night and doesn't get enough sleep.

Two years prior to hospitalization, at the age of 15, the patient had his first seizure. As stated by his mother, after two days of not sleeping much, at 5 am, while sleeping, he straightened his upper body up, screamed and then started reaching for his mouth with his both hands 'as if he wanted to open it' while speaking incomprehensibly. A simultaneous stiffening and then shaking of all four extremities followed this event. After one year, at the age of 16, the patient had his second seizure. As stated by his friends, it happened in a café while drinking coffee when his whole body suddenly stiffened and then his arms and legs started shaking. The third seizure happened two months before hospital admission and it was described by his brother according to whom this seizure was identical to the first one. As reported by his family and friends, the seizures lasted less than 2 minutes, the patient was unresponsive during the seizures and seemed confused in a period shorter than 1 hour after the seizures.

The neurological examination of the patient showed normal head size, no obvious dysmorphic body characteristics and normal sensory and motor functions. The routine blood tests were also normal. The two routine EEG studies that were brought along were made after the two first seizures and their evaluation showed different interictal findings. The first study revealed occasional sharp waves and occasional spikes above both central-parietal and temporal regions of the scalp but with a slight right accentuation, and the second study was normal.

A sleep-deprived EEG study was made at our Clinic and an interesting pathologic finding was discovered. During awake state, frequent epileptiform discharges consisted of spike and wave complexes were registered. The discharges were present in both hemispheres but occurred independently right and left with frontal maximum above the right hemisphere and temporal maximum above the left hemisphere (Figures 1 and 2). During sleep, the spike and wave epileptiform discharges were mostly bilateral and synchronous.

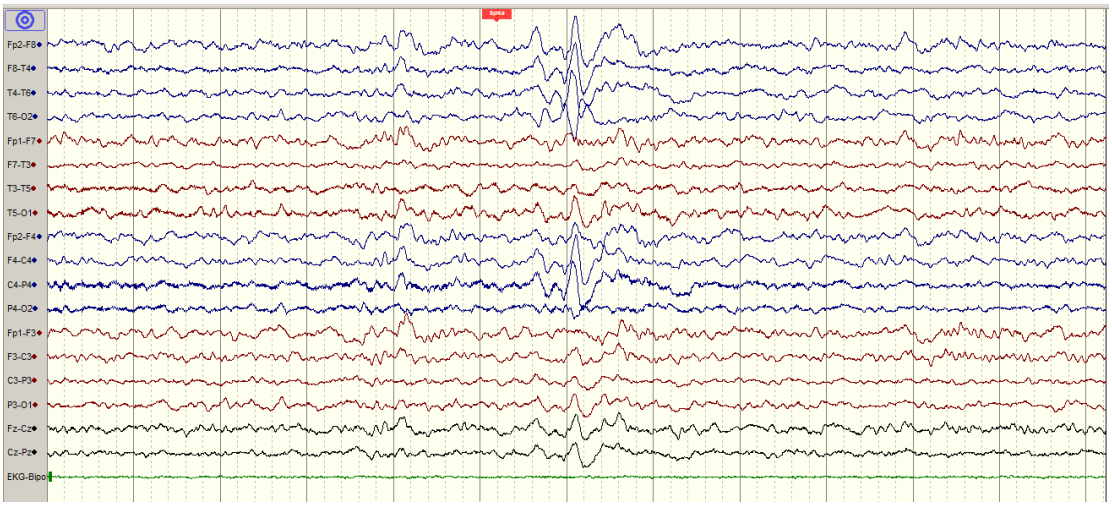

Fig. 1. Epileptiform discharges above the right hemisphere (independent from the left discharges noticed later in the recording)

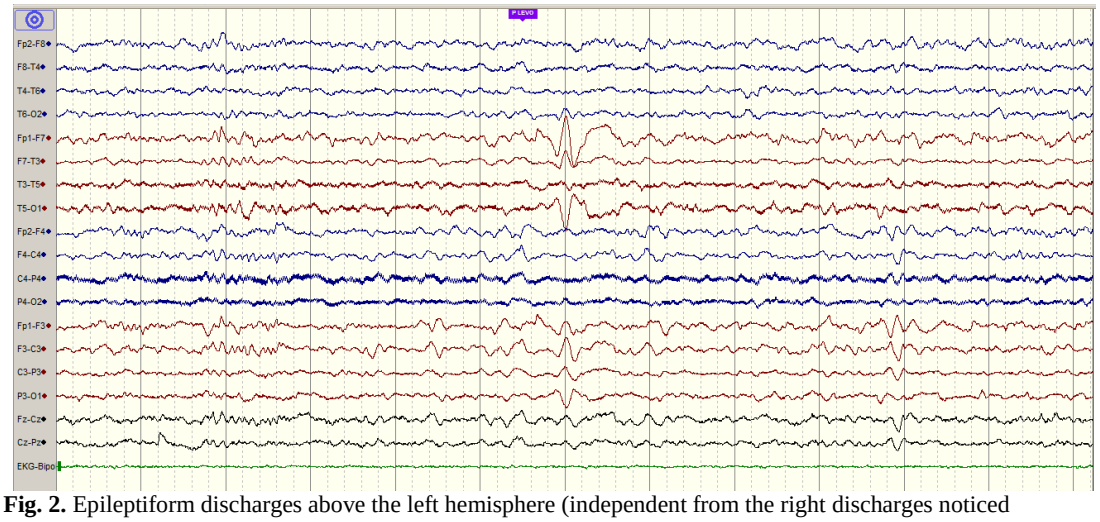

Fig. 2. Epileptiform discharges above the left hemisphere (independent from the right discharges noticed earlier in the recording)

The two MRI studies of the brain that were evaluated next revealed ependymal and subependymal abnormalities located in both lateral ventricles, mostly alongside the temporal horns, the trigonums and the posterior horns of the ventricles. These abnormalities were isodense with normal cortex and represented unmigrated cortex-gray matter heterotopia; periventricular nodular heterotopia that was bilateral and asymmetric (Figures 3, 4 and 5).

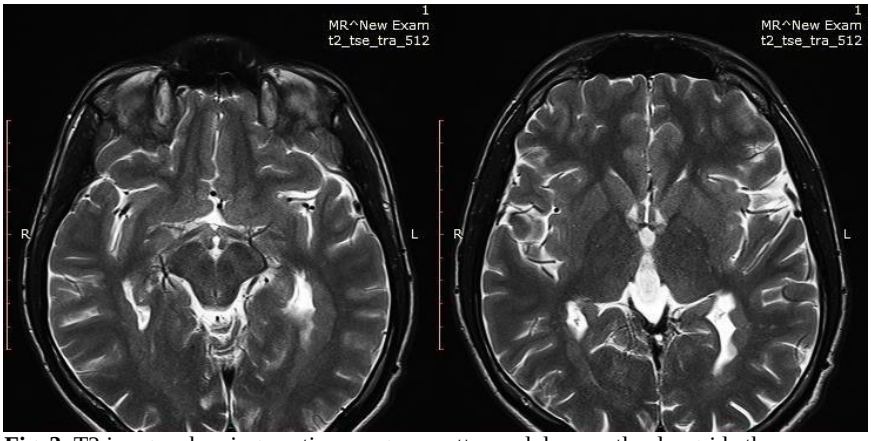

Fig. 3. T2 images showing continuous gray matter nodules mostly alongside the posterior horn of the left ventricle (axial view)

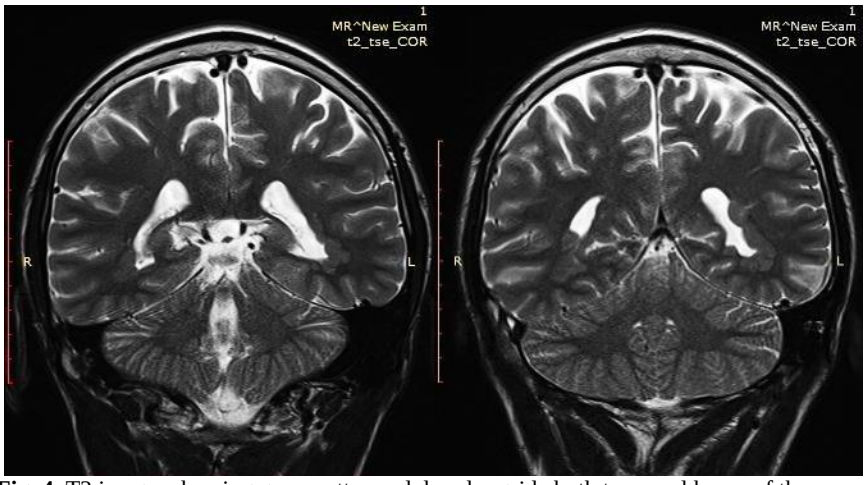

Fig. 4. T2 images showing gray matter nodules alongside both temporal horns of the lateral ventricles (coronal view)

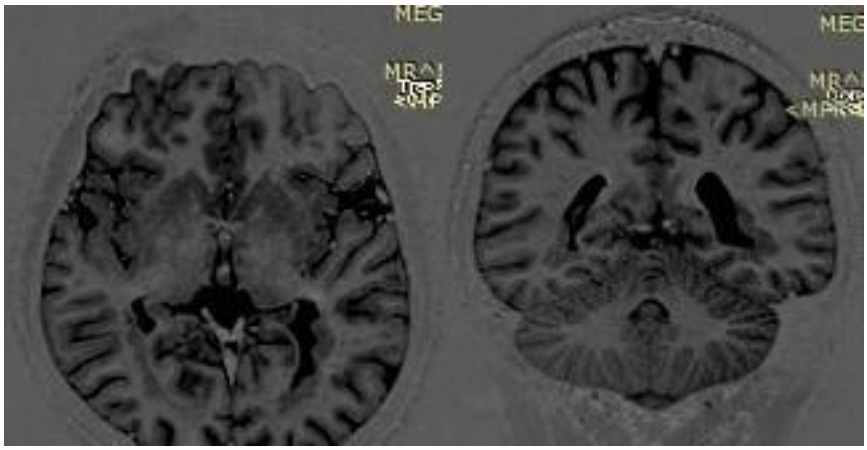

Fig. 5. T1 images showing periventricular gray matter nodules that are isodense with the normal cortex (axial view left and coronal view right)

The neuropsychological examination of the patient consisted of non-verbal test that measured his general intelligence using Raven's progressive matrices. The test showed borderline intellectual functioning with an IQ of 76.

Further in the evaluation process the patient was referred for genetic testing for mutations of the genes associated with periventricular nodular heterotopia. A targeted massive parallel sequencing of the exons of the protein-coding genes of the genome of the patient and bioinformatic analysis of the genes associated with his condition were made. The analysis revealed a heterozygous variant of uncertain clinical significance: c. 812A>C, p.Glu271Ala in the exon 10 of the NEDD4L gene.

During the hospitalization at the Clinic, the patient was regularly observed, no seizures were detected and monotherapy with broad-spectrum anti-seizure drug-valproic acid was initiated. At the follow-up visit after three months, no seizures and no adverse effects of the drug were reported. The patient has been seizure-free for one year now at the time of publishing this case report.

Discussion

The clinical presentation of the patient predominantly included seizures. According to the reported descriptions of the three seizures, the assumed semiology of two of them was highly suggestive of focal onset seizures with impaired awareness that progressed to bilateral tonic-clonic seizures, while not enough information regarding the onset of one of the seizures was provided [4]. The routine EEG findings were uninformative in detecting a focal epileptiform activity while the sleep-deprived EEG findings revealed possible epileptic focuses in both hemispheres. This focal epileptiform activity consisted of spike and wave complexes that occurred independently in the right and left hemisphere during awake state, while the discharges during sleep were mostly bilateral and synchronous. Classifying the seizures and classifying the epilepsy are an important part of the diagnostic process from an etiological point of view [4,11]. When seizure semiology and EEG findings suggest focal seizures, neuroimaging is indicated because focal epilepsies are usually associated with structural brain abnormalities and that information carries therapeutic and prognostic implications [11]. Indeed, the MRI studies of the patient revealed bilateral periventricular nodular heterotopia, a malformation of cortical development that is associated with focal epilepsy in 80-90% of the cases [4].

The prognostic value of this comes from the fact that cortical malformations are an important cause of drug-resistant epilepsy in 40% of the cases [12]. On the other side, there is the therapeutic value of the search for such malformations due to the fact that some patients may be eligible for epilepsy surgery, while for the others neuromodulation may be considered [3].

Periventricular nodular heterotopias may be acquired due to an intrauterine insult or may be genetic [7]. Taking into consideration the medical history and the neurological examination of the patient, a causing intrauterine infection, trauma or vascular accident was excluded and a genetic cause came to mind. The negative family history of the patient implicated that the genetic cause was probably due to a sporadic (de novo) mutation and not due to an inherited one.

In the Online Mendelian Inheritance in Men (OMIM) database, there are nine genotype-phenotype correlation entries regarding periventricular nodular heterotopia (PVNH) [8].

PVNH1 is X-liked dominant heterotopia associated with cardiovascular anomalies and is caused by heterozygous mutation in the FLNA gene on the Xq28 chromosome. This is the most common mutation and most of the affected individuals are females presenting with epilepsy but normal intelligence while males tend to die *in utero*. PVNH4 was previously regarded as Ehlers-Danlos variant and now is considered to be the same as X-linked dominant PVNH1. Several patients with PVNH and FLNA mutations have been reported to have combinations of cardiovascular, cutaneous and joint-related disorders [8].

PVNH2 is autosomal recessive heterotopia associated with microcephaly and is caused by homozygous mutation in the ARFGEF2 gene on the 20q13 chromosome [8].

PVNH3 is associated with anomalies of the chromosome 5p, and PVNH5 is associated with deletions of the chromosome 5q [8].

PVNH6 is autosomal dominant heterotopia caused by heterozygous mutation in the ERMARD gene on the 6q27 chromosome. PVNH7 is autosomal dominant heterotopia caused by heterozygous mutation in the NEDD4L gene on the 18q21 chromosome. PVNH8 is autosomal dominant heterotopia caused by heterozygous mutation in the ARF1 gene on the 1q42 chromosome. PVNH9 is autosomal dominant heterotopia caused by heterozygous mutation in the MAP1B gene on the 5q13 chromosome [8].

The genetic testing of our patient consisted of targeted sequencing of the protein-coding genes associated with PVNH and it revealed a heterozygous variant of uncertain clinical significance in the NEDD4L gene on the exon 10. The NEDD4L gene is located on the 18q21 chromosome, has 38 exons and encodes an ubiquitin protein ligase (E3) [8,13]. This enzyme is expressed in the brain, heart, lungs and the kidneys and takes roles in the function of the voltage gated sodium channels, epithelial sodium channels and the sodium-chloride co-transporters [14]. Patients with PVNH and known NEDD4L mutations may have variable dysmorphic facial features, optic nerve atrophy, strabismus, hearing impairment, micrognathia, cleft

palate, truncal hypotonia, contractures, cutaneous or toe syndactyly, cryptorchidism, hypospadias and many central nervous system disorders [8]. The neurological disorders also include delayed psychomotor development, intellectual disability, absent or poor speech, absent or poor walking, seizures but only in some patients and finally, periventricular nodular heterotopia that in some patients may be associated with focal cortical dysplasia or corpus callosum atrophy [8].

Having in mind the normal medical history of our patient and his normal neurological examination on one hand and the fact that the genetic testing did not detect any of the known mutations in the NEDD4L gene on the other hand, the question is: what does this variant of uncertain significance mean for the clinician and for the patient?

The American College of Medical Genetics and Genomics (ACMG) categorizes gene variants into five categories: pathogenic, likely pathogenic, variants of uncertain significance, likely benign and benign [15]. When there is insufficient or conflicting evidence for a gene variant to be associated with some diseases, that variant is categorized as variant of uncertain significance (VUS). A basic understanding of VUS is important for test interpretation and clinical management so that the presence of VUS, even in a relevant gene, does not confirm a genetic diagnosis [10].

The interpretation of a gene variant is influenced by the frequency at which the variant is found in the general population, the previous observations of that variant in other patients with the same clinical features and the predicted impact of the variant. As time passes by and more people get tested, the categorization of a VUS may change into pathogenic or benign. However, only few VUS become suspicious over time and are later considered diagnostic. While testing the general population is important, testing the parents may also help the process because if a child has a VUS that is not inherited from the parents, this information may be enough to upgrade that variant to a likely pathogenic variant. Finally, VUS should not dictate the decision-making process regarding clinical management or reproduction [10].

Conclusion

Reporting patients with malformations of cortical development and variants of uncertain significance is important because that may help us understand the connection between the malformation and the variant and even upgrade that variant to a pathogenic one over time.

We reported a patient with heterozygous variant of uncertain significance (c.812A>C, p.Glu271Ala) in the exon 10 of the NEDD4L gene, with asymmetrical bilateral periventricular nodular heterotopia presented with focal to bilateral tonic-clonic seizures and borderline intellectual functioning. Genetic testing of the healthy parents of the patient may prove that this is a de novo heterozygous variant, which may be categorized as a likely pathogenic variant.

Finally, while waiting to find out the significance of gene variants, we encounter with patients with seizures which may be hard to control in the future and that gives cortical malformations enough significance to follow these patients carefully.

Conflict of interest statement. None declared.

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ММР

Case report

POSTACUTE TREATMENT OF CEREBRAL VENOUS SINUS THROMBOSIS WITH RIVAROXABAN AND CARBAMAZEPINE: A CASE REPORT AND LITERATURE REVIEW

ПОСТАКУТЕН ТРЕТМАН НА ЦЕРЕБРАЛНА ВЕНСКА СИНУС ТРОМБОЗА СО РИВАРОКСАБАН И КАРБАМАЗЕПИН: ПРИКАЗ НА СЛУЧАЈ И РЕЗИМЕ НА ЛИТЕРАТУРАТА

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Abstract

Introduction. Cerebral venous sinus thrombosis (CVST) is a medical emergency in pregnancy and the postpartum period. The currently recommended treatment for CVST is low molecular weight heparin (LMWH) followed by oral anticoagulant therapy. Various data suggest that the use of novel anticoagulant therapy (NOACs) is associated with a lower risk of bleeding. Epileptic seizures are also common in CVST. Acute symptomatic seizures occur in about 35-50% of cases. In absence of precise guidelines, the decision on the timing, type, dose and duration of the antiepileptic treatment in patients with CVST is often individualized.

Case presentation. A previously healthy 27-year-old woman, gravida 4, para 3, delivered by caesarean section 15 days ago, presented to the Department of Emergency Neurology with a left-sided headache accompanied by multiple secondary generalized focal seizures. Neurological examination showed weakness of the right upper extremity. Magnetic resonance imaging revealed thrombosis of several cerebral venous sinuses as well as cortical vein thrombosis. Treatment with LMWH and Carbamazepine was started. No seizures and no further deterioration were observed during hospital stay. The patient was discharged with oral anticoagulant (Rivaroxaban) and anticonvulsant (Carbamazepine) therapy. A 6-month follow-up showed no neurological deficit, no seizures and no other cerebrovascular complications, with the exception of a slightly increased menstrual bleeding.

Conclusion. Outpatient treatment of postpartum CVST with Rivaroxaban appears to be effective and reduces the risk of bleeding. Treatment with Carbamazepine during the next 2.5 years showed no thromboembolic accidents or seizures in our patient.

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Keywords: cerebral venous sinus thrombosis, seizures, postpartum, Rivaroxaban, Carbamazepine

Апстракт

Вовед. Церебрална синус венска тромбоза (ЦВСТ) е итна состојба во бременоста и постпарталниот период. Моментално препорачаниот третман за ЦВСТ е со нискомолекуларен хепарин, а потоа со орална антикоагулантна терапија. Употребата на новите антикоагуланси е поврзана со помал ризик од крварење. Епилептичните напади се чести кај ЦВСТ. Кај 35-50% од пациентите се појавуваат акутни симптоматски напади. Во отсуство на прецизни упатства, одлуката за почетокот, видот, дозата и времетраењето на антиепилептичната терапија е честопати индивидуализирана.

Презентација на случај. Претходно здрава 27 годишна жена, гравида 4, став 3, породена со царски рез пред 15 дена, беше хоспитализирана на Одделот за ургентна неврологија поради левострана главоболка и повеќе секундарно генерализирани фокални напади. Невролошкиот преглед покажа слабост на горниот десен екстремитет. Магнетната резонанца откри тромбоза на повеќе церебрални венски синуси и тромбоза на кортикалните вени. Пациентката беше третирана со нискомолекуларен хепарин и карбамазепин, а по престојот, за време на кој не се регистрираа напади, беше испишана со орална антикоагулантна (ривароксабан) и антиепилептична (карбамазепин) терапија. На контролниот преглед по 6 месеци пациентката беше без невролошки дефицит, не пријави напади и други цереброваскуларни компликации, туку лесно зголемено менструално крварење.

Заклучок. Амбулантскиот третман на постпарталната ТЦВС со ривароксабан е ефикасен и го намалува ризикот од крварње. Терапијата со карбамазепин во текот на следните 2.5 години не предизвика

тромбоемболични настани, ниту повторна појава на епилептичните напади.

Клучни зборови: тромбоза на церебрални венски синуси, напади, постпартум, ривароксабан, карбамазепин

Introduction

Cerebral venous sinus thrombosis (CVST) is an uncommon form of venous thromboembolism (VTE) with an estimated annual incidence of 3 to 4 cases per million in adults and 7 cases per million in children [1]. CVST is characterized by a wide range of clinical presentations, diverse etiology, frequent misdiagnosis, and an uncertain prognosis. While there is no conclusive evidence to support its relationship with puerperium in developing countries, it is intriguing that it may be linked to more variables, including poor prenatal care, metabolic disorders, and infections related to childbirth. The incidence was calculated to be 11.6 cases per 100,000 births [2].

In younger individuals, the spectrum of CVST may include venous congestion that may or may not be identified on neuroimaging, parenchymal cortical or subcortical ischemia, and subarachnoid and subdural hemorrhages, frequently reported in conjunction with CVST [3].

One typical complication of cerebral venous sinus thrombosis are seizures. Acute symptomatic seizures are frequently linked to supratentorial lesions and focal neurological deficits. A history of acute symptomatic seizures is strongly correlated with post-CVST epilepsy. Prophylactic therapy following the initial acute symptomatic seizure is advised in certain situations, although there is not enough reliable data to support it [4].

Our aim was to present a case of a young patient who underwent postpartum CVST and acute symptomatic seizures and was treated with Rivaroxaban and Carbamazepine. We analyzed the course of Carbamazepine treatment and its duration in the discussion, drawing on information and firsthand data from the available literature.

Case presentation

We present a case of a 27-year-old nursing mother who was admitted to the Department of Urgent Neurology 15 days after giving birth to her third child. She has had four pregnancies, has given birth to two healthy full-term children, and had a prematurely terminated last pregnancy that resulted in a stillborn infant (no medical documentation was available). She did not report any food or drug allergies, previous or other coexisting diseases, or a family history of neurological diseases. Due to placental abruption, the baby was delivered via caesarean section. Rehydration, analgesics, uterotonics, anticoagulants, and symptomatic medication were administered to the patient during the uneventful postpartum period, and she was discharged in a stable condition and a good general health. Following the recommendations of her gynecologist, she was taking Bromergon tablets (2.5 mg). She visited a neurologist nine days after giving birth because of a headache that had persisted for two days, despite having a normal neurological examination. Additionally, hyperdense venous sinuses (sagittal, confluence, transverse, and superior anastomotic veins; Trolard vein) were identified on a native CT scan, indicating cerebral venous thrombosis (Figure 1).

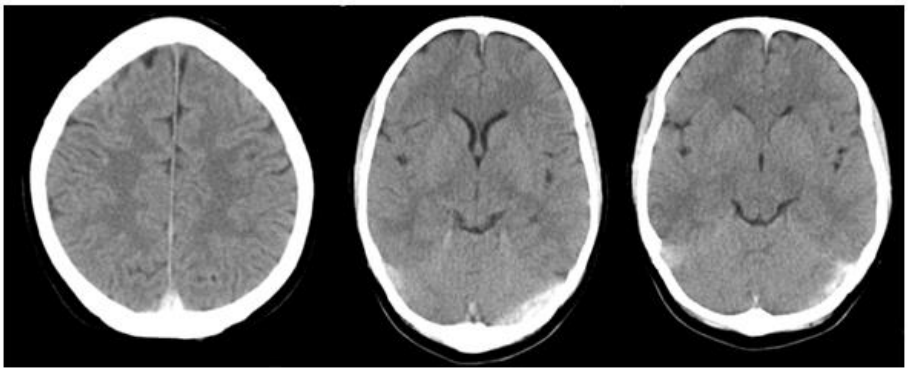

Fig. 1. Native CT scan with hyperdense venous sinuses (sagittal, confluence, transverse, and superior anastomotic veins; Trolard vein)

The next day, the patient complained of persistent headaches and weakness on the right upper extremity. Following birth, she was placed on Fraxiparine (0.6 ml 1x1) to maintain hemostasis within normal ranges for 14 days, and D-dimers of 1873. In addition to the headache and the weakness during these two postpartum weeks, three epileptic seizures with a focal onset and secondary generalization had occurred, due to which the patient was referred to the University Clinic for Neurology in Skopje. Upon admission, the patient was somnolent with a GCS of 14/15 and exhibited mild right hemiparesis. Intramuscular Diazepam was administered, and treatment with LMWH and Carbamazepine tablets (200 mg 2x1) was initiated. A new CT scan was performed and it indicated CVST (Figure 2).

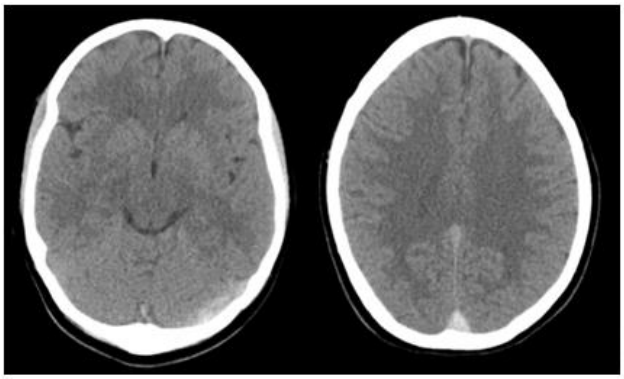

Fig. 2. Native CT scan indicating CVST

Somatic status: conscious, afebrile, and with limited mobility. Body temperature of 37.6°C. Auscultation revealed bilateral vesicular breath sounds. Rhythmic cardiac action with a frequency of 79 bpm. From a neurological standpoint, on the day after admission, she was conscious, responsive, and oriented in time, place and people. Pupils were isochoric and properly reactive to light, accommodation, and convergence. Bulbs were centrally placed, with neatly preserved conjugate movements. A mild degree of right monoparesis of the hand (Todd's paresis) was noted, without a positive Babinski sign. She properly performed the coordination tests with her left hand but did not perform them properly with her right hand. Sensitivity in all qualities was nurtured. Sphincters were properly controlled. Scores: mRS 2, NIHSS 2, GCS 14/15 (M6, E3, V5).

Lab analyses provided reference values for all findings, with the exception of CRP 14.4 mg/dl and D-dimers at 14 ng/ml (1953).

No *ex utero* hemorrhage was discovered during the gynecological check-up. The wound has healed per primum. Ongoing treatment with antibiotics.

Echocardiographic examination showed regular findings, except for light mitral regurgitation and registered light shadowing of fossa ovalis without L-D shunt.

Electroencephalogram was within normal limits.

Genetic analyses for thrombophilia using the real-time PCR technique to isolate DNA from peripheral blood were performed, and the following results were obtained: heterozygosity in F7, ITGA2, MTHFR 1298, MTRR, PAI-1, PC 111, and PS 73, with 37 AFA 4. Lupus anticoagulant was negative, and LA screening was 37 AFA 4.

MRI of the brain: A brain MRI using standard pulse sequences in three planes was performed. On the left sections of the brain, an absence of a normal flow void can be seen, and on the sections with the P1 pulse sequence, a slight hyperintensity is present, a finding that indicates thrombosis in the sinus. On the proximal sections starting from the confluence of the superior and inferior sinuses, as well as within the sagittal sinus itself, a flow void can be seen, which also indicates thrombosis. Proximal sections, especially those taken in FLAIR left and right parietal lobes posteriorly, show irregular linear hyperintensities in the cortex, suggestive of thrombosis in the small cortical veins (Figure 3).

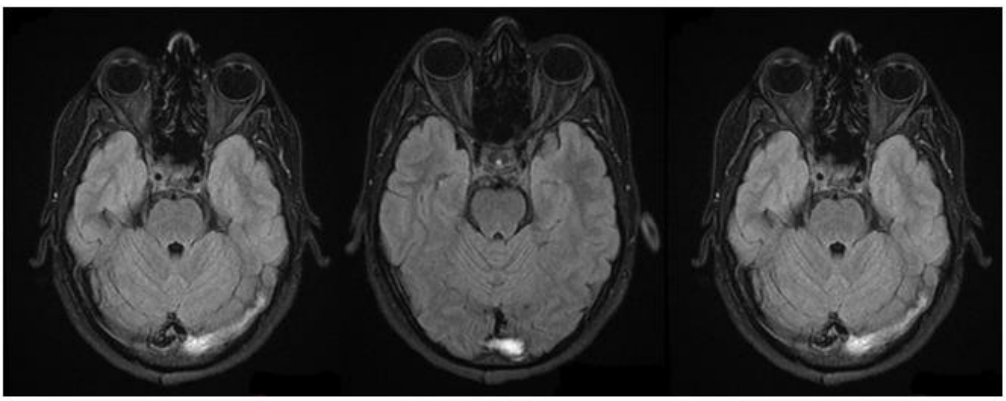

Fig. 3. MRI of the brain showing CVS

During the hospital stay, the patient was treated with antiedematous, anticoagulant, antiseizure, and other symptomatic therapies. Following a 12-day hospital stay, the patient was discharged home in a stable overall health condition (mRS 0, NIHSS 0, GCS 15) with the following treatment: tabl. Rivaroxaban 20 mg 1x1,

tabl. Carbamazepine 400 mg, drag. Diazepam 2 mg 1x1 as needed. During the follow-up visit one month after hospital admission, the patient reported minor latent right hemiparesis and sporadic headaches in the left temporal and occipital regions. A follow-up MRI was performed three months after the acute episode, and the results were assessed as normal (Figure 4). The patient did not experience weakness on the right side of her body and does not suffer from seizures. She is treated with antiseizure medication and NOAC on a regular basis. Her complaints were limited to somewhat increased menstrual bleeding. Rivaroxaban was discontinued one year after the event, and she is continuing on antiepileptic medication.

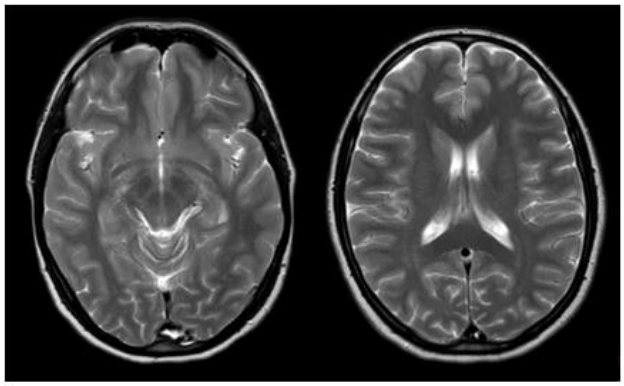

Fig. 4. Control MRI of the brain after 3 months showing normal findings

Discussion

Based on data from the International Study of Cerebral Vein and Dural Sinus Thrombosis (ISCVT), the frequency of CVST locations is as follows: transverse sinus 86%, superior sagittal sinus 62%, straight sinus 18%, cortical veins 17%, jugular veins 12%, vein of Galen 11%, and internal veins of the brain 11% [5]. CVST is a multifactorial condition with gender-specific causes [6]. As with any thrombotic process, risk factors are associated with the Virchow's classic triad of thrombogenesis: hypercoagulability, vessel wall damage, and blood stasis [7-8]. So far, in developed countries, the most commonly associated factors with homozygous mutations are the prothrombin gene G20210A, mutations of methylene-tetra-hydro-folate-reductase 677TT, protein C and S deficiencies, lack of anti-thrombin III, and positive anti-cardiolipin or anti-phospholipid antibodies [9-10]. There are also acquired causes of CVST, such as brain tumors, head trauma, intracranial hypotension, internal jugular vein abnormality, local head infection, extracerebral neoplasms, dural fistulas, hematological diseases, nephrotic syndrome, systemic vasculitis, CNS infections (bacterial meningitis, cerebral malaria), drugs (cisplatin, methotrexate, steroids, oral contraceptives), neurosurgery, lumbar puncture, pregnancy, and puerperium (Table 1) [11].

Table 1. Risk factors associated with CVST [11]

Inherited	Acquired
Homocysteinemia	Brain tumors
Factor V Leiden homozygous mutation	Head trauma
G20210A prothrombin gene	Intracranial hypotension
Methylene-Tetra-Hydro-Folate-Reductase 677TT mutations	Internal jugular vein abnormalities
Protein C and S deficiency	Local head infection
Anti-thrombin III deficiency,	Extracerebral neoplasias
Positive anti-cardiolipin or anti-phospholipid antibodies	Dural fistulas
	Hematological conditions
	Nephrotic syndrome
	Systemic vasculitis
	CNS infections
	Bacterial meningitis
	Cerebral malaria
	Medicaments
	Cisplatin
	Methotrexate
	Steroids
	Neurological surgery
	Lumbar puncture
	Pregnancy
	Puerperium

Alvis-Miranda HR, Milena Castellar-Leones S, Alcala-Cerra G, Rafael Moscote-Salazar L. Cerebral sinus venous thrombosis. J Neurosci Rural Pract. 2013; 4(4): 427-38. [11]

CVT usually begins in the large venous sinuses and may extend into the cortical veins. In a small number of cases, thrombosis is limited to the cortical or internal cerebral veins. Clinical symptoms depend on the localization and expansion of the thrombus and are very diverse. When the thrombus is confined to the main sinuses or jugular veins, the main problem is intracranial hypertension due to reduced venous drainage and reduced absorption of cerebrospinal fluid. Intracranial hypertension causes headaches, often accompanied by papilledema, and sometimes diplopia as a result of sixth cranial nerve palsy. Severe papilledema, although rare, can result in blindness. In patients with widespread thrombosis of multiple sinuses or when the cortical veins are obstructed, additional symptoms often develop. Collateral venous drainage in the brain is limited, and venous obstruction causes increased capillary pressure, cerebral edema, and ultimately, venous infarcts [7]. These focal cerebral lesions can result in seizures and neurological signs, such as hemiparesis, aphasia, hemianopia, and cognitive impairment. If left untreated, large venous infarcts can be life-threatening, and death can occur within hours in these patients due to cerebral herniation [12].

Pregnancy and puerperium

Pregnancy and the puerperium are common causes of transient prothrombotic states. Approximately 2% of pregnancy-related strokes are attributable to CVT. The frequency of CVT in the puerperium is estimated at 12 cases per 100,000 deliveries, and is only slightly lower than puerperal arterial stroke. In a study from Mexico, 50% of CVTs occurred during pregnancy or the puerperium. Most pregnancy-related CVTs occur in the third trimester or puerperium. Seven to eight CVTs among 50,700 deliveries in Canada occurred postpartum. During pregnancy or 6 to 8 weeks postpartum,

women are at an increased risk of venous thromboembolism. Pregnancy induces several prothrombotic changes in the coagulation system that persist into the early puerperium. Hypercoagulability worsens after delivery as a result of blood volume depletion or trauma. During the puerperium, additional risk factors include infection and instrumental delivery or cesarean section. One study reported that the risk of peripartum CVT increases with increasing maternal age, cesarean delivery, hypertension, infections, or excessive vomiting during pregnancy. It was recently reported that in pregnant women, hyperhomocysteinemia was associated with an increased risk of puerperal CVT in a study of 60 cases and 64 control subjects [7].

Treatment plans target the removal of the clot, managing seizures or focal impairments brought on by cerebral edema and ICH, as well as controlling or curing the underlying condition. Further treatments for CVST complications include surgery for hemorrhagic stroke, ICH, and hydrocephalus, among other conditions [13].

Warfarin, a long-term vitamin K antagonist, should be administered with an international normalized ratio (INR) goal of 2-3 after emergency care of CVT. The etiology determines the duration of anticoagulation. According to the AHA/ASA guidelines, anticoagulation is advised for 3-6 months in cases of provoked CVT, 6-12 months in cases of unprovoked CVT, and possibly lifelong in cases of recurrent CVT, VTE following CVT, or CVT linked to severe thrombophilia [7]. More recently, the American College of Chest Physicians (ACCP) has recommended prolonging anticoagulation for three months in cases of induced VTE, revisiting after three months for unprovoked VTE, and evaluating bleeding risk yearly thereafter [14].

It is unclear if this holds true for CVT, which has a somewhat different risk factor profile. Since recanalization only occurs in the initial few months following CVT, and there is no evidence linking it to clinical outcomes, it does not appear to affect the decision regarding the duration of anticoagulation [15].

Direct oral anticoagulants (DOACs) are important to discuss. Research has demonstrated that when it comes to treating VTE and preventing recurrence, DOACs are comparable to Warfarin [16].

There are very few observational studies available for CVT, and one of them included only fifteen patients [17]. In the absence of clear evidence, DOACs remain a potential treatment option for patients in whom Warfarin is not appropriate, but they would not be recommended as a first-line therapy. Geisbusch *et al*. reported data on 16 patients with cerebral venous and sinus thrombosis, comparing the recanalization status, complications, and clinical outcomes of Rivaroxaban with Warfarin [18]. The overall outcome was excellent in 93.8%, and all patients showed at least partial revascularization. No statistically significant differences were found between the groups, except for the use of heparin before the start of oral anticoagulation (p=0.03). One patient on Warfarin and two patients on Rivaroxaban had minor bleeding (p= 0.55) within a median follow-up of 8 months. Anticoli *et al*. reported data on six patients with CVT treated with Rivaroxaban, showing an excellent outcome in 100% of patients and complete or partial recanalization in 83% at three-month follow-up. At 12 months, they observed an excellent outcome in 100% of patients and complete (33%) or partial recanalization (67%) in all cases. There were no bleeding complications (major, clinically relevant non-major, or minor) or recurrent thrombotic events during follow-up [19].

Recently, Shankar *et al*. published a case study of 21 patients treated directly with Rivaroxaban without bridging heparin therapy [20]. An excellent clinical outcome was observed in 95% of patients when assessed after three months of treatment, with complete and partial recanalization observed in 60% and 40% of participants, respectively. According to various studies, because of their decreased risk of bleeding, new oral anticoagulants (NOACs) such as Rivaroxaban have been introduced as alternatives to traditional vitamin-K antagonists for the long-term management of thrombotic events [21].

However, there is insufficient data to support safety and effectiveness of Rivaroxaban in treating cerebral venous thrombosis (CVT). A study aimed to compare the effectiveness and bleeding risk of Rivaroxaban to Warfarin in treating CVT. Thirty-six individuals with a diagnosis of CVT participated. Patients were monitored for a minimum of 12 months after admission, during which clinical and baseline data were evaluated. The modified Rankin scale (mRS), major or mild bleeding, and indications of recanalization on contrast-enhanced magnetic resonance venography (MRV) were used as outcome measures. Patients were divided into two groups according to the type of oral anticoagulant (Rivaroxaban *vs.* Warfarin). The groups were compared in terms of final outcomes and side effects. Thirteen patients received Warfarin and 23 received Rivaroxaban. An optimal mRS score (0-1) was achieved in 9 out of 10(90%) of patients treated with Rivaroxaban, and 19 out of 22(86.36%) of patients treated with Warfarin. MRV showed complete or partial recanalization in 12 of 14 (85.71%) patients treated with Rivaroxaban, and in all patients in the Warfarin group. There was no significant difference between the two groups in terms of major and minor bleeding. This study concludes that rivaroxaban shows promise in the management of CVT.

SECRET is investigating the safety of rivaroxaban *versus* standard care for the treatment of symptomatic cerebral venous thrombosis initiated within 14 days of diagnosis. SECRET is an open-label, randomized, controlled Phase II study that will evaluate the safety of Rivaroxaban, a non-vitamin K antagonist oral anti-

coagulant (NOAC), compared to the standard of care (unfractionated or low-molecular-weight heparin with a transition to Warfarin [INR 2.0-3.0], or continuous low molecular weight heparin) for cerebral venous thrombosis. Patient recruitment is at 17 stroke research centers across Canada over 3 years. During the pilot phase, 50 adult patients within 14 days of diagnosis of symptomatic cerebral venous thrombosis will be randomized to receive Rivaroxaban 20 mg daily *versus* the standard approach (Warfarin or low molecular weight heparin). Patients will be followed up for one year. The feasibility of recruitment will be tested during the pilot phase, and the results will be refined for future Phase III testing. Two case reports of patients with CVT, treated with Rivaroxaban, have been published [22].
Rivaroxaban is a direct factor Xa inhibitor approved for venous thromboembolism and non-valvular atrial fibrillation. It is a drug that is taken once a day and does not require INR monitoring. A study presents results obtained from two CVT patients who elected to undergo anticoagulation with Rivaroxaban instead of Warfarin. One patient, a 39-year-old lawyer and smoker, experienced sigmoid sinus thrombosis on the left side after taking oral contraceptives for a year (MRV). The second patient, who was a thirty-two years old employee, reported having a headache that felt like lightning. MRV revealed internal jugular vein, right lateral sinus, and sigmoid sinus thrombosis. Hypercoagulable states of both individuals were examined and found to be normal. After three months of taking Rivaroxaban, partial to total recanalization of the left lateral sinus occurred in the second patient's MRV follow-up, while the right lateral and sigmoid sinuses were partially to completely recanalized. They did not report any bleeding complications. After three to four months, Rivaroxaban was discontinued in both situations. During the 6- and 12-month follow-up, there was no recurrence.
In a brief series, Geisbüsch *et al.* reported on the use of Rivaroxaban in CVT. Seven of sixteen individuals with CVT received Rivaroxaban treatment. At a mean follow-up of eight months, all patients exhibited at least partial recanalization without significant internal bleeding. When used as an anticoagulant strategy, Rivaroxaban may be beneficial for people with benign or simple CVT who do not require long-term anticoagulation [23].

Management of seizures in CSVT

Numerous inconsistent data exist with regard to seizures in cases of cerebral venous sinus thrombosis (CVST). An analysis of all papers and data regarding this medical condition during the period of 1966 to 2016 was conducted. After examining 63 articles, seizures were categorized as post-CVT Seizures (PCE), which occurred after 14 days, and acute symptomatic seizures (ASS), which occurred during the first 14 days. Patients with loss of consciousness, focal neurological impairments, supratentorial lesions, and thrombosis in the cortical veins, rectus sinus, and superior sagittal sinus were frequently affected by ASS. PCE was predisposed by the occurrence of ASS, motor deficits, and supratentorial lesions, especially hemorrhage. Most experts believe that primary prophylaxis with antiepileptic drugs in the acute phase is not indicated. However, initiation of prophylaxis after the first attack should be recommended in patients with supratentorial lesions or focal neurologic deficits. The quality of the current evidence is low, and most conclusions are based on expert opinion. More accurate reports of seizure semiology, detailed antiepileptic treatment plans, and outcomes are necessary to answer existing questions. Acute symptomatic seizures occur in about 35-50% of all patients, with a higher incidence (76%) in peripartum CVST [24-27].
In some studies, only 61% of seizures were focal, with or without secondary generalization [28-30]. In 2004, Masuhr *et al.* described Todd's paresis following CVST-induced seizures and introduced it as an indication of CVST, particularly if the paresis becomes bilateral [31]. In a follow-up study in 2006, Todd's palsy was reported in 54.7% of patients after ASD [32].
On the other hand, a higher frequency of generalized seizures was reported in some trials [33-35]. As stated by Price *et al*., there is no well-designed randomized controlled trial regarding primary or secondary seizure prevention in CVST, and there is also no solid evidence to demonstrate the efficacy of prophylactic use of ASMs for seizure prevention [36].
Therefore, decision-making regarding the timing, type, dose, and duration of antiepileptic treatment in patients with CVST is often individualized. The results of a study by Coutinho *et al.* showed that 8% of experts initiated prophylactic ASM in all patients, 21% prescribed prophylactic therapy only in patients with focal cerebral lesions, and the rest (71% did not recommend prophylaxis ($p<0.001$) [37].
Despite the lack of sufficient data, the AHA/ASA guidelines suggest that in patients with CVST and no supratentorial lesion or focal neurologic deficits who experience a seizure, early initiation of an ASM for a period to prevent further seizures is probably recommended [7].
However, there are no suggestions made for these patients in the ESO/EAN guidelines. There are fewer proponents of starting antiepileptic medication just in individuals who have had recurring seizures during the acute period [38].
In addition, there is no consensus on the duration of treatment. As PCE is more common in patients with ASS and usually occurs within the first 6-12 months after the acute phase, ASM treatment for one year is recommended in patients with ASS and previously indicated risk factors [39-44]. Nevertheless, in patients

without these risk factors who have received ASM, tapering ASMs after the acute phase seems reasonable [45].

For these individuals, a period of about three months may be contemplated [46]. However, it is advised to continue antiepileptic medication for an additional one to two years or more if seizures occur during or after tapering [47].

It is interesting that the ESO/EAN guidelines state that no recommendations for PCE prevention can be offered at this time because therapy with ASMs during the acute phase has no function in preventing PCE [48].

Initially, our patient was started on Carbamazepine. Nevertheless, we chose to keep her on the medication as a prophylactic measure in order to prevent further seizures. As stated in the medication directions, Carbamazepine and Rivaroxaban taken together can cause an interaction in the liver. Potent substrates of Pgp/BCRP efflux transporters and cytochrome P450 (CYP) enzymes include RIV. In the meantime, CBZ is well recognized for being a potent inducer of several transporters and enzymes. Therefore, it is anticipated that CBZ and RIV will have a drug-drug interaction (DDI). When given in combination with RIV in humans (900 mg/day of CBZ and 20 mg/day of RIV), CBZ is anticipated that CBZ will greatly reduce RIV exposure. Following the first dose, it drops correspondingly by 52.3% and 68.5% in AUC inf. and 41.0% and 49.8% in Cmax at steady state. In order to ascertain the success or failure of extrapolating PK parameters from rats to humans, the extrapolated parameters were compared to empirically observed clinical values. When the projected human PK parameters (AUC inf and C max) were doubled from the experimental values, the extrapolation was deemed successful. Other values were deemed ineffective [49-52].

These outcomes are consistent with those achieved through the use of the PBPK model-based methodology. In order to fully understand the safety and effects of RIV and CBZ, human DDI studies are necessary [53].

In one study, 28 individuals were prescribed anticonvulsant medication, with six on Valproate, six on Levetiracetam, one on Carbamazepine, and one on Phenobarbital, and there were no significant differences in their levels of effectiveness, with the exception of one patient who was prescribed additional ASM during follow-up due to seizures occurring later in the course of observation. Typically, treatment is initiated during hospitalization [54].

Additionally, we found a case study in the literature about a 55-year-old man who underwent 12 years of treatment with Carbamazepine, Sodium valproate, and Phenobarbital. He experienced neurological symptoms, including a severe headache, nausea, momentary diplopia, minor cognitive impairment, and postural tremor. An empty delta sign and uneven contrast agent pooling around the superior sagittal sinus were visible on the contrast-enhanced CT scan of the head. A head MRI revealed that there was no lack of flow in the superior sagittal sinus. However, cerebral angiography revealed well-developed collateral channels and partial blockage of the superior sagittal sinus. The patient was diagnosed with superior sagittal sinus thrombosis and started on anticoagulant and antiplatelet therapy. The patient lacked any additional risk factors. However, he did receive treatment with anticonvulsants, such as Carbamazepine, which was linked to lower extremity venous thrombosis. Thus, there is a possibility of a correlation between long-term Carbamazepine medication and thrombosis of the superior sagittal sinus. This is the first case report of anticonvulsant-associated cerebral venous thrombosis. It implies that a risk factor for cerebral venous thrombosis could be a long-term treatment with Carbamazepine [55].

Conclusion

The clinical results and the rate of recanalization in our patient treated with Rivaroxaban validate the medication efficacy and attenuated adverse effects compared to conventional anticoagulants. During concurrent use of Rivaroxaban and Carbamazepine, our patient did not experience any additional thrombotic events. However, there was a minor side effect of increased menstrual bleeding. The results of the treatment are satisfactory compared to those of Warfarin. Therefore, when compared to traditional medications, Rivaroxaban may be regarded as a safe alternative for the treatment of CVST with good outcomes in terms of clinical improvement. In our opinion, large prospective randomized controlled trials are necessary to assess and validate the safety and suitability of Rivaroxaban for the treatment of CVT. In terms of seizure management, our patient is taking Carbamazepine, and during the 2.5-year follow-up, there have been no reports of seizures, thromboembolic events (such as recurrence of CVST), or any additional clinical decline. A comprehensive evaluation of the literature indicates that the necessity of antiseizure medication treatment is debatable, and no reliable randomized clinical trials exist.

Conflict of interest statement. None declared.

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Case report

CONGENITAL CARDIAC RHABDOMYOMA AND EPILEPSY ASSOCIATED WITH GENETIC REVEALED PATHOGENIC VARIANT OF THE TSC 1 GENE

КОНГЕНИТАЛЕН СРЦЕВ РАБДОМИОМ И ЕПИЛЕПСИЈА АСОЦИРАНА СО ГЕНЕТСКИ ОТКРИЕНА ПАТОГЕНА ВАРИЈАНТА НА TSC 1 ГЕНОТ

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Abstract

Introduction. Tuberous sclerosis complex (TSC) is a rare genetic disease with an autosomal dominant inheritance. TSC is caused by genetic mutations on either TSC1 or TSC2 gene. The TSC1 and TSC2 genes regulate cell growth in the body by suppressing a group of proteins, one of which is called mechanistic target of rapamycin, or mTOR. These proteins are expressed in many different organs of the body, which is why multiple organ systems are often affected by TSC.
Case presentation. We present a case of a two-year-old girl, with prenatal diagnosed olygohydramnios and multiple cardiac tumors suspected for cardiac rhabdomyomas. After birth, molecular genetic tests revealed likely pathogenic variant of the TSC 1 gene, inherited from her father and confirmed the diagnosis of tuberous sclerosis. Postnatal transthoracic echocardiogram confirmed the presence of multiple cardiac tumors. At the age of eight months, the first afebrile partial seizure with EEG changes was manifested, and treatment with AEDs was started. Last brain MRI revealed presence of multiple subcortical and cortical tubers. Based on brain MRI, clinical features and intractable epilepsy, we are planning to include Everolimus (mTOR inhibitor) in the therapy.
Conclusion. Prenatal diagnosis is important for timely detection and recognition of TSC, as well as early initiation of therapy with mTOR inhibitors in order to improve the life of affected children.

Keywords: tuberous sclerosis, mTOR inhibitors, rhabdomyomas, epilepsy

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Апстракт

Вовед. Комплексот на туберозна склероза (ТСК) е ретко генетско заболување со автосомно доминантен начин на наследување. ТСК е предизвикан од генетски мутации или на TSC1 или TSC2 генот. Гените TSC1 и TSC2 го регулираат растот на клетките во телото со супресија на група протеини, од кои еден се нарекува цел на активноста на рапамицин или мТОР. Овие протеини се изразени во многу различни органи на телото, поради што повеќе органски системи често се засегнати од ТСК.
Презентација на случај. Презентираме случај на двегодишно женско дете,со пренатално дијагностициран олигохидроамнион и мултипни кардијални тумори суспектни за рабдомиоми. После раѓање, молекуларните генетски тестови открија патогена варијанта на TSC 1 генот, наследена од нејзиниот татко и ја потврдија дијагнозата Туберозна склероза. Постналталната ехокрадиографија го потврди присуството на мултипните срцеви тумори. На осум месечна возраст, манифестираше прва атака на афебрилни конвулзии со присутни промени во ЕЕГ, поради што се започна со антиепилептична терапија. Последниот реализиран МРИ на мозок откри присуство на мултипни субкортикални и кортикални тубери. Базирајќи се на промените на последната магнетна резонанца на мозокот, клиничките манифестации и резистентната епилепсија, во план е вклучување на Еверолимус (мТОР инхибитор) во терапијата.
Заклучок. Пренаталната дијагноза е особено значајна за навремено откривање и препознавање на ТСК, како и за рано започнување со терапија со мТОР инхибитор, со цел да се подобри квалитетот на живот на афектираните деца.

Клучни зборови: туберозна склероза; мТОР инхибитори; рабдомиоми; епилепсија;

Introduction

Tuberous sclerosis (TS) was initially described approximately 150 years ago by von Recklinghausen in 1862 [1]; then in 1880, Bourneville coined the term "sclerose tubereuse" based on the pathological features of the sclerotic tubers found in the postmortem investigation of patients with epilepsy and mental retardation [2]. TS is a rare genetic disorder of autosomal-dominant inheritance with a prevalence ranging from one in 6,000 to one in 12,000. Both sexes and all ethnic groups can be affected. It is a multisystem disorder involving the brain, skin, heart, kidneys, eyes, lungs, and liver, which manifests only in late childhood [3]. The two gene loci that code for TSC are TSC1, located on chromosome 9q34, and TSC2 on 16p13.3 [4]. TSC1 encodes TSC1 (hamartin), a 140-kD protein with no homology to TSC2. TSC2 encodes TSC2 (tuberin), a 200-kD protein with a GAP domain near the carboxy terminal. TSC1 and TSC2 are coexpressed in cells within multiple organs, including the kidney, brain, lung, and pancreas. TSC2 has been demonstrated to be localized in the Golgi apparatus and the nucleus, and TSC1 in the centrosome. The TSC1-TSC2 complex interacts with several proteins. One of the first mechanistic clues to the roles that TSC1 and TSC2 have in cell function was the finding that mutations in the drosophila Tsc1 and Tsc2 homologues increased cell and organ size. In normal cells, direct phosphorylation and inactivation of TSC2 by protein kinase B (AKT) leads to mTOR activation. In a serine-threonine kinase, mTOR has a central role in the control of cell growth and proliferation through the regulation of ribosome biosynthesis and protein translation. Rheb (RAS-homologue expressed in brain), a member of the RAS superfamily, is a specific GTPase downstream of TSC2 that functionally links the TSC1-TSC2 complex to the mTOR pathway [5]. Antagonism of the mTOR pathway with rapamycin and related compounds may provide new therapeutic options for TSC patients [6].

TSC can be diagnosed by the presence of clinical criteria and by genetic testing. Two major features or 1 major and 2 minor features are needed for a definite clinical diagnosis (Table 1) [7]. One of the major goals of the conference was to revisit the clinical diagnostic criteria published subsequent to the first International TSC Consensus Conference in 1998. Since 1998, one additional manuscript regarding the diagnostic criteria has been published that was designed to provide more guidance to practitioners by including pictures of the major and minor findings. At the 2012 meeting, the most significant change recommended to the diagnostic criteria was the incorporation of genetic testing. The recommendation of the Genetics Panel was to make identification of a pathogenic mutation in TSC1 or TSC2 an independent diagnostic criterion, sufficient for the diagnosis or prediction of TSC regardless of the clinical findings [1].

Possible diagnosis: Either one major feature or $\geq$ 2 minor features [1].

Table 1. Diagnostic criteria for tuberous sclerosis complex, 2012

Genetic diagnostic criteria	Pathologic mutation in TSC1 or TSC 2 gene
	10% to 25% of TSC patients have no mutation identified by conventional genetic testing, and a normal result does not exclude TSC, or have any effect on the use of clinical diagnostic criteria to diagnose TSC.
Major diagnostic criteria	Hypomelanotic macules (≥ 3, at least 5 mm diameter)
	Angiofibromas (≥3) or fibrous cephalic plaque
	Ungual fibromas (≥ 2)
	Shagreen patch
	Multiple renal hamartomas
	Cortical dysplasias (≥3) includes tubers and white matter radial migration lines
	Subependymal nodules (≥ 2)
	Subependymal giant cell astrocytoma
	Cardiac rhabdomyoma
	Lymphangioleiomyomatosis (LAM)
	Angiomyolipomas
	(AMLs) (≥ 2)
Minor diagnostic criteria	Confetti skin lesions
	Dental enamel pits (≥ 3)
	Intraoral fibromas (≥ 2)
	Retinal achromatic patch
	Multiple renal cysts
	Nonrenal hamartomas

Case report

We present the case of a two-year-old girl, from first regular and properly monitored pregnancy. At 36 weeks of gestation, olygohydramnios and multiple cardiac tumors suspected for cardiac rhabdomyomas were diagnosed by ultrasonography. The baby was delivered at

37+6 gestational week, by caesarean section, with birth weight 2530g, birth length 44 cm and Apgar score 6/8.
After birth, an ultrasonographic examination of the heart, kidneys and brain was performed and a genetic analysis for tuberous sclerosis was sent to the Macedonian Academy of Sciences and Arts.
The ultrasonographic examination of the brain and urogenital tract showed normal finding, but the performed transthoracic echocardiogram of the heart showed the presence of many tumorous formations in the right atrium, left and right ventricles. The largest tumorous formation was with dimensions of 1.2x0.6 cm and located in the left ventricle without cardiac inflow or outflow obstruction.
The child has been hospitalized several times at the University Clinic for Pediatric Diseases; for the first time at the age of 4 months, due to high fever, bronchial obstruction and first attack of febrile convulsions. The implemented PCR test for SARS Cov 2 in the mother and child was positive.
At the age of 4.5 months, the realized ultrasonographic examination of the brain showed hypertrophy of plexes and physical examination showed the presence of a small growth of skin in the area of the left mammillary region.
Molecular genetic tests revealed likely pathogenic variant c.1734_1738delinsT (p.Ile580SerfsTer48) in the exon 15 of the TSC 1 gene, inherited from her father. This variant c.1734_1738delinsT (p.Ile580SerfsTer48) in TSC1 gene has not been previously described in the literature. According to the criteria of the American College of Medical Genetics (ACMG) is interpreted as a likely pathogenic.
At the age of 8 months, a short-term convulsive attack occurred again, with shaking of the upper right limb and stiffness of the jaw. On physical examination, a nevus was noted in the posterior cervical region. Initial electroencephalogram (EEG) (Figure 1) revealed bihemispheral epileptiform discharges with dominant left-sided foci and a tendency for generalization. During the hospital stay, antiepileptic therapy was started with Vigabatrin. At follow-up examination, one month after hospitalization, she was still having short-term seizures. That's why we added another antiepileptic drug (Levetiracetam) in the therapy. In the following period, she still had short-term seizures and Rufinamide was included in the therapy.

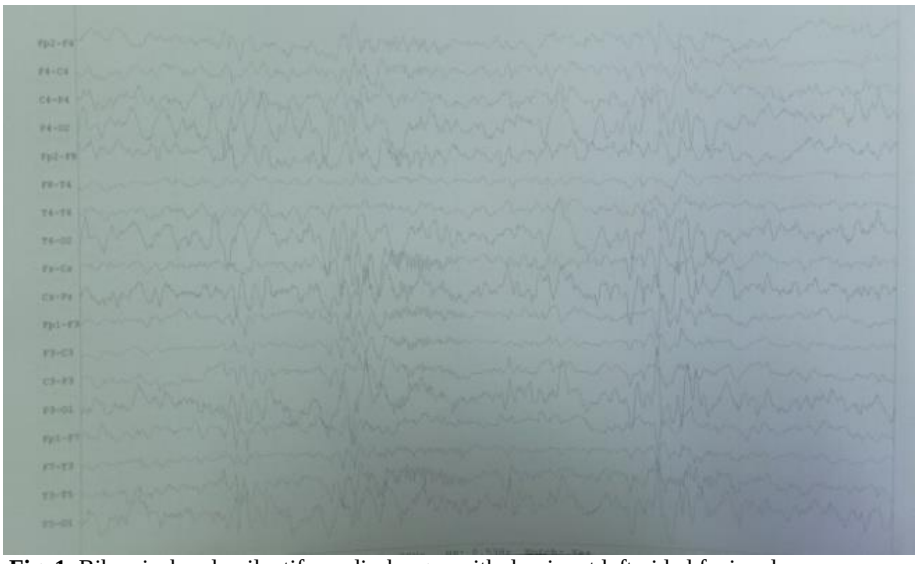

Fig. 1. Bihemispheral epileptiform discharges with dominant left-sided foci and a tendency for generalization

At the age of 10 months, abdominal MRI and MRI of the brain were performed and revealed no abnormalities, except for the finding of a small arachnoid cyst on the left temporally with a proportion of about 8 mm.
The echocardiogram of the heart showed that some of the tumorous formations were completely missing, some reduced and visualized in the right ventricle towards the top and one on the back wall of the left atrium.
A control examination at the age of 13 months showed that the child reacted to sound and light, began to sit and developed fine motor skills. The therapy with Vigabatrin tablets, Levetiracetam and omega fatty acids was continued.
After 6 months of observation, the child still had seizures, and the control EEG showed diffuse cerebral dysfunction (Figure 2). Therefore, the therapy continued with Vigabatrin and Carbamazepine. After few months the patient was seizure-free.

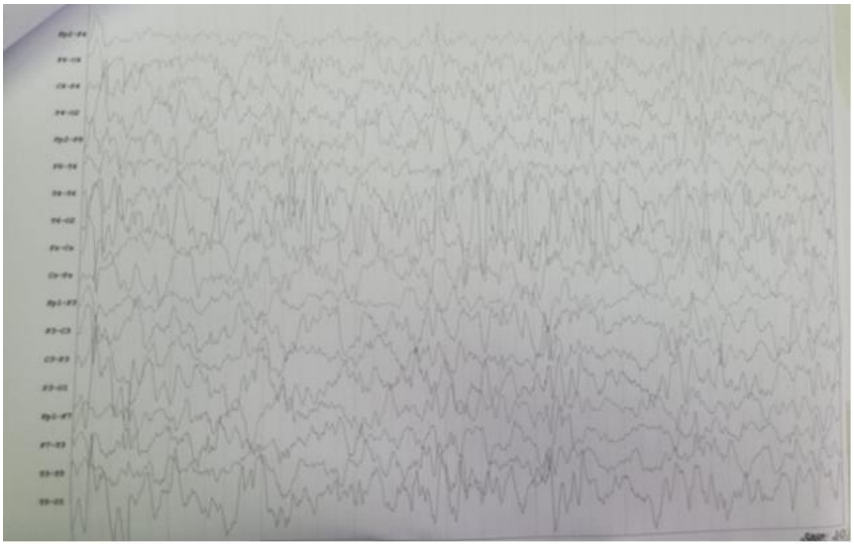

Fig. 2. EEG - Diffusely changed with appearance of polymorphic discharges

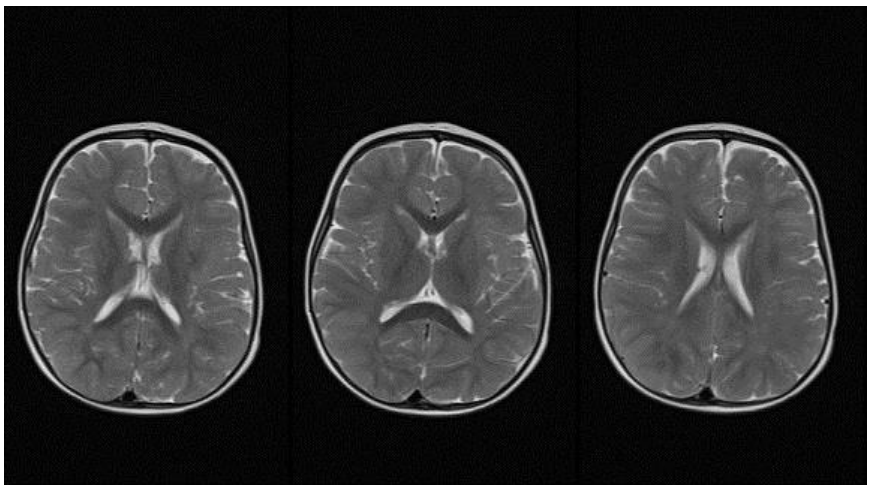

Fig. 3. Subcortical and cortical tubers

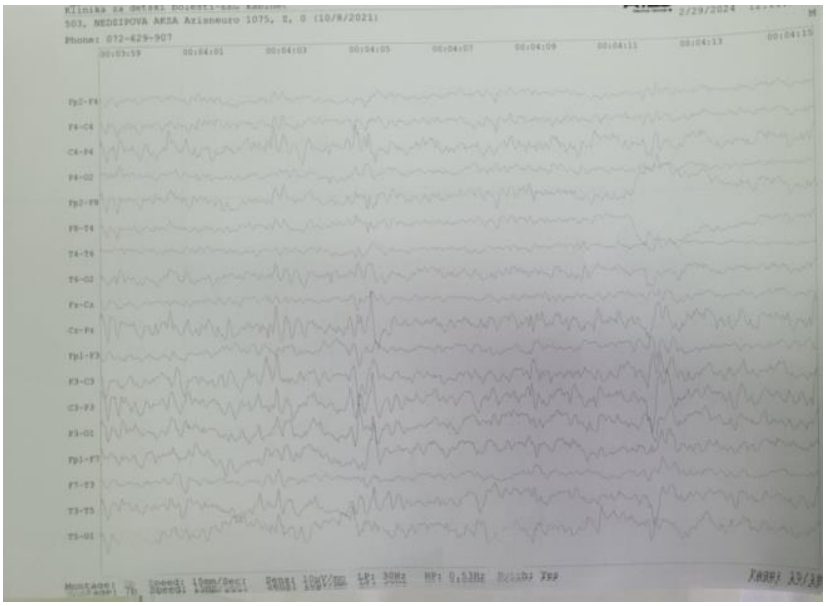

Fig. 4. Follow-up EEG: Basic brain activity, present alpha rhythm with dominant left- sided foci

The control magnetic resonance imaging (Figure 3) of the brain showed bilaterally diffuse corticosubcortical hyperdensities in the white matter of T2 and the flair sequences that went in favor of cortico subcortical tubers, without diffusion restriction.
After 6 months of observation, multidisciplinary treated and seizure-free, she was hospitalized again because of destabilization in terms of attacks and short-term seizures which resolved spontaneously. The follow-up EEG (Figure 4) showed basic brain activity, present alpha rhythm with dominant left-sided foci. The therapy with two ASDs was continued, but seizures still persisted. Considering the condition of the child, it is planned to start therapy with Everolimus (mTOR inhibitor) as a next step to manage the disease.

Discussion

Cardiac rhabdomyomas are benign tumors of the heart that are rarely observed in non-TSC-affected individuals.These lesions usually do not cause serious medical problems, but they are highly specific to TSC and often the first noted manifestation of the disease, and therefore remain a major feature [1]. Rhabdomyomas can be detected in 20 to 30 weeks' prenatal ultrasound [8]. In our case, rhabdomyomas were detected in 36 weeks of gestation. They are most frequently located in the ventricles, where they can compromise ventricular function and on occasion interfere with valve function or result in outflow obstruction. These tumors are frequently observed in TSC-affected individuals during fetal life, but after birth they often regress and in some individuals may no longer be detectable by echocardiographic examination. The prenatal presence of a cardiac rhabdomyoma is associated with a 75-80% risk of TSC, with multiple rhabdomyomas conveying an even higher risk [1]. After birth, genetic tests that were performed on our patient proved the presence of a pathogenic variant inherited from her father. At the beginning, only the presence of cardiac rhabdomyomas was proven, but then in infancy she manifested epileptic seizures, which were reststent of antiepileptic monotherapy. Epilepsy is one of the most common manifestations of TSC, associated with significant morbidity and mortality and, as such, the management of seizures is an important treatment goal. Treatment is particularly challenging because seizures are generally refractory to standard anti-seizure drugs (ASDs). TSC is a common cause of West syndrome, an epileptic syndrome characterised by IS (infantile spasms), hypsarrhythmia in an electroencephalogram (EEG) and developmental delay that generally presents in the first year of life. In the European Union (EU), vigabatrin (VGB) is indicated as monotherapy for infantile spasms, and in combination with other ASDs for patients with refractory focal epilepsy, with no age specifications [9]. Because of that, to control the seizures in our patient we added another antiepileptic drug. Medical problems related to the brain result in the greatest morbidity and mortality in TSC, and hence, three panels at the 2012 Consensus Conference devoted their efforts to central nervous system-related findings of TSC. The panels were: (3) brain structure, tubers, and tumors; (4) epilepsy; and (5) TSC-associated neuropsychiatric disorders [1]. The control magnetic resonance imaging of the brain showed bilaterally diffuse cortico-subcortical tubers. TSC is caused by a mutation in either the TSC1 or the TSC2 gene, the loss of which triggers constitutive activation of the mTOR signaling pathway, leading to an abnormal cell growth/proliferation and the subsequent formation of hamartomatous lesions. The discovery of the relationship between TSC1/TSC2 and mTOR has resulted in important clinical advances in the use of mTOR inhibitors, particularly sirolimus and its analog everolimus, for the treatment of several TSC manifestations [10]. Few months before brain changes, our patient was seizures-free, taking the regular prescribed antiepileptic therapy (combination of two ASDs). After that, she was manifested short-term seizures, refractory to prescribed anti-seizure drugs which resolved spontaneously. Treatment with everolimus, a mTOR inhibitor, has been shown to be of great benefit to TSC patients, both in reducing tumor growth and as a treatment for intractable epilepsy. Everolimus works as an antiseizure medication or antiepileptogenic agent, or as both. Up to 40% of TSC patients with intractable epilepsy show a clinically relevant seizure response to everolimus. If epilepsy is mainly the result of the focal cortical dysplasia that formed during the prenatal phase of neuronal migration and differentiation, then it may be that the epileptogenic process has already started prenatally, and epilepsy may be inevitable. In TSC patients, the epileptic focus is often associated with a tuber and removal of such a tuber and the perituberal tissue by epilepsy surgery has a high chance of inducing seizure remission. This may support the hypothesis that the cortical dysplasia is central in epileptogenesis. This was highly significant and led to the registration of everolimus in the treatment of intractable epilepsy in patients with TSC aged 2 years and older [11]. After all procedures, changes in control MRI and intractable epilepsy, in the following period, we are planning to start with everolimus in the therapy to control the seizures and to improve the quality of our patient's life.

Conclusion

TSC is one of the neurocutaneous syndromes, characterized by an autosomal dominant mode of inheritance, which means there is no curative treatment of this disease. It affects many organ systems.
Prenatal diagnosis is one of the most important factors in early diagnosis of TSC. Timely diagnosis of TSC

before seizure onset is feasible and it is becoming pivotal for epilepsy management and improvement of cognitive outcome. After the diagnosis is confirmed, genetic counseling is recommended.

Multidisciplinary approach and earlier use of mTOR inhibitor may improve the quality of patients' life. The prognosis is individual.

In conclusion, tuberous sclerosis is a lifelong condition that requires careful monitoring and follow-up because many symptoms may take years to develop.

Conflict of interest statement. None declared.

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УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски медицински преглед"(ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.
Списанието ги има следниве рубрики и категории на трудови:

1. **Изворни трудови**
2. **Соопштувања за клинички и лабораториски искуства**
3. **Прикази на случаи**
4. **Од практика за практика**
5. **Едукативни статии**
6. **Вариае (**писма од редакцијата, општествена хроника**,** прикази на книги, извештаи од конгреси, симпозиуми и други стручни собири, рубриката „Во сеќавање„ и др).

Изворните трудови имаат белези на научни трудови, додека трудовите категоризирани во рубриките 2-5 имаат белези на стручни трудови.
Во ММП се објавуваат трудови на членовите на МЛД или на членови на други стручни здруженија. Авторите се одговорни за почитувањето на етичките начела при медицинските истражувања, а изнесените ставови, изведени од анализата на сопствените резултати, не се нужно и ставови на Редакцијата на ММП.
Редакцијата ги испраќа ракописите на стручна рецензија; рецензентот (ите) и Редакцијата ја определуваат дефинитивната категоризација на ракописот кој е прифатен за печатење. Редакцијата го задржува правото ракописите да ги печати според рецензираниот приоритет.
Упатството за соработниците на ММП е во согласност со Ванкуверските правила за изедначени барања за ракописите кои се праќаат до биомедицинските списанија.

1. ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (е-маил) на МЛД-ММП, со двоен проред и најмногу 28 редови на страница. Трудот се поднесува на англиски јазик латиничен фонт Тимес Нењ Роман големина 12 и апстракт на македонски јазик. Лево, горе и долу треба да се остави слободна маргина од најмалку 3 см, а десно од 2,5 см.. Редниот број на страниците се пишува во десниот горен агол.
Ракописот на трудот треба да е придружен со писмо на првиот автор, со изјава дека истиот текст не е веќе објавен или поднесен/прифатен за печатење во друго списание или стручна публикација и со потврда дека ракописот е прегледан и одобрен од сите коавтори, односно со придружна декларација за евентуален конфликт на интереси со некој од авторите.

Насловната страна треба да има: наслов на македонски и англиски, имиња и презимиња на авторите, како и институциите на кои им припаѓаат, имињата на авторите и насловот на установата се поврзуваат со арапски бројки; автор за кореспондеција со сите детали (тел. е-маил); категорија на трудот; краток наслов (до 65 карактери заедно со празниот простор); како и информација за придонесот за трудот на секој коавтор (идеја, дизајн, собирање на податоци, статистистичка обработка, пишување на трудот).

Насловот треба концизно да ја изрази содржината на трудот. Се препорачува да се избегнува употреба на кратенки во насловот.

Изворните трудови и **соопштувањата** го имаат следниов формален редослед: насловна страна, извадок на македонски јазик (вовед, методи, резултати, заклучок) со клучни зборови, извадок на македонски јазик со клучни зборови, вовед, материјал и методи, резултати, дискусија и заклучоци, литература и прилози (табели, графици и слики) и легенди за прилозите во еден фајл.

Приказите на случаи треба да содржат вовед, детален приказ на случајот, дискусија со заклучок и литература со прилози.

Извадокот на македонски јазик треба да содржи најмногу 250 зборови и да биде структуриран со сите битни чинители изнесени во трудот: вовед со целта на трудот, методот, резултати (со нумерички податоци) и заклучоци. Заедно со извадокот, треба да се достават и до 5 клучни, индексни зборови.

Извадокот на англиски јазик мора да е со содржина идентична со содржината на извадокот на македонски јазик. Клучните зборови треба да се во согласност со МеСХ (Медицал Сибјецт Хеадингс) листата на Индеџ Медицус.

Воведот треба да претставува краток и јасен приказ на испитуваниот проблем и целите на истражувањето, со наведување на етичкиот комитет односно институцијата која го одобрила испитувањето (клиничка студија која се работи според принципите на Хелсиншката декларација за пациентите и нивните права).

Методите треба да бидат точно назначени, за да се овозможи повторување на прикажаното истражување. Особено е важно да се прецизираат критериумите за селекција на опсервираните случаи, воведените модификации на веќе познатите методи, како и идентификација на употребените лекови според генеричното име, дозите и начинот на администрација.

Резултатите треба да се прикажат јасно, по логичен редослед. Резултатите се изнесуваат во стандардните СИ единици. Во текстот треба да се назначи оптималното место каде ќе се вметнат табелите и илустрациите, за да се избегне непотребното повторување на изнесените податоци. Значајноста на резултатите треба да се обработи статистички, со детален опис на употребените статистички методи на крајот на делот *методи*.

Дискусијата треба да ги истакне импликациите од добиените резултати, споредени со постојните сознанија за испитуваниот проблем.

Заклучоците треба да не бидат подолги од 150 зборови.

2. ПРИЛОЗИ

Како прилог-документација на трудовите предложени за печатење, може да се достават до 5 прилога (табели, фигури,/слики - илустрации).

Табелите се доставуваат на крајот на трудот во истиот фајл. Секоја табела треба да има свој наслов и реден број кој ја поврзува со текстот. Хоризонтални и вертикални линии на табелата не се дозволени; ознаките на колоните во табелата се пишуваат скратено или со симбол, а нивното објаснување се пишува на дното на табелата, во вид на легенда.

Илустрациите се доставуваат со реден број како слика во црно-бела техника, а секоја слика треба да е придружена со легенда (опис).

Микрофотографиите може да содржат посебни ознаки во вид на стрелки или симболи. Покрај описот на сликата, мора да се наведе и зголемувањето и видот на боењето на препаратот (ако тоа веќе не е направено во секцијата *материјал и методи*).
Сите ознаки на фотографиите мора да бидат доволно големи, за да може јасно да се распознаат и по смалувањето во печатницата, при нивното вклучување во печатената страница на списанието.

3. ЛИТЕРАТУРА

Цитираната литература се пишува на крајот на трудот по заклучоците, со редни броеви според редоследот на појавувањето на цитатот на текстот на трудот ставени во средни загради и без простор меѓу нив (ако се последователни треба да се поврзани со цртичка, на пр. Ш3-6Ќ).
Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени во Индеџ Медицус):
а) ***статија во списание*** (се наведуваат сите автори, ако ги има до 4 или помалку; ако ги има повеќе од 4 се наведуваат првите 3 автори и се додава: *и сор.*) Неглиа ЈП Меадоњс АТ, Робисон ЛЛ *ет ал*. Сецонд неопласмс афтер ацуте лзмпхобластиц леукемиа ин цхилдхоод. Н Енгл Ј Мед 1991; 325:1330-6.
б) заеднички автор
ГИВИО (Интердисциплинарс гроуп фор цанцер царе евалуатион). Редуцинг диагностиц делас ин бреаст цанцер. Поссибле тхерапеутиц имплицатионс. *Цанцер* 1986; 58: 1756-61.
в) ***без автор*** **-** анонимно. Бреаст сцреенинг: нењ евиденце. (*Едиториалл Ланцет* 1984; и :1217-8).
г) ***поглавје во книга или монографија***
Њеинстеин Л, Сњартз МН. Патхогениц пропертиес оф инвадинг мицроорганисмс. Во: Содеман ЊА Јр, Содеман ЊА, Ед. Патхогениц пхсциологс: мецханисмс оф дисеасе. Пхиладелпхиа; Њ Б Саундерс, 1974: 457-72.

Првите отпечатоци на трудовите им се праќаат на авторите за корекција: авторите се должни коригираниот отпечаток да и го вратат на Редакцијата на ММП во рок од 2 дена.

Уплата за испечатен труд во списанието ММП изнесува 3.000, 00 денари и се уплаќаат на жиро сметката на: Македонско лекарско друштво
300000000211884 Ѣ Комерцијална банка
со цел на дознака : уплата за стучен труд

Адресата на Редакцијата
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Авторите што сакаат да објавуваат трудови во списанието треба да ја имаат уплатено членаринатa за тековната година во висина од 1440 денари и за тоа да ја информираат стручната служба на Македонско лекарско друштво, писмено или преку телефон.

Детални информации можете да добиете на телефонот на Друштвото 02 3 162 557.

Известување за рецензентите за ММП

Во склад со правилникот на УКИМ рецензентите што навремено и одговорно ќе ја одработат рецензијата ќе добијат 0.4 бода кои се собираат за унапредување во академските звања. Бодовите можат да се добијат и ретроградно преку побарување во МЛД - 3162 557.