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Original article

^{64}CU-COMPLEX OF THE BIFUNCTIONAL CHELATOR WITH PSMA TARGETING MOLECULE IN *EX VIVO* BIODISTRIBUTION STUDY IN HEALTHY RATS

СТУДИЈА ЗА EX-VIVO БИОДИСТРИБУЦИЈА КАЈ ЗДРАВИ СТАОРЦИ НА КОМПЛЕКС НА БИФУНКЦИОНАЛЕН ХЕЛАТОР СО ПРОСТАТА СПЕЦИФИЧЕН МЕМБРАНСКИ АНТИГЕН (PSMA) РАДИООБЕЛЕЖАН СО ^{64}CU

Maja Chochevska[1,2], Toni Tripunoski[3], Shasho Nikolovski[1,4], Filip Jolevski[1], Jasmina Shumkovska-Dimitrova[4] and Ana Ugrinska[1,3]

[1]University Institute for Positron Emission Tomography, Skopje, [2]Faculty of Medical Sciences, University "Goce Delchev", Shtip, [3]Institute of Pathophysiology and Nuclear Medicine, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, [4]Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

The development of new diagnostic radioactive biochemical products and effective therapeutic radiopharmaceuticals is very important in the era of a personalized approach in the management of cancer patients. Consistent expression of prostate specific membrane antigen (PSMA) in metastatic prostate cancer helps us in finding the relevant radioactive prostate-specific membrane antibody. The radiopharmaceuticals used in molecular imaging require a simple radiolabeling (radiosynthesis) procedure, good target-to-organ sensitivity, and metabolic stability.

The aim of this study was to label bifunctional chelator, DOTA, covalently bound to a PSMA targeting vector (DOTAGA-(I-y)fk(Sub-KuE)) with Copper-64 (^{64}Cu) and to examine its physicochemical properties and pharmacokinetic characteristics. Healthy adult rats of Wistar strain were used to carry out experiments through *ex vivo* biodistribution with intravenous radiopharmaceutical administration of (7±1.5) MBq. Target organs and tissues were harvested 60 min post-injection.

Also, *in vitro* radiochemical stability of radiolabeled molecule ^{64}Cu-DOTA-PSMA was studied with TLC method, 24 h post preparation of the product.

The obtained data showed a low uptake (≤0.45 % IA g^{-1}) in the majority of organs, except for the liver (1.21± 0.48 % IA g^{-1}) and the kidneys (6.10±0.98 % IA g^{-1}), indicating them as clearance organs.

The *in vitro* stability study confirmed a relatively high stability and the product can be safely used during that period.

Keywords: radiopharmaceuticals, biodistribution, PSMA, rats, stability

Correspondence to: Maja Chochevska, University Institute for Positron Emission Tomography, Skopje, Republic of North Macedonia, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia; E-mail: maja.chochevska@gmail.com

Апстракт

Развојот на нови радиофармацевтски препарати за дијагноза и за терапија е клучен во стремежот кон персонализиран пристап во третманот на болните со малигни заболувања. Конзистентната експресија на простата специфичниот мембрански антиген (PSMA) кај метастатски карцином на простата ни дава можност за истражување со кое ќе се пронајде релевантна радиоактивно обележана молекула специфична за простатата. Радиофармацевтските препарати што се користат во клиничката пракса бараат едноставна процедура за радиообележување (радиосинтеза), добра чувствителност кон целниот орган и метаболичка стабилност.

Целта на оваа студија беше да се обележи радиоизотопот бакар-64 (^{64}Cu) со бифункционалниот хелатор, DOTA ковалентно врзан за PSMA молекулата (DOTAGA-(Iy)fk(Sub-KuE)) и да се испитаат физичко-хемиските и фармакокинетичките карактеристики на таквиот радиофармацевтски препарат. За спроведување на експериментот со ex-vivo биодистрибуција беа користени здрави стаорци од сојот Wistar, во кои радиофармацевтскиот препарат беше инјектиран интравенски (7±1,5) MBq. Животните беа жртвувани а органите и ткивата од интерес беа извадени 60 минути по инјектирање. In-vitro радиохемиската стабилност на ^{64}Cu-DOTA-PSMA беше проучувана со TLC метод, 24 часа по радиобележувањето на радиофармацевтикот.

Студијата од биодистрибуција покажа ниска акумулација (≤0,45% IA g-1) во органите, освен во црниот дроб (1,21±0,48 % IA g-1) и бубрезите (6,10 ±0,98% IA g-1) што ни посочува дека елиминацијата на овој радиофармацевтски препарат се одвива главно преку овие органи. In-vitro студијата за ста-

билност покажа висока стабилнот што значи дека препаратот може безбедно да се користи во тој период.

Клучни зборови: радиофармацевтски препарати, биодистрибуција, PSMA, стаорци, стабилност

Introduction

Although positron-emission tomography (PET) imaging with 18-fluorodeoxyglucose (^{18}F-FDG) is one of the most powerful tools for detecting and monitoring numerous oncological diseases, the development of different more specific diagnostic radiopharmaceuticals is an important endeavor that can lead to better management of patients with malignant diseases [1-6]. During the development of new radiopharmaceuticals, biodistribution studies are essential experiments. It is a critical step that could provide safety and dosing information for radiolabeled molecules designed for *in vivo* application in PET.

Radiopharmaceuticals (RFs) labeled with various copper radioisotopes, especially ^{64}Cu-based RPs, have been very attractive for PET molecular imaging in the last decade. The special interest in ^{64}Cu is due to its physical properties ($T_{1/2}$=12.7 h, 17.4% ß$^+$, E(ß$^+$)max=0.656 MeV; 39% ß$^-$, E(ß$^-$) max=0.573 MeV) showing that this positron emitter has a longer half-life than ^{18}F, enabling central production, the possibility of shipment and suitability for visualization at different time points up to 48 h. Low positron energy is another favorable opportunity for imaging with better spatial resolution, similar to Fluorine-18 but better than Galium-68, which is the most commonly used positron-emitting isotope for clinical management of patients with prostate cancer. On the other hand, ^{64}Cu has a low sensitivity due to its low positron decay fraction (17.4%) in comparison with ^{18}F (97%) and ^{68}Ga (88%) [7-11].

Another research interest for ^{64}Cu is its immense potential to react with different types of chelators considering good radiometal coordination chemistry [12-14]. Different bifunctional chelators (BFCs) have been labeled with ^{64}Cu as simple chelators with reactive functional groups that can be covalently bonded to a targeting molecule (peptide, antibody, nanoparticle) and can form kinetically stable complexes with the radiometal in order to avoid an accumulation of radioactivity in the background tissues [15-24]. These chemical properties of ^{64}Cu-complexes of the bifunctional chelator also present an opportunity for radiochemists to elaborate the pharmacokinetics and control the metal complex properties (overall charge, lipophilicity, size, different target-specific binding and biodistribution) [3,11,25].

PSMA, as a transmembrane protein significantly expressed in the prostate cancer cells compared to benign prostate tissue cells and normal prostate cells, can be used as a marker of tumor biological aggressiveness [26]. A large number of publications over the last few years presented the possibilities for modifications of the PSMA peptide labeled to a different radiotracer. In this literature, there are many studies that describe improving the success of radiopharmaceutical development related to its efficacy, safety, and metabolic stability. The latest preclinical and clinical research in the field were ^{18}F-labelled-PSMA [27-31], ^{68}Ga-PSMA [32-36] for PET imaging or for SPECT, ^{99m}Tc-PSMA [37-43]. The most prominent designed matched pair for imaging and therapy of prostate cancer for PSMA-inhibitor, till now is ^{68}Ga-PSMA/^{177}Lu-PSMA as a DOTA-conjugated complex, due to its availability as a generator product [43,44-50]. A literature review has shown that ^{64}Cu is a promising radionuclide for PSMA inhibitors and offers further improvements in diagnostics and therapy of prostate cancer in different types of tumors [7,10,18,20,24,40,51-60]. Finally, having in mind all strengths of ^{64}Cu mentioned above, future efforts might be aimed at transforming ^{68}Ga/^{177}Lu in ^{64}Cu/^{67}Cu matched pair or only copper-64 as a theragnostic agent [9,56,61-63].

Methods

General

All chemicals and solvents used were obtained from commercial sources with analytical grade and were used as received without any further purification. Radiopharmaceutical precursor which contains $^{64}Cu^{2+}$, a radionuclide of copper, as copper (^{64}Cu) chloride ([^{64}Cu]$CuCl_2$) in HCl 0,1N solution was obtained by Sparkle S.r.l, Montecosaro, Italy. Commercially available chemical precursor as a linear peptide with five amino acids and a covalently bound chelator DOTA (Glu-CO-Lys[(Sub)DLys-DPhe-DTyr(3I)-DOTAGA] trifluoroacetate) was purchased from piCHEM, Austria. All other chemicals and solvents were purchased from Sigma-Aldrich.

Preparation - Radiolabeling

DOTA-PSMA precursor (250 µg peptide) was dissolved in acetate buffer (2 ml; pH 5.5). Afterwards, radiolabeling of 400 µL DOTA-PSMA solution with [^{64}Cu] Cu^{2+} radionuclide was performed. The maximum amount of 64-copper radioactivity was 370 MBq. The reaction mixture was kept at room temperature (24°C±2°C) for labeling reaction at least for 60 min. Radioactivity measurements were made with a VDC 505 dose calibrator (COMECER, Netherlands).

Quality control of the radiolabeled complex

Quality control analysis for the sample included measu-

rement of pH, visual inspection, radiochemical purity, sterility and bacterial endotoxin test. Radiochemical purity and yield of the radiolabeled complex were performed with Raytest miniGita Star TLC instrument. Five µL aliquots of the radiolabeled solution was analyzed on a silica gel glass coated TLC plate using methanol/ aqueous ammonium acetate 10% (1:1) as a mobile phase. Monitoring of the radiochemical stability by TLC method was conducted 24 h post preparation, keeping it at 24±2°C.

Ex vivo biodistribution

Two anesthetized rats with diethyl ether (weight 300± 15 g) were i.v. injected via the tail vein with 100 µL ^{64}Cu-DOTA-PSMA (7±1.5 MBq). At 1 h p.i., the rats were sacrificed by ether anesthesia; organs and tissues of interest were harvested, assayed for radioactivity and weighed. The tail was cut above injection site and was taken into calculations. The radioactivity accumulation data were reported as a percentage of injected activity per gram of tissue (%ID/g) assessed with a calibrated dose calibrator, Capintec (Mirion Technologies, USA). The percentage of injected dose per gram (%ID/g) for different organs was calculated by dividing the decay corrected activity of each tissue (A) with the mass of each tissue/organ in grams.
The animal experimental methods were approved by the Institutional review board, and were conducted adhering to the ethical principles for the care and use of laboratory animals in accordance with the Law on Protection and Welfare of Animals in the Republic of North Macedonia.

Results

Radiochemistry

A cold kit of the chemical precursor was directly labeled with $^{64}Cu^{2+}$. Using a direct labeling method, a suitable labeling condition of $^{64}Cu^{2+}$ was achieved at room temperature. Visual inspection showed that the solutions remained clear, particulate-free, and pH values were within the acceptable limits of 4-9. The measurements showed more than 95% of radiochemical yield. Radiochemical stability of the radiolabeled complex using TLC at 1h, 6h, 12h, 18h, and 24h post preparation was analyzed. The results showed that the stability of the product did not decrease during that period.

Ex vivo biodistribution

Biodistribution data for rats are presented in Figure 1. Results are expressed as a percentage of injected radioactivity dose per gram of tissue. A small sample of blood, skeletal muscle, and bone were taken and weighed. All other organs were measured in their entirety.
The distribution results 60 min post-injection of ^{64}Cu-DOTA-PSMA in rats for the majority of organs (blood,

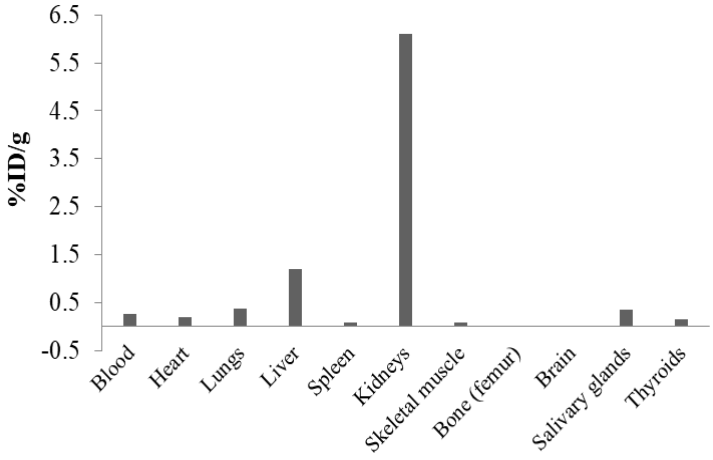

Fig. 1. Ex-vivo biodistribution of ^{64}Cu-DOTA-PSMA in healthy rat tissue

heart, lungs, spleen, skeletal muscle, bone (femur), brain, salivary glands and thyroid) showed no significant accumulation of the radiotracer (≤0.45% IA g^{-1}), higher uptake in the liver (1.21±0.48% IA g-1) and the highest in the kidneys (6.10±0.98% IA g-1).

Discussion

Radiochemical stability results demonstrate that ^{64}Cu-DOTA-PSMA has good stability as a result of non-specific binding of copper to peptide, which is rela

tively easily controlled by maintaining pH value between 4.5 and 5.5. Also, the complexation kinetics of Cu^{2+} is such that complex formation is rapid, while dissociation is slow enough for a good *in vivo* kinetic stability [60,64-66].

Radioactivity accumulation measurements in our study showed a rapid clearance from most normal tissues, such as blood, heart, lungs, spleen, skeletal muscle, bone (femur), brain, salivary glands and thyroid. Higher uptake was observed in the liver (1.21±0.48% IA g^{-1}) and the highest in the kidneys (6.10±0.98% IA g^{-1}) (Figure 1). It indicated that the ^{64}Cu-DOTA-PSMA is mainly metabolized through hepatobiliary and kidney tissues. Renal uptake of the ^{64}Cu-DOTA-PSMA is partially due to the route of excretion of these radiotracers [53,64,67].
Distribution pattern of radioactivity in this experiment was consistent with the available preclinical data, taken from literature for the same or similar sequences of PSMA molecule. Biodistribution studies performed in normal mice showed also high uptake in kidneys and relatively high uptake in the liver [25,53,68-71]. Further studies with slightly modified peptide-conjugated compounds have to demonstrate their potential for lower accumulation in these two organs. Depending on the amino acid sequence, peptides can greatly differ in pharmacokinetic properties. In most cases, improvement of these properties is necessary.
DOTA covalently bound to a PSMA targeting vector (DOTAGA-(I-y)fk(Sub-KuE)) with Copper-64 (64Cu) can become a candidate for future clinical trials.

Conclusions

^{64}Cu-complex of the bifunctional chelator with prostate-specific membrane antigen (^{64}Cu-DOTA-PSMA) as a new compound was readily obtained in a single reaction step by standard labeling with good yields. Altogether, our stability results have shown that this novel radiotracer complex with PSMA molecule is an interesting alternative to the existing bifunctional chelators, such as DOTA for ^{64}Cu-based radiopharmaceuticals. *Ex vivo* biodistribution using healthy rats, 60 min post-injection showed low radioactivity accumulation in healthy tissues with slightly higher accumulation in the liver and the kidney. The percentage of radioactivity in the kidney per gram of tissue was not more than 7% and, in the liver, not more than 1.5%.

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Abbreviations

%IA/g: Percent of injected activity per gram; ^{64}Cu: Copper-64; BFC: Bifunctional chelator; DOTA: 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid; RFs – Radiopharmaceuticals; PSMA - Prostate-specific membrane antigen: DOTAGA-(I-y)fk(Sub-KuE))

Conflict of interest statement. None declared.

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Original article

BLEPHAROPLASTY - OUR EXPERIENCE

БЛЕФАРОПЛАСТИКА - НАШЕ ИСКУСТВО

Boro Dzonov, Lazo Noveski, Elizabeta Mirchevska, Margarita Peneva, Sofija Pejkova, Gordana Georgieva, Blagoja Srbov, Mirjana Nacka and Darko Daskalov

University Clinic for Plastic and Reconstructive Surgery, Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia, N. Macedonia, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

One of the most popular aesthetic surgery procedures is blepharoplasty. Preoperative planning and tissue resection can reduce complications and improve outcomes. Although some patients want blepharoplasty to address age-related changes in the skin of their eyelids, the procedure is more of a sculpture and contouring of the overall aesthetic unit. In this paper we present the history, basic anatomy, indications and surgical technique of upper and lower blepharoplasty. The importance of the preoperative patient evaluation for blepharoplasty has also been stated. We present our experience in blepharoplasty surgery done at the University Clinic for Plastic and Reconstructive Surgery in a three-year period, along with the complication rate and outcome. We have briefly described the techniques of standard upper and lower eyelid blepharoplasty. Practically, the rejuvenation of this complex anatomical area requires a combination of therapies including fat excision, repositioning or transfer, simultaneous brow or midface lift, and adjunctive treatment for skin resurfacing and periorbital hollows.

Keywords: blepharoplasty, upper eyelid, lower eyelid, complications

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Апстракт

Една од најпопуларните процедури во естетска хирургија е блефаропластиката. Предоперативно планирање и ткивна ресекција може да помогнат да се намалат компликациите и да се подобрат резултатите. Иако некои пациенти сакаат блефаропластиката да ги реши промените поврзани со возраста и стареење на кожата на нивните очни капаци, постапката е повеќе од оформување и контурирање на една цела естетска единица. Во овој труд ја презентираме историјата, основната анатомија, индикациите и хируршката техника на горната и долната блефаропластика. Наведена е и важноста на предоперативната евалуација на пациентот за блефаропластика. Ви го претставуваме нашето искуство од операцијата на блефаропластика направена на нашата клиника во период од три години, стапката на компликации и исходот. Накратко ги опишавме техниките на стандардна блефаропластика на горните и долните очни капаци. Практично, подмладувањето на оваа сложена анатомска област бара комбинација од терапии, вклучувајќи отстранување на масно ткиво како и негово репозиционирање или трансфер, симултано подигнување на веѓите или средината на лицето и дополнителен третман за обновување на површината на кожата и периорбиталните вдлабнатини.

Клучни зборови: блефаропластика, горен очен капак, долен очен капак, компликации

Introduction

The history of blepharoplasty dates back to more than 2000 years ago when Susruta described eyelid surgery in the Susruta-tantra. Also, about 25 A.D. Aulus Cornelius Celsus, a first century Roman philosopher, described the excision of upper eyelid skin for the “relaxed eyelid” in his *De re Medica*. The first medical illustration of the aging eyelid was published in 1817 by Beer. One year later Von Graefe first used the term ‘blepharoplasty” to describe a case of eyelid reconstruction following a cancer resection. In 1844 Sichel described “ptosis adiposa” as a condition by which the excessive upper eyelid skin fold was filled with fat. Fuchs later correctly recognized the role of the fascia attachments between skin, orbicularis, tarsus, and the levator in the development of the supratarsal skin fold and the importance of its recreation. In 1907, the American surgeon Conrad Miller wrote one of the first books on cosmetic surgery entitled *Cosmetic Surgery in the Correction of Facial Imperfection*. Suzanne Noel,

*Correspondence to:*Boro Dzonov, University Clinic for Plastic and Reconstructive Surgery, 1000 Skopje, R. N. Macedonia; E-mail: bdzonov@yahoo.com

a Parisian surgeon, wrote a book on cosmetic eyelid surgery. In the 1950s Castanares detailed the anatomy of the eyelids and made a contribution by identifying the role of orbicularis resection. Flowers in the 1970s introduced the supratarsal fixation for the low eyelid crease [1].

One of the most popular aesthetic surgery procedures is blepharoplasty. Preoperative planning and tissue resection can reduce complications and improve outcomes. Although some patients want blepharoplasty to reduce age-related changes in the skin of their eyelids, the procedure is more of a sculpture and contouring of the overall aesthetic unit. Skin texture changes with loss of elasticity and wrinkle formation, fat redistribution, enophthalmos, and anterior fat displacement with a lower eyelid orbital fat prolapse characterize the aging process in the eyelid complex. A local examination and surgical treatment strategy can achieve best results once the origin of the deformity and the accompanying periorbital anatomy are identified [2].

This surgery is a procedure of treating abnormalities, deformities, and disfigurations of the eyelids for functional, aesthetic, or both purposes. The most prevalent reason is cosmetic one, in which extra skin and fat are removed and/or redistributed to improve appearance [3]. Upper eyelid blepharoplasty is often paired with a ptosis surgery since the upper eyelids are drooping or ptotic in many situations. The levator aponeurosis is relocated on the tarsal plate and the upper eyelid height is titrated. Excess skin and fat are removed once the upper eyelid height is determined to produce a more youthful appearance. Upper blepharoplasty, on the other hand, may be recommended for functional reasons, such as upper eyelid fat, orbicularis hypertrophy or laxity, or skin laxity. In most cases, levator dehiscence is the cause of upper eyelid ptosis. Infections, trauma, tumors, and inflammation can all lead to dehiscence. Myasthenia gravis, trauma, orbital or eyelid tumors, congenital ptosis, third nerve palsy, or Horner syndrome are some of the less prevalent reasons of upper eyelid ptosis. Before performing a correction, it's critical to make sure the ptosis is caused by levator dehiscence. Advancement of the levator will not provide adequate lift if there is inadequate levator function (less than 4 mm), hence a frontalis sling technique is required to elevate the eyelid [4]. Shortly, we will give a quick review of the anatomy of the upper and lower eyelid.

Anatomy

Anatomy of the upper eyelid [5] (Figure 1):

1. The 4 layers contribute to the fascial framework of the upper eyelid.
2. The first is the orbicularis (superficial) fascia.
3. The second layer is the deep fascia of the upper lid and acts to retain the orbital fat.
4. The third layer is the levator aponeurosis.
5. Tarsus.

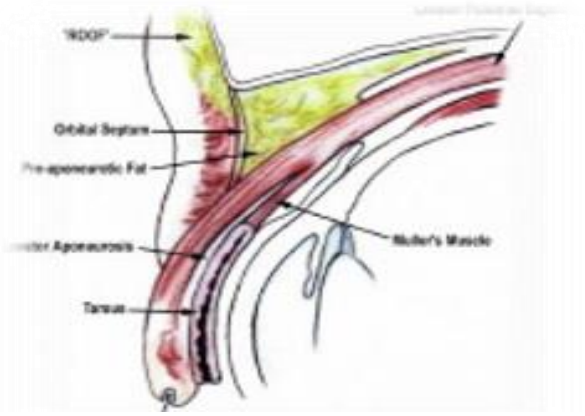

Fig. 1. Anatomy of upper eyelid

Lower eyelid anatomy [5] (Figure 2):

1. The lower lid margin commonly rests 1-2 mm above the inferior border of the limbus, making a gentle S curve.
2. The delicate skin of the lower eyelid is closely adherent to the underlying tarsus superiorly.
3. The orbicularis oculi muscle is closely adherent to the overlying periorbital skin and is designated into three zones: pretarsal, preseptal, and orbital regions.
4. Orbital septum.

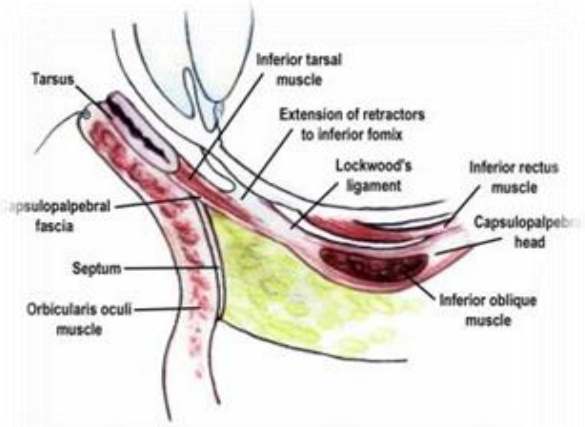

Fig. 2. Anatomy of lower eyelid

Material and methods

This study was performed at the University Clinic for Plastic and Reconstructive Surgery in Skopje, North Macedonia. In a three-year period, from 2018 to 2020, there were a total of 118 patients, of whom 79% were females and 21% were males, with mean age of 44.5 years (range 35-60).

Surgical technique

The upper lids should be injected superficially, with 2% lidocaine with 1:100,000 epinephrine using a 27 to

30-gauge needle. Skin incision can be made either with a No 15 Bard Parker blade or the radiofrequency monopolar cautery. Conservative fat excision can be performed as part of upper lid blepharoplasty. Retro-orbicularis oculi fat can be accessed beneath the lateral orbicularis overlying the superior orbital rim. Resection has been described to help decrease heaviness of the upper lid and lateral brow. The sub-brow fat pad can be repositioned during wound closure with use of eyelid suspension sutures. This can be done with two to three absorbable sutures that incorporate the orbicularis from the lower and upper edge of the incision along with the superolateral arcus marginalis. These sutures might result in early over-correction of the upper eyelid leading to lagophthalmos, which improves within days after the surgery. The skin incision can be closed using running or interrupted sutures with various absorbable or permanent materials [6]. As for transcutaneous lower lid blepharoplasty, the 'skin pinch' technique is ideal for skin laxity alone, with no fat prolapse. It is approached through a subciliary incision with the skin elevated off the orbicularis. The amount of skin to be resected can be estimated with a skin pinch between forceps. Redundant skin can be removed conservatively and redraped without disturbing the underlying orbicularis. The more aggressive 'skin-muscle flap' method is also approached through a subciliary incision, undermining the skin and orbicularis. The pretarsal orbicularis fibers should remain intact, and the skin and preseptal orbicularis are elevated as one flap. Dissection can be continued along the orbital septum to the level of the orbital rim. Periorbital fat is approached through small incisions in the septum. Orbicularis muscle fibers and skin can be excised at closure; however, damage to the orbicularis may lead to lower lid malposition and orbicularis denervation [7].

Results

The study included a total of 118 patients, of whom 79% were females and 21% were males (Figure 3), with the mean age of 44.5 years (range 35-60). The incidence of complications was 8.5 % (n=10). The complications (Figure 4) were 1 patient with hematoma (10%) that was treated with conservative care. Six patients had chemosis (60%); 70% of patients were with canthopexy, which at the end spontaneously resolved. Two patients had asymmetry (20%) and both of them were managed with retraction. Only 1 patient was with ectropion (10%), which was surgically treated.

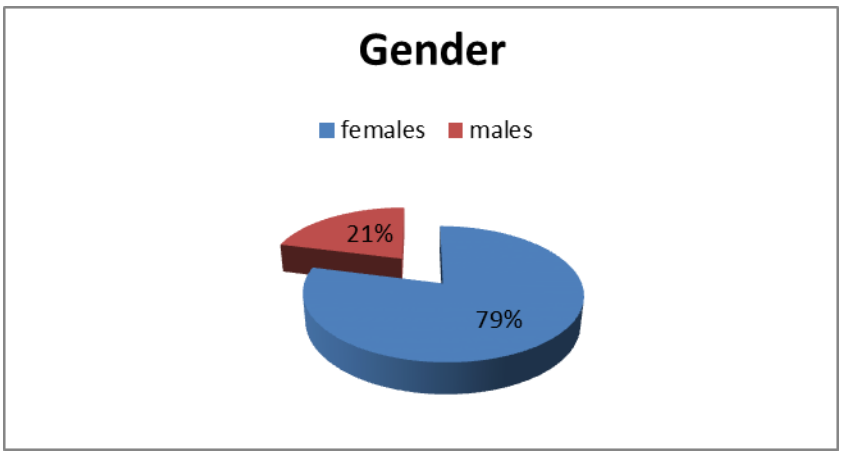

Fig. 3. Gender distribution among patients with blepharoplasty

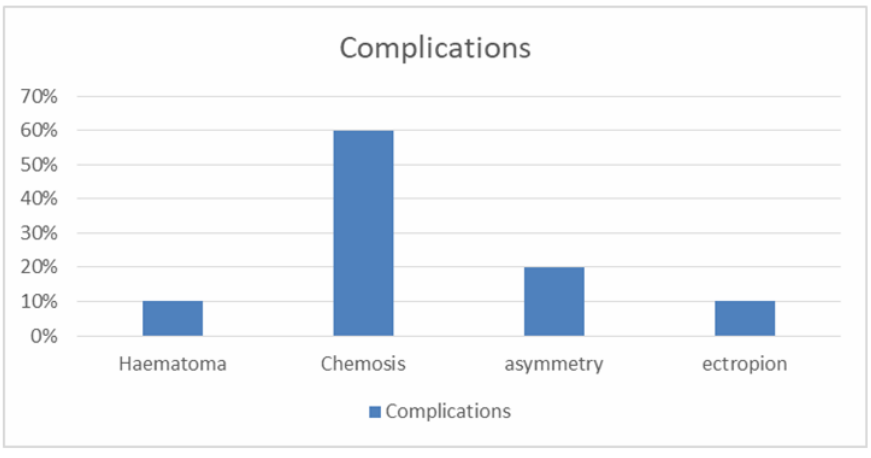

Fig. 4. Percentage complications in patients with blepharoplasty

Discussion

Preoperative patient evaluation for blepharoplasty should document medical and ophthalmologic history. Ophthalmologic history should include vision, corrective lenses, trauma, glaucoma, allergic reactions, excess tearing, and dry eyes. Cahill KV *et al.* [8], in their study state that preoperative indications for upper blepharoplasty should include margin reflex distance 1 (MRD(1)) of 2 mm or less, superior visual field loss of at least 12 degrees or 24%, down-gaze ptosis impairing reading and other close-work activities, a chin-up backward head tilt due to visual axis obscuration, symptoms of discomfort or eye strain due to droopy lids, central visual interference due to upper eyelid position, and patient self-reported functional impairment. Upper eyelid dermatochalasis is the loss of elasticity and support in the skin. It can create a fold of excess upper eyelid skin, which can impair the function of the eye, including supero-lateral visual field obstruction. Evaluation of pre-septal and eyebrow fat pads is important in redefining the superior sulcus. Assessment of patient's old photographs aids the surgeon in restoring the youthful look. Upper eyelid ptosis should also be noted, since it can be corrected simultaneously. As Naik MN *et al.* [9] state that lower eyelids should be assessed for skin excess and fat herniation, which typically presents as medial, central, and lateral fat pads. Downward displacement of the lateral canthus can result from disinsertion, laxity, or the presence of a prominent eye. Lower lid distraction test can determine the degree of laxity and guide lower eyelid canthal repositioning. The posterior displacement of the orbital rim in relation to the anterior cornea and lower lid margin, a negative vector, should be noted preoperatively. Brow ptosis is assessed by evaluating the position of the eyebrow in relation to the superior orbital rim. Asymmetry in the upper and lower eyelids and brow position is common and should be recognized and addressed individually.

Lyon DB *et al.* [10], in their paper state that upper lid blepharoplasty is a procedure associated with a high level of patient and surgeon satisfaction. New insights into the anatomic underpinnings of the periorbital aging process have enabled more successful and reproducible surgical results. In this paper, we have given a detailed description of the relevant anatomy and integrated it into the surgical philosophy for upper lid blepharoplasty. Preoperative markings should be made with the patient sitting upright in neutral position with the brow properly positioned. The eyelid crease is situated above the ciliary margin approximately 8 to 9 mm in women and 7 to 8 mm in men. The lower limit of excision should be along the eyelid crease, and the lateral extent of the marking should be limited by an imaginary line joining the lateral end of the brow to the lateral canthus. Possible complications include upper eyelid retraction with scleral show from anterior lamellar inadequacy, lagophthalmos, acquired diplopia and corneal exposure. The most common complication of cosmetic surgery is failure to meet patient's expectations. This can be avoided by preoperative counseling and identifying reasonable expectations. There have been no long-term outcomes studies for upper eyelid blepharoplasty. However, because of natural aging, the benefits of an upper blepharoplasty should endure at least a decade. If fat is removed properly, very likely pseudoherniation of orbital fat will not occur in a long period. As previously said, the opposite consequence, hollowing, will pose the greatest problem in the coming years. The descent of the brows is a significant aspect in determining the lifespan of an upper lid blepharoplasty. This condition will increase hooding by creating a pseudoredundancy of upper eyelid skin. Aging can lead to a number of aesthetic changes in the lower eyelid. Common complaints include eyelid bags, circles under the eye, wrinkles around the eye, or a tired look. Anatomically, relaxation of the orbital septum, orbicularis muscle, and skin can cause protrusion of intraorbital fat leading to eyelid bags. The traditional procedure in lower eyelid blepharoplasty was to remove the pseudoherniated fat via skin incision. A recent, more conservative approach has included repositioning of the herniated fat in cases of tear through deformity into the subperiosteal space. Both these approaches may be accompanied by strengthening procedures for the atenuated septum or septorrhaphy [11]. Severe complications, such as visual loss from orbital hemorrhage, orbital injection, or posterior optic nerve infarction are extremely rare, but have been described. Other possible complications are lower eyelid retraction with scleral show, lagophthalmos, corneal exposure and acquired strabismus [12].

Mack WP *et al.* [13] in their paper *Blepharoplasty complications* clearly state that when we do upper and lower lid blepharoplasty, it is recommended to be done with preservation of orbicularis muscle and its innervations. Regarding cosmetic eyelid surgery, the surgeon should strive to avoid skeletonization and hollowing deflation by repositioning and reinforcing tissue with an emphasis to restore fullness to achieve facial aesthetic balance between the forehead, eyelids, and mid-face.

A combination therapy is also possible when applying hyaluronic acid fillers, botulinum toxin or autologous fat transfer in this area.

Conclusion

We have briefly described the techniques of a standard upper and lower eyelid blepharoplasty. Practically, the rejuvenation of this complex anatomical area requires a combination of therapies including fat excision, repositioning or transfer, simultaneous brow or midface lift, and adjunctive treatment for skin resurfacing

and periorbital hollows. Patients with pseudoherniated fat pads in the upper and lower eyelids, as well as excess skin and hooding in the upper eyelids, might consider blepharoplasty. While the results of the device used - cold steel *vs.* laser - are not conclusive, it appears that the transconjunctival technique is favored in the case of lower lid. Patients should anticipate to look less tired after these treatments, and should be informed about the risks and recovery time associated with blepharoplasty.

This type of surgery is a common and very successful procedure to erase the aging changes of the upper periorbital region. A precise preoperative evaluation, thorough understanding of the patient's goals and careful planning and execution of a procedure that involves an appropriate lifting and resection of the soft tissue from the eyelid and eyebrow are used to restore a more youthful upper facial appearance. Serious complications of upper blepharoplasty are rare and are minimized by proper patient preparation and education.

Conflict of interest statement. None declared.

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ММР

Original article

HETEROGENEITY OF ENDOMETRIUM – AN INCREASED RISK FACTOR FOR ENDOMETRIAL MALIGNANCY

ЕНДОМЕТРИЈАЛНАТА ХЕТЕРОГЕНОСТ - ЗГОЛЕМЕН РИЗИК ФАКТОР ЗА ЕНДОМЕТРИЈАЛЕН МАЛИГНИТЕТ

Valentina Tofiloska[1], Goran Dimitrov[1], Sasha Jovcevski[1], Drage Dabeski[1], Jadranka Georgievska[1], Elena Dzikova[1], Megi Micevska[1], Katerina Nikoloska[1] and Maja Bojadzioska[2]

[1]University Clinic for Gynecology and Obstetrics, [2]University Clinic of Rheumatology, Faculty of Medicine, Ss. Cyril and Methodius University, Skopje, Republic of North Macedonia

Abstract

Introduction. The normal postmenopausal endometrium should appear thin, homogeneous and echogenic. Endometrial cancer causes the endometrium to thicken, appear heterogeneous, have irregular or poorly defined margins, and show increased color Doppler signals.
Aim. To examine the correlation between endometrial echogenicity and the risk of endometrial malignancy in postmenopausal women.
Methods. This was a prospective clinical study involving 120 postmenopausal patients treated at the University Clinic for Gynecology and Obstetrics-Skopje, divided into two groups: control and examined. The control group included 40 postmenopausal patients, hospitalized and operated on due to urogenital pathology. The examined group consisted of 80 patients divided into two subgroups – a group with uterine bleeding and the other one without uterine bleeding. According to the ultrasound verified thickness of the endometrium, the two subgroups were divided according to endometrial thickness into: the first group with endometrial thickness from 5-8 mm; the second from > 8-11mm and the third group above 11 mm. We made ultrasound examination by measuring the echogenicity of the endometrium in both groups of patients as well as in subgroups, which were also divided into other subgroups according to endometrial thickness.
Results. The probability of endometrial malignancy was significantly increased by 4,938 in heterogenous endometrium.
Conclusion. There are many examples of intratumor heterogeneity in endometrial malignancy, either at the morphologic or the molecular level. Attention should be paid so as not to miss minor subpopulations of tumor cells with diagnostic and prognostic relevance

Keywords: postmenopause, echogenicity, endometrial malignancy

Correspondence to: Valentina Tofiloska, University Clinic for Gynecology and Obstetrics, 1000 Skopje, R. N. Macedonia; E-mail: valentinatofiloska@yahoo.com

Апстракт

Вовед. Нормалниот постменопаузален ендометриум ултрасонографски изгледа тенок, хомоген и ехоген. Абнормалниот ендометриумот предизвикува згуснување на ендометриумот, изгледа хетерогено, има неправилни или слабо дефинирани маргини и покажува зголемени колор доплер сигнали.
Цел. да се испита корелацијата помеѓу ендометријална ехогеност и ризикот од ендометријален малигнитет кај жени во постменопауза.
Методи. Ова е проспективна клиничка студија која вклучува 120 постменопаузални пациентки третирани на Универзитетската клиника за гинекологија и акушерство-Скопје, поделена во две групи: контролна и испитувана група. Контролната група вклучува 40 постменопаузални пациенти, хоспитализирани и оперирани поради урогенитална патологија. Испитуваната група се состоеше од 80 пациенти поделени во две подгрупи-групата со крварење на матката и другата без крварење на матката. Според ултразвук проверена дебелина на ендометриумот, двете подгрупи со и без крварење ги поделивме според дебелината на ендометриумот во: првата со дебелина на ендометриум од 5-8 мм; втората од> 8-11мм и третата над 11мм. Направивме ултразвучен преглед со следење на ехогеноста на ендометриумот на обете групи на пациенти и нивните подгрупи.
Резултати. веројатноста за ендометријална малигност е значително зголемена за 4,938 пати кај пациентки со зголемена ехогеност на ендометриумот.
Заклучок. Постојат многу примери на интратуморна хетерогеност кај ендометријален малигнитет, било на морфолошки или на молекуларно ниво. Треба да се посвети внимание за да не се пропуштат малите субпопулации на клетките на туморот со дијагностичка и прогностичка важност.

Клучни зборови: постменопауза, ехогеност,

ендометријална малигност

Introduction

Endometrial carcinoma (EC) shows intertumoral heterogeneity at the morphologic level, with different histologic types that have different morphologic features. The two most frequent types are endometrioid EC, and serous carcinoma, which have different risk factors, prognosis, patterns of metastasis, and microscopic appearance [1,2]. There is also intratumoral morphologic heterogeneity. There is evidence that morphologic and molecular features are heterogeneously present in different tumor cell clones in a high proportion of ECs. There is a wide spectrum of heterogeneous tumors, ranging from EEC, with subtle variations in cytologic patterns, to mixed tumors, composed of 2 different histologic types (i.e., endometrioid EC and serous carcinoma) in the same tumor. Tumor heterogeneity may have an important clinical impact, since it can be a challenge to identify minor tumor cell populations that may have an impact on diagnosis, prognosis, and therapeutic decisions. Endometrial cancer is the most common gynecologic cancer and 90% of cases occur in women over 50 years of age [3]. Ultrasound signs that are more specific for endometrial cancer are a heterogeneous endometrium with color flow or an indistinct endometrial-myometrial interface [4]. Cystic areas may be observed in cases of atrophic endometrium, hyperplasia, polyps, or cancer and the presence of cysts in the endometrium is nonspecific [5].

Aim

The purpose of the study was to investigate the predictive role of heterogeneous endometrium in the early onset of endometrial malignancy in postmenopausal patients.

Material and methods

This was a prospective clinical study involving 120 postmenopausal patients treated at the University Clinic for Gynecology and Obstetrics-Skopje, divided into two groups: control and examined. The control group included 40 postmenopausal patients, hospitalized and operated on due to urogenital pathology. The examined group consisted of 80 patients divided into two subgroups-a group with uterine bleeding and the other one without uterine bleeding. According to the ultrasound verified thickness of the endometrium, the two subgroups were divided according to endometrial thickness into: the first group with endometrial thickness from 5-8 mm; the second from >8-11 mm and the third above 11 mm. We made ultrasound examination by measuring the echogenicity of the endometrium in both groups of patients as well as in subgroups, which were also divided into other subgroups according to endometrial thickness.

The study excluded patients in generative reproductive age, patients who were not able to do fractional exploratory curettage, patients with a personal history of malignant disease (past or current), patients with a personal anemia for benign or malignant tumors of the ovary, breast cancer patients treated with tamoxifen, patients with any pelvic surgery due to other gynecological pathology.

Statistical analysis

The data during the survey were analyzed with the statistical package SPSS 20.0. The Pearson Chi square homogeneity test was used to establish an association between certain attributive dichotomies of the two groups of patients. The Shapiro-Wilk W test was used to determine the frequency distribution of certain variables. To test the significance of difference between two and more numerical variables with regular or irregular distribution of frequencies the Student's T-test for independent samples, the Mann Whitney U test and the Kruskal-Wallis ANOVA test were used. A significance level of $p < 0.05$ was used to determine the statistical significance.

Results

According to the echogenicity of the endometrium, patients in the study were divided into two groups: a) homogeneous and b) heterogeneous (Table 1). There were 43(53.7%) patients in the study group with homogeneous endometrial echogenicity while heterogeneous were 37 (46.3%). In the control group, all 40 (100%) patients had homogeneous endometrial echogenicity. For $p<0.05$, a statistically significant difference was observed between the two groups in terms of endometrial echogenicity (Fisher exact two tailed test: p=0.00001).

Table 1. Descriptive analysis of the sample by groups and endometrial echogenicity

Endometrial echogenicity		Group		Total
		Examined	Control	
Homogeneous	**N**	43	40	83
	%	53.75%	100%	
Heterogeneous	**N**	37	0	37
	%	46.25%	0%	
Total value	**N**	80	40	120
	%	66.67%	33.33%	100%

Fisher exact two tailed test: p=0.00001*, * significant for p<0.05

Analysis of the examined group according to the thickness of endometrium and endometrial echogenicity

In the first group with endometrial thickness of 5 mm-8 mm, 21(58.3%) patients were with homogeneous en-

dometrial echogenicity and 15(41.7%) with heterogeneous echogenicity.

In the second group with endometrial thickness <8 mm -11 mm, 10(58.8%) patients were with homogeneous

Table 2. Analysis of the examined group according to endometrial thickness and endometrial echogenicity

Echogenicity		Subgroups (endometrial thickness)			Total
		5 mm-8 mm	<8 mm-11 mm	<11 mm	
Homogeneous	**N**	21	10	12	43
	%	58.33%	58.82%	44.44%	
Heterogeneous	**N**	15	7	15	37
	%	41.67%	41.18%	55.56%	
Total value	**N**	36	17	27	80
	%	45.00%	21.25%	33.75%	100.00%

Pearson Chi-square: 1.4207. df=2. p=0.49147, *significant for p<0.05

endometrial echogenicity while the remaining 7 (41.2%) had heterogeneous echogenicity (Table 2).

In the third group with endometrial thickness <11 mm, 12(14.4%) patients were with homogeneous endometrial echogenicity, while in the remaining 15 (55.6%) the endometrial echogenicity was heterogeneous (Table 2).

For p>0.05, there was no statistically significant difference between the groups in terms of endometrial echogenicity (Pearson Chi-square: 1.4207, df=2, p=0.4915) (Table 2).

Analysis of the examined group according to uterine bleeding and endometrial echogenicity

In the group without uterine bleeding, 21(52.5%) patients were with homogeneous endometrial echogenicity while in the group with uterine bleeding 22(55%)

Table 3. Analysis of the examined group according to uterine bleeding and endometrial echogenicity

Endometrial echogenicity		Uterine bleeding		Total value
		No	Yes	
Homogeneous	**N**	21	22	43
	%	52.50%	55%	
Heterogeneous	**N**	19	18	37
	%	47.50%	45%	
Total value	**N**	40	40	80
	%	50%	50%	100%

Pearson Chi-square=0.0503. df=1. p=0.82257, *significant for p<0.05

patients had homogeneous echogenicity of the endometrium. For p>0.05, there was no statistically signifycant difference between the two groups in terms of echogenicity of endometrium (Pearson Chi-square= 0.0503, df =1, p=0.8226) (Table 3).

Analysis of groups without/with uterine bleeding according to the thickness of endometrium and endometrial echogenicity

The analysis of the group without bleeding according to the thickness of endometrium and endometrial echogenicity (Table 4). The analysis indicated that the homogeneous echogenicity of the endometrium was most frequently present-in 11 (53.4%) patients with an endometrial thickness of 5 mm to 8 mm, followed by 6 (28.6%) patients with endometrial thickness < 8 mm - 11 mm. In relation to heterogeneous echogenicity of the endometrium, the majority of patients, 8(42.1%), had an endometrial thickness of 5 mm-8 mm, followed by 6(31.6%) patients with endometrial thickness of < 11mm. In patients without uterine bleeding, for p> 0.05, there was no statistically significant difference between the groups with homogeneous/heterogeneous echogenicity of the endometrium relative to the thickness of endometrium (Pearson Chi-square=0.867, df=2, p=0.6483).

Table 4. Analysis of the group without bleeding according to the thickness of endometrium and endometrial echogenicity

Endometrial thickness		Echogenicity		Total value
		Homogeneous	Heterogeneous	
5 mm-8 mm	**N**	11	8	19
	%	52.38%	42.11%	
< 8 mm-11 mm	**N**	6	5	11
	%	28.57%	26.32%	
< 11 mm	**N**	4	6	10
	%	19.05%	31.58%	
Total value	**N**	21	19	40
	%	52.50%	47.50%	100%

Pearson Chi-square=0.8667. df=2. p=0.64832, *significant for p<0.05

In patients with uterine bleeding, an analysis was performed according to endometrial thickness and endometrial echogenicity (Table 4). Homogeneous echogenicity of the endometrium was most common, found in 10 (45.5%) patients with an endometrial thickness of 5 mm to 8 mm, followed by 8(36.4%) patients with an

endometrial thickness of <11 mm. Heterogeneous echogenicity of endometrium was encountered in 9(50%) patients with an endometrial thickness of <11 mm, followed by 7(38.9%) patients with an endometrial thickness of 5 mm-8 mm.

In the group of uterine bleeding, for p>0.05, there was no statistically significant difference between subgroups with homogeneous/heterogeneous echogenicity of endometrium relative to endometrial thickness (Pearson Chi-square =0.863; df=2, p=0.6493 (Table 5).

Table 5. Analysis of a group with uterine bleeding according to the thickness of endometrium and endometrial echogenicity

Endometrial thickness		Homogeneous	Heterogeneous	Total value
5 mm-8 mm	**N**	10	7	17
	%	45.45%	38.89%	
<8 mm -11 mm	**N**	4	2	6
	%	18.18%	11.11%	
<11 mm	**N**	8	9	17
	%	36.37%	50%	
Total value	**N**	22	18	40
	%	55%	45%	100%

Pearson Chi-square=0.8635. df=2. p=0.64936, *significant for p<0.05

Echogenicity of the endometrium was a significant predictor of endometrial malignancy (p<0.05). Women with heterogeneous endometrial echogenicity had 4.938 times [p=0.023, 95% CI=1,243-19,619] likelihood of endometrial cancer compared to women with homogeneous endometrial echogenicity (Table 6).

Table 6. Binary logistic regression analysis of the predictive role of certain parameters in relation to endometrial malignancy - examined group

Variable	B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B) Lower	Upper
Echogenicity - homogeneous								
Heterogeneous	1.597	.704	5.149	1	.023*	4.938	1.243	19.619

*significant for p<0.05

Discussion

According to Bourne, endometrial cancer is always accompanied by pronounced ultrasound hyperechoic transonicity [6].

Opolskiene *et al*. concluded that heterogeneous endometrium was superior in prediction of endometrial cancer [7]. In two studies, Opolskiene and Epstain [7,8] confirmed that endometrial thickness and heterogeneous endometrial echogenicity increased the risk of malignancy. However, some published models require highly experienced ultrasound examiners to evaluate the echogenicity of the endometrium [7,8] and the morphology of endometrial vessels [8].

Conclusion

There are many examples of intratumoral heterogeneity in endometrial malignancy, either at the morphologic or the molecular level. Attention should be paid so as not to miss minor subpopulations of tumor cells with diagnostic and prognostic relevance. Interestingly, endometrial aspirates are able to capture the molecular heterogeneity of EC, probably because they contain cells from different areas of the tumor. Finally, a conventional sampling approach, with 1 tissue seems to be enough for the detection of pathologic parameters (levels of myometrial invasion and lymphovascular space invasion) in a hysterectomy specimen of endometrial carcinoma.

Conflict of interest statement. None declared.

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Original article

FIRST AND SECOND TRIMESTER MEDICAL ABORTION - PILOT STUDY IN REPUBLIC OF NORTH MACEDONIA

МЕДИКАМЕНТОЗЕН АБОРТУС ВО ПРВ И ВТОР ТРИМЕСТЕР - ПИЛОТ СТУДИЈА ВО РЕПУБЛИКА СЕВЕРНА МАКЕДОНИЈА

Jadranka Georgievska[1], Gligor Tofoski[1], Ana Daneva[1], Beti Zafirova[2], Viktorija Jovanovska[1], Ljupcho Petrovski[1], Adela Stefanija[1], Ivo Kjaev[1], Arta Bina[1], Sasho Dimitrovski[1], Adriana Buklioska[1], Eva Sozovska[1], Katerina Dudeska[1] and Adrijana Shterjovska[1]

[1]University Clinic for Gynecology and Obstetrics, [2]Institute of Epidemiology and Biostatistics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Introduction. Medical abortion is a procedure in which medication is used to end a pregnancy up to 22 weeks of gestation. The aim of this study was to assess the efficiency, safety and acceptance of medical abortion in the first and second trimester using a regimen of medications, mifepristone followed by misoprostol.
Methods. In a prospective study, conducted at the University Clinic for Gynecology and Obstetrics in Skopje in the period from March to November 2021, women that came for medical abortion in the first and second trimester were enrolled. They were divided into two groups: Group I (5 to 12 weeks of gestation) and Group II (12 to 22 weeks of gestation). Participants in Group I were given 200 mg mifepristone for peroral application and after 24 hours instructed for sublingual application of 800 μg misoprostol at home. Participants in Group II were hospitalized and treated with the same regimen. Additional dosage of misoprostol was given to complete abortion.
Results. A total of 208 women with medical abortion up to 22 weeks of gestation (173 in the first group and 35 in the second group) were enrolled in the study. In the first group a complete uterine evacuation was achieved in 168 women (97.1%) and in 25 women (71.4%) in the second group. Acceptability of the method was high among both groups (95.14 % in Group I and 82.14 % in Group II). All doctors/clinicians who participated in this pilot study were satisfied with the method.
Conclusions. Medical abortion is effective, safe and acceptable option for women in the first and second trimester. Acceptability of the method was high among both groups. All doctors/clinicians who participated in this pilot study were satisfied with the method and would offer it as an option to their patients in the future.

Correspondence to: Jadranka Georgievska, University Clinic for Gynecology and Obstetrics, 1000 Skopje, R.N. Macedonia; Phone: +389 70305256; Email: jadrankageo@yahoo.com

Keywords: medical abortion, efficacy, acceptance, first and second trimester

Абстракт

Вовед. Медикаментозен абортус е терминација на бременост до 22-та гестациска недела со користење на медикаменти. Целта на студијата беше да се процени ефикасноста, безбедноста и прифатливоста на медикаментозниот абортус во прв и втор триместар со кристење на режим со мифепристон следено со мизопростол.
Методи. Во проспективна студија изведена на Универзитетската клиника за гинекологија и акушерство во Скопје во период од Март до Ноември 2021 беа вклучени жените кои дошле за медикаментозен абортус во прв и втор триместар. Тие беа поделени во две групи: Група 1 (од 5. до 12. гестациска недела) и Група 2 (од 12. до 22. гестациска недела). Партиципантките од првата група добија 200 мг мифепристон за перорална апликација и по 24 часа беа инструирани за сублингвална апликација на 800 мгр мизопростол дома. Партиципантките од Група 2 беа хоспитализирани и третирани со истиот режим. Дополнително дозирање на мизопростол беше дадено за комплетирање на абортусот.
Резултати. Во студијата беа вклучени 208 жени со медикаментозен абортус до 22. гестациска недела (173 во првата група и 35 во втората група). Во првата група комплетна утерина евакуација беше постигната кај 168 жени (97.1%) и кај 25 жени (71.4%) во втората група. Прифатливоста на методата беше голема во двете групи (95.14 % во Група 1 и 82.14 % во Група 2). Сите клиничари кои партиципираа во оваа пилот студија беа задоволни со методата.
Заклучоци. Медикаментозниот абортус во првиот и вториот триместар е ефективна, безбедна и прифатлива опција за жените. Прифатливоста на методата беше голема во двете групи. Сите клиничари вклучени во оваа пилот студија беа задоволни од

методата и ќе ја понудат на нивните пациентки во иднина.

Клучни зборови: медикаментозен абортус, ефикасност, прифатливост, прв и втор триместар

Introduction

Medical abortion is a procedure for termination of pregnancy by using medications to induce a process similar to a miscarriage. Even though overall rates of abortion decline, the use of medical abortion has grown significantly since its approval by the FDA in 2000. While some countries continue to impose regulations that restrict access, others are exploring ways of increasing access by allowing non-physician clinical providers to prescribe mifepristone and testing the effectiveness of telemedicine to expand access to abortion services for those who seek them.

Medical abortion is an alternative to surgical abortion [1]. It is an effective and acceptable option for abortion care [2-3]. Given the few medical requirements for safe provision of medical abortion drugs, and that the abortion process may generally be managed by the woman, a growing proportion of induced abortions in the United States (US) and internationally are medical abortions [4-5]. Improved access to medical abortion that includes expanding the gestational ages at which it can safely be used is one strategy to reduce unsafe abortion, particularly where trained surgical providers are limited.

The most effective medical abortion regimen combines mifepristone with misoprostol; however, variation exists in dose, timing and route of administration of the two drugs. A large body of evidence, practice internationally, and recommendations by the World Health Organization (WHO) supports the efficacy of a 200 mg dose of mifepristone, followed by 800 μg of misoprostol in pregnancies up to 63 days gestational age [6-7] and recent data supports extending its use to 70 days gestation [8]. Misoprostol, taken 24-48 hours after mifepristone, works to empty the uterus by causing cramping and bleeding, similar to an early miscarriage. A follow-up visit is typically scheduled a week or two later, to confirm that the pregnancy was terminated via ultrasound or blood test. Gestational age is known to affect the efficacy of all regimens, with decreasing efficacy after nine weeks of gestation, which is why regimens recommend routinely repeating misoprostol doses starting in the late first trimester. Home administration of misoprostol has similar effectiveness as clinical administration up to 63 days of gestation and is endorsed as a safe and acceptable practice [9,10]. Studies of later gestational age ranges would need also to demonstrate similar efficacy, acceptability and rates of adverse events with home administration of medical abortion drugs [11].

As abortion provision has streamlined and improved, several resource-rich countries have experienced increased utilization of medication abortion as compared with aspiration abortion, improved access to medical abortion methods, and a decline in medical abortion complications [12]. In countries where abortion medications (mifepristone/misoprostol) are available directly from pharmacies rather than requiring dispensation by a physician in an office, clinic, or hospital, abortion provision happens at substantially earlier gestations [13]. In the United States, medical abortion accounted for approximately 40 percent of all abortions in 2018 and the majority of these abortions were performed at or before 9 weeks of gestation [14-16]. Medical abortion is a safe method of termination of pregnancy when performed as per guidelines. Whereas multiple studies investigate acceptability of medical abortion, much less has been published in the last 15 years about factors influencing women's satisfaction with surgical abortion. Medical abortion studies show very high acceptability; the data can be assessed in aggregate to simply mean that women who are able to access abortion services are generally happy because they were able to get an abortion [17].

Abortion in the Republic of North Macedonia has been legal since 1972 and medical abortion became legalized for use in May 2019 (Law on pregnancy termination R North Macedonia) [18]. Following the adoption of Clinical guidelines on safe abortion in December 2020, we conducted a pilot project on medical abortion in the first and second trimester of pregnancy [19]. Although medical abortion drugs are not registered in the country, due to favorable political climate for reproductive rights in the Republic of North Macedonia in the past several years, the budget for procurement of drugs to introduce medical abortion services at the University Clinic for Gynecology and Obstetrics has been allocated from the Government Preventive Program for mother and child health care.

Aim of the pilot study

We conducted a clinical study to examine the effecttiveness, safety and acceptance of introducing medical abortion in the first and second trimester of pregnancy applying a combination of 200 mg mifepristone followed by 800 μg sublingual dosing of misoprostol and repeated doses of misoprostol.

Materials and methods

This pilot study was a prospective study conducted at the University Clinic for Gynecology and Obstetrics, Faculty of Medicine in Skopje, Republic of North Macedonia between March and November 2021. Women that came for first and second trimester termination of pregnancy (5 to 22 gestational weeks of pregnancy) due to personal or socio-economic reasons and desired

medical abortion (MA) were enrolled in the study. Women were divided into two groups: Group I (5 to 12 weeks of gestation) and Group II (12 to 22 weeks of gestation). Clinicians with experience in providing surgical abortion in the Clinic were trained in medical abortion regimen and study implementation. Exclusion criteria were: hypersensitivity to prostaglandins, suspicion for ectopic pregnancy, coagulation disorders, cardiac disorders, current uncontrolled hypertension, porphyry and acute renal disease. Information about patients enrolled in the study were recorded in special questionnaires (information for socioeconomic status, reproductive history, hemoglobin levels before and after medical abortion, gestational age after ultrasonographic evaluation, doses of mifepristone and misoprostol that were given to patients, results of medical abortion on control examination, need for surgical evacuation of residual masses in uterus after medical abortion, side effects, etc.). Written informed consent was obtained from all patients included in pilot study prior to procedure.

All doctors/clinicians that participated in the study were interviewed for the study purpose. They were asked about satisfaction of the medical abortion training they received, previous experience in providing medical abortion, the change of attitude towards medical abortion after the study finished, the preference between medical and surgical abortion and whether they would offer medical abortion as a safe method to their patients in the future.

After gynecological and ultrasonographic evaluation for confirmation of gestational age, women seeking medical abortion were given 200 mg mifepristone for peroral application and after 24 hours 800 μgr misoprostol for sublingual application at home. Additional dosage of 400 to 800 μg misoprostol was given in women in the first trimester of pregnancy to complete abortion. Group II were patients for the second trimester termination of pregnancy because of socioeconomic reasons with gestational age between 12 and 22 weeks. They were hospitalized and treated at the Department for High-risk Pregnancy at the University Clinic for Gynecology and Obstetrics in Skopje. Women were given 200 mg mifepristone for peroral application and after 24 hours 800 μgr misoprostol for sublingual application. Often, after expulsion of fetus, additional doses of 400 μg misoprostol were given sublingually.

Women in both groups were given 200 mg ibuprofen for pain relief. In case of increased pain in women who were hospitalized (Group II), 50 mg tramadol was given. All patients enrolled in the pilot study were interviewed by a social worker in the Clinic regarding their experience with medical abortion and satisfaction of the procedure.

Statistical analysis

Statistical analysis of data was made by using the statistical program SPSS 23,0. Kolmogorov-Smirnov and Shapiro Wilks tests were used for testing of normal distribution of data. Results of statistical analysis are presented in tables and figures. Attributive variables are shown with absolute and relative numbers. Quantitative variables are shown with average, standard deviation, minimal-maximal, median, interquartile rank. For comparison of the results between both groups of patients, parametric and nonparametric tests for independence (Chi-square test, Student t-test for independent samples, Mann-Whitney test) were used.

Statistical significance was defined as $p<0.05$.

Results

In this pilot study for the first and second trimester medical abortion, a total of 208 women with medical abortion up to 22 weeks of gestation (173 in the first group and 35 in the second group) were enrolled. In the first group, the median age was 31.4±7.0 (15-47 years), and in the second group, the median age was 27.7±7.1 (14-43). Women in the first group were significantly older than women in the second group ($p<0.0001$) (Table 1).

Table 1. Age distribution of patients with MA in both groups

Variable	Gestational week		p-level
	< 12	12 – 22	
Age			
mean ±SD	31.4±7.0	27.7±7.1	t=61.7
min – max	15-47	14-43	***p=0.00000
Age groups n (%)			
15 – 20	10(5.78)	5(14.29)	X^2=7.93
21 – 25	35(20.23)	9(25.71)	p=0.094
26 – 35	73(42.2)	17(48.57)	
36 – 40	36(20.81)	3(8.57)	
>40	19(10.98)	1(2.86)	

X^2(Chi-square test); t(Student t-test); ***p<0.0001

The majority of women were aged between 26-35 years-42.2% in the first group and 48.6% in the second group. There were 5.8% and 14.3% patients aged between 15-20 years in the first and second group, respectively, whereas there were 11% patients older than 40 years in the first group and one woman in the second group. Most of the patients had completed secondary school - 100 (48.1%) and were employed in a private sector – 83 (39.9%) or were unemployed - 64 (30.8%).

Table 2. Distribution of patients in both groups according to gestational age

Gestational age (G.W.)	n	G.W.	
		<12 n(%)	12-22 n(%)
до 9	162	162(93.64)	0
9-12	11	11(6.36)	0
12-18	27	0	27(77.14)
19-22	8	0	8(22.86)

According to nationality, most of the patients-141 (67.8%) were Macedonians, 33(15.9%) were Albanians and 34 (16.3%) were from other ethnicities.
The vast majority of women (88.9%) did not use any contraception method at the time of their visit to the Clinic.
The distribution of patients in both groups according to gestational age is given in Table 2.
In the first group, most of the women were pregnant up to 9 weeks of gestation-162(93.64%). In the second group, most of the women were pregnant between 12-18 weeks of gestation-27(77.14%).
In regards to reproductive health history of the participants, most of the patients had two previous life birth-66(31.7%), with two newborn children-68(32.7%), one caesarean delivery-17(8.2%). Twenty-five (12%) women had one artificial abortion and 23(11.1%) one spontaneous abortion.
At the follow-up visit, there was no significant decrease in hemoglobin levels in both groups. The average hemoglobin level before MA was 126.8±7.3 gr/l in the first group and 123.7±9.1 gr/l in the second group. The average hemoglobin level after MA was 121.9±8.6 in the first group and 119.6±10.4 gr/l in the second group. Before MA, all participants had hemoglobin levels higher than 100 gr/l, after MA 1.7% of women in the first group and 5.7% of women in the second group had hemoglobin levels lower than 100 gr/l, but without a statistical significance (p=0.16). These results are presented in Table 3.
Pain and need for analgesics were reported as side effects by 5.2% women in the first group and 28.6% in the second group. Statistical analysis showed that women in the second group had pain more often in comparison with women in the first group (p=0.00019). The remaining early side effects after MA were: bleeding, nausea, vomiting, fever, allergic reaction.
Additional doses of misoprostol were given in both groups of patients. In the first group, 57.8% of patients used an additional dose of 400 to 800 μg misoprostol. In the second group, 21 patients (60%) used an additional dose of 400 to 1200 μg misoprostol.
Success rate of medical abortion was defined as abortions completed without surgical intervention at any point during the pilot study. A success rate of 97.1% was achieved with mifepristone and misoprostol in the first trimester medical abortion patients (5 to 12 weeks of gestation). As length of pregnancy increased, the success rate decreased, with 71.4% of women with pregnancies of 12 to 22 weeks of gestation having successful abortions with mifepristone and misoprostol regimen. In the first group, 100 women (57.8%) took additional doses of 400 to 800 μg misoprostol because of incomplete abortion and only 5 women (2.89%) further required surgical intervention for complete uterus evacuation. On the other side, 21 women in the second trimester (60%) took additional doses of 400 to 1200 μg misoprostol because of incomplete abortion and 10 women (28.6%) further required surgical intervention for complete uterus evacuation. Additional surgical interventions were required in the second trimester medical abortion and the difference was statistically significant (p<0.00001). These results are presented in Table 4 and Figure 1.

Table 3. Comparison of Hg levels before and after MA in both groups

Hgb (gr/L)		G.W. < 12	G.W. 12 – 22
before MA	mean ±SD	126.8 ± 7.3	123.7 ± 9.1
	min – max	102 – 152	105 – 141
after MA	mean ±SD	121.9 ± 8.6	119.6 ± 10.4
	min – max	73 – 149	97 – 140
p-level		t=16.1 ***p=0.000	t=3.02 **p=0.0047
before MA	> 100 gr/l	173 (100)	35 (100)
after MA	до 100 gr/l	3 (1.73)	2 (5.71)
	> 100 gr/l	170 (98.27)	33 (94.29)
p-level		X^2=1.96 p=0.16 ns	

X^2(Chi-square test); t(Student t-test); ***p<0.0001

Table 4. Success rate of MA in both groups

Success rate of MA in both groups	n	Gestational week < 12 n (%)	Gestational week 12 – 22 n (%)	p-level
Success rate of MA in both groups				
abortion after tbl. Mifepristone	4	1(0.58)	3(8.57)	X^2=47.78
abortion after tbl. Misoprostol	66	65(37.57)	1(2.86)	p=0.0000 sig
abortion after additional Misoprostol	121	100(57.8)	21(60)	
unsuccessful MA completed with instrumental revision	15	5(2.89)	10(28.57)	
repeated MA	2	2(1.16)	0	
Surgical intervention				
No	193	168(97.11)	25(71.43)	X^2= 28.7
Yes	15	5(2.89)	10(28.57)	p<0.00001 sig

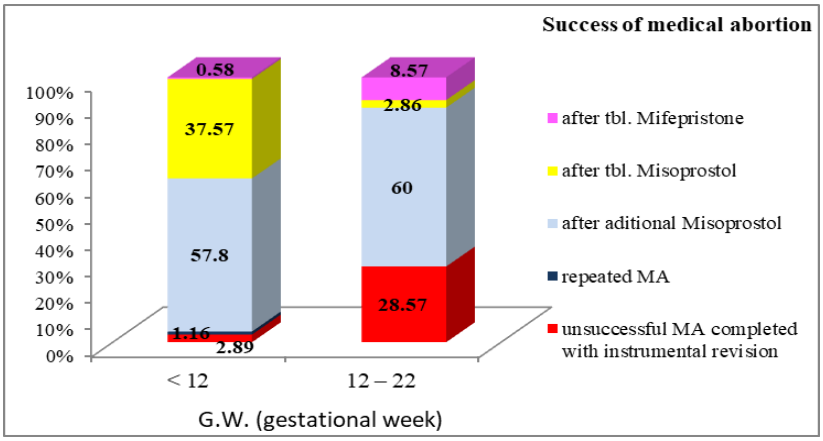

Fig. 1. Success of medical abortion in both groups

About 90% of women from the first group and 93.5% from the second group visited the clinic for the scheduled follow-up care and were given post-abortion contraceptive information. The remaining women were controlled by their gynecologists.

At the final interview, 173 participants were interviewed for satisfaction from medical abortion (144 in the first group and 28 in the second group). About 99.3% of patients in the first group were satisfied or very satisfied with the method and about 100% in the second group. These results are presented in Table 5.

Table 5. Satisfaction of patients from MA

Satisfaction of patients from MA	n	Gestational week (G.W.)		p-level
		< 12 n (%)	12 – 22 n (%)	
very satisfied	148	130(90.28%)	18(64.29%)	X^2=19.7 p=0.0006
satisfied	23	13(9.03%)	10(35.71%)	
not satisfied	1	1(0.69%)	0	

The majority of women preferred the medical abortion over surgical abortion if they needed another abortion. Medical abortion would be recommended to a friend by about 95.14% of participants in the first group and about 82.14% of participants in the second group. Women were asked to say something about the best and worst features of the medical abortion. The majority of participants answered that the best feature was to avoid surgery and anesthesia, followed by having a successful abortion without complications. More than half (65%) felt that the pain and cramps was the worst feature of medical abortion. The majority of participants (98.6 %) answered that they had got detailed and accurate information from the medical team for medical abortion, complications, side effects etc. in the first group and about 92.86 % in the second group. Because of the coronavirus pandemic, all women in the first group took pills for home use, which was more comfortable, more intimate for them. The majority of women (96%) reported they would prefer medical abortion over surgical abortion if they had to terminate another pregnancy.

All doctors/clinicians that participated in this pilot study were interviewed about their satisfaction with the medical abortion method. Most of them had experience and were familiar with medical abortion before entering the study. Clinicians believed that medical abortion had less percentage of complications than surgical abortion and is a safer method of pregnancy termination. They all reported that would offer medical abortion to their patients in the future as an option for women to end their pregnancy.

Discussion

In the Republic of North Macedonia medical abortion became legal for use in May 2019 (Law on pregnancy termination R. North Macedonia). Medications for this kind of abortion were not available until 2020 when the budget for procurement of drugs to introduce medical abortion services at the University Clinic of Gynaecology and Obstetrics was allocated from the Government Preventive Program for mother and child health care. We decided to start a pilot study for medical abortion in the first and second trimester of pregnancy to evaluate effectiveness, success rate and safety of medical abortion. Most of the doctors/clinicians that participated in this pilot study had previous experience with some medical abortion regimens, but before starting the project, they received additional education about the procedure.

A success rate of 97.1% was achieved with mifepristone and misoprostol regimen in the first trimester medical abortion patients (5 to 12 weeks of gestation). The study of Ashok PW *et al*. from 1998 found 96.7% of complete abortion rate among women in the 7-9 weeks of gestation [20]. As length of pregnancy increased, success

rate decreased, with 71.4% of women with pregnancies of 12 to 22 weeks of gestation having successful abortions with mifepristone and misoprostol regimen. A systematic review reported average success rates of 96.7% in the 8th week, 95.2% in the 9th week, and 93.1% in the 10th week of gestation [21].

Medical abortion in our study was less effective in the group with higher gestational age (second group), requiring surgical uterine evacuation in a higher percenttage than in the first group with early gestation (28.6% *vs.* 2.89%) respectively. In the first group, two of the patients had ongoing pregnancy (1.16%) and after repeated medical abortion complete uterine evacuation was achieved. In one WHO multicentre study from 1993, the continuing pregnancy rate was 0.4% [22]. In the first group, 100 women (57.8%) took additional doses of 400 to 800 μg misoprostol in order to complete the abortion procedure. In the other group, 21 women in the second trimester (60%) took additional doses of 400 to 1200 μg misoprostol because of incomplete abortion. Because of the increasing size and volume of the uterus and larger fetus, the amount of progesterone to be blocked by the same dose of mifepristone is increased and a larger fetus requires the uterus to generate more force and the cervix to be softer and more dilatated to allow expulsion. Therefore, additional doses of misoprostol to the regimen will improve success rates.

Most common side effects reported by participants in this pilot study were pain and need for analgesic, reported by 5.2% in the first group and 28.6% in the second group. Statistical analysis showed that women in the second group had pain more often in comparison with the women in the first group. At the follow-up visit, in both groups there was no significant decrease in hemoglobin levels. According to the study of Grossman D *et al.*, the second trimester medical abortion was associated with higher rates of complications compared to the first trimester medical abortion, but absolute risk was low. They reported excessive bleeding in 0.9% of patients, and 0.2% required blood transfusion [23].

The results obtained in our pilot study showed that medical abortion is a safe method for pregnancy termination in the first and second trimester of pregnancy. These results are similar with the results presented by Schummers L *et al.* They reported an incidence of abortion complications of 0.76% and incidence of uterine evacuation of 4.5% in patients with the first trimester medical abortion [24].

Most of the participants (98.6% in the first group and 92.86% in the second group) answered that they had got detailed and accurate information by the clinicians and considered that very important information was given regarding the course of the medical abortion (timing of pills, type of bleeding, type of pain, complications, side effects etc). Because of the coronavirus pandemic, all women in the first group received and took misoprostol at home, which was more comfortable and intimate for them. All women in the second group were hospitalized at the Clinic because of risks of prolonged bleeding and abdominal pain, which sometimes required more analgesics compared to the first group. Similar results were obtained in the study of Louie KS *et al.* from 2015. They reported that 94.8% of participants in their study thought that abortion counseling they received from their providers was sufficient regarding the amount of pain, bleeding and time to complete abortion [25]. In our study, the majority of women (96%) reported they would prefer medical abortion over surgical one if they had to terminate another pregnancy.

Clinicians who participated in this pilot study were satisfied with the medical abortion and believed it is a safer method of pregnancy termination. They would continue offering medical abortion to their patients in future as an option for termination of pregnancy up to 22 weeks of gestation.

Conclusions

The first and second trimester medical abortion with 200 mg mifepristone followed by 800 μg misoprostol for sublingual administration 24 hours later and repeated doses of misoprostol is effective, safe and acceptable option for women. Evidence suggested that the first trimester medical abortion is more effective than the second trimester medical abortion applying the same regimen. Adjusting regimen of medical abortion in both first and second trimesters should be considered in the future in order to decrease the repeated dosage of misoprostol and to increase efficiency. Medical abortion has been proven as a feasible option for women and staff in the Clinic during Covid-19 pandemics and in the future medical practice.

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Conflict of interest statement. None declared.

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Original article

CLINICAL PATHOLOGICAL CHARACTERISTICS AND FREQUENCY OF ALK MUTATIONS IN NON-SMALL CELL LUNG CANCER (NSCLC)

КЛИНИЧКО ПАТОЛОШКИ КАРАКТЕРИСТИКИ И ФРЕКВЕНЦИЈА НА ALK МУТАЦИИ КАЈ НЕСИТНОКЛЕТОЧЕН БЕЛОДРОБЕН КАРЦИНОМ (НСКБК)

Irfan Ismaili[1], Marija Zdraveska[1], Dejan Todevski[1], Aleksandra Tatabitovska[1], Sava Pejkovska[1], Dimitar Karkinski[1], Irina Angelovska[1], Bojan Stoshevski[1], Zekirja Shaini[1], Rabije Mustafi[1], Simonida Crvenkova[2], Magdalena Bogdanovska-Todorovska[3] and Gordana Petrusevska[3]

[1]University Clinic for Pulmonology and Allergology, [2]University Clinic for Radiotherapy and Oncology, [3]Institute of Pathology, Skopje, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Introduction. Lung cancer is a malignant tumor originating from the respiratory epithelium of the lungs: the alveolar epithelium and the epithelium of the tracheobronchial stem. It is the most common cause of death associated with malignant diseases in the world. The incidence of lung cancer varies depending on gender and race. It is twice as high in men, although in recent years there has been a significant decline in the incidence in men, while the rate in women has risen in the last decade. About 80-85% of all lung cancers are of the non-small cell lung cancer (NSCLC) type, while about 15% are small cell lung cancers. Smoking is the most common risk factor. In 5% of patients with NSCLC, an inversion of chromosome 2 consisting of a juxtaposition of the 5' end echinoderm microtubule associated with protein 4 (EML4) gene with a 3' end of anaplastic lymph node with oncogene EML4-ALK is detected. Screening for this fusion gene in NSCLC is particularly important because "ALK-positive" tumors are highly sensitive to targeted therapy with ALK inhibitors.
Objectives. 1. To determine the distribution of non-small cell lung cancer (NSCLC) by gender and age, smoking status, histological subtype; 2. To determine the stage of the disease determined by radiological TNM classification; 3. To determine the frequency of ALK mutations in patients with NSCLC.
Methods. This is a prospective study conducted at the PHI University Clinic for Pulmonology and Allergology and the Institute of Pathology, Faculty of Medicine - Skopje over a period of 1 year. In all patients included in the study, tissue samples obtained from lung biopsy (bronchobiopsy or transthoracic biopsy) were analyzed. The microscopic description included histological type definition of the tumor and the degree of histological differentiation. The study also processed clinical patient data and disease stage with radiological TNM classification (determined by contrast CT of the chest and mediastinum). To determine the mutation of the ALK gene, tissue samples were processed with FISH using an identical DNA probe.
Conclusion. ALK mutations were noted in 3 of a total of 52 patients, or 5.77%, which was very close to the global trend of mutations of about 5%. Given the small sample of participants, it is possible that the general population of patients with NSCLC follows exactly the world trend. According to the available studies, screening with low-dose computed tomography of the lungs is recommended for all current heavy smokers or individuals who have smoked 15 or more years, aged 55 to 80 years. All this coincides with our research, in which most of the patients diagnosed with NSCLC were smokers and over 50 years of age.

Keywords: NSCLC, ALK, TNM, EML4, histopathology

Correspondence to: Irfan Ismaili, University Clinic for Pulmonology and Allergology, 1000 Skopje, R. N. Macedonia; E-mail: irfan.ismaili2@gmail.com

Апстракт

Вовед. Карциномот на белите дробови е малиген тумор со потекло од респираторниот епител на белите дробови: алвеоларниот епител и епителот на трахеобронхалното стебло. Преставува најчестиот причинител за смрт поврзана со малигни болести во светот. Инциденцијата на белодробниот карцином варира зависно од полот и расата. Двојно е поголема кај мажи, иако во последните години се забележува значителен пад на инциденцијата кај мажите, додека стапката кај жени во последната деценија расте. Околу 80-85% од сите белодробни карциноми се од типот на неситноклеточен белодробен карцином (НСКБК), додека околу 15% отпаѓаат на ситноклеточните карциноми на белите дробови. Како најчест ризик факатор се вбројува

пушењето. Група на пациенти со неситноклеточен белодробен карцином имаат тумор кој содржи инверзија во хромозом 2 која се состои од јукстапозициониран на 5' крајот ехинодермниот микротубул асоциран протеин 4 (EML4) ген со 3' крајот од анапластичниот лимфома киназа ген (ALK), резултирајќи со новодобиен фузионен онкоген EML4-ALK. Се среќава кај околу 5% од НСКБК. Скрининг за овој фузионен ген кај НСКБК е од особено значење бидејќи „ALK-позитивни" тумори се високо сензитивни на таргетирана терапија со ALK инхибитори.

Цели. 1. да се одреди дистрибуцијата на неситноклеточен белодробен карцином (НСКБК) според: пол и возраст, пушечки статус, хистолошки поттип. 2. да се утврди стадиумот на болеста одреден со радиолошка ТНМ класификација 3. да се одреди фрекфенцијата на ALK мутации кај пациенти со НСКБК.

Методи. Студијата беше реализирана на ЈЗУ Универзитетска клиника за пулмологија и алергологија и Институтот за патологија, Медицински факултет -Скопје и истата претставува проспективна студија и се одвиваше во период од 1 година. Кај сите пациенти вклучени во студијата, беа анализирани ткивните примероци добиени од биопсија на бел дроб (бронхобиопсија или трансторакална биопсија). Микроскопскиот опис опфаќаше дефинирање на хистолошкиот тип на туморот и степенот на хистолошка диференцијација. Во студијата беа обработени и клинички податоци за пациентот и одредување на стадиумот на болеста со радиолошка ТНМ класификација (одредена со КТ на граден кош и медијастинум со контраст). За одредување на мутација на ALK генот, ткивните примероци беа обработени со FISH за кои се користи идентична ДНК проба.

Заклучок. ALK мутациите се нотирани кај 3 од вкупно 52 пациенти, или во проценти 5,77%, што е многу блиску до светскиот тренд на мутациите околу 5%. Со оглед на малиот примерок на испитаниците, можно е генералната популација на болни со НСКБК да потпаѓа точно во светскиот тренд. Според достапните студии се препорачува скрининг со ниско-дозирана компјутеризирана томографија на бели дробови, кај сите актуелни тешки пушачи или индивидуи кои пушеле 15 години и повеќе, на возраст од 55 до 80 години. Сето ова се совпаѓа и со нашето истражување, каде што најголемиот број на дијагностицирани пациенти со НСКБК беа пушачи и со возраст над 50 години.

Клучни зборови: НСКБК, ALK, ТНМ, EML4, Хистопатологија

Introduction

Lung cancer is a malignant tumor originating in the respiratory epithelium of the lungs: the alveolar epithelium and the epithelium of the tracheobronchial stem. It is the most common cause of death associated with malignant diseases in the world [1,2]. According to data obtained from GLOBOCAN 2018 [3], lung cancer is the most common cancer in men, while in women it is in the third place, right after breast cancer and colorectal cancer. With approximately 2 million new cases per year in 2018, lung cancer is the most commonly diagnosed cancer in the world [4]. The incidence of lung cancer varies depending on gender and race. It is twice as high in men, although in recent years there has been a significant decline in the incidence in men, while the rate in women has risen in the last decade. About 80-85% of all lung cancers are of the type of non-small cell lung cancer (NSCLC), while about 15% are small cell lung cancers [5]. The analysis of the incidence of lung cancer by histological type shows a trend for reduction of small cell carcinoma, more in men than in women. This is probably due to the decrease in the smoking rate of the population in the last decade, but also to the change in the production, composition and design of cigarettes [6]. In small cell carcinoma, squamous cell carcinoma is present with about 25-30%, large cell carcinoma with 10-15%, while adenocarcinoma accounts for about 40% of NSCLC, and its incidence is growing in most developed countries. A change in the location of the occurrence of primary lung cancer has been documented, with increased predilection to peripheral zones, associated with the histological predominance of adenocarcinoma [7,8]. The average age of patients with lung cancer is 70 years, although many of the newly discovered cases are between 50 and 60 years old.

The most common risk factor is smoking (90% of lung cancers are attributed to smoking); tobacco burning produces at least 60 known carcinogens; smokers are 13 times more likely than non-smokers to develop lung cancer [9].

Only 7% to 13% of patients are asymptomatic at the time of initial diagnosis because lung cancer (LC) is usually a diagnosis made during its later stages [10,11]. Only 26% of patients have stage I and 8.3% have stage II, while 27.6% have stage III and 38.1% have metastatic disease [12]. Patients often have symptoms for months before diagnosis [13,14]. Symptoms, especially those associated with localized disease include cough, shortness of breath, and dyspnea, and symptoms often attributed to comorbidities and associated diseases by both patients and their physicians.

Basic non-invasive imaging methods are: chest X-ray in PA and profile (X-ray); ultrasound examination of the chest; computed tomography of the chest (CT); positron emission tomography with low-level computed tomography (PET-CT). Invasive sampling procedures may be performed by a pulmonologist and radiologist (trained in invasive diagnostics) or a surgeon. The number of specimens taken by endoscopy (bronchobiopsies, tran-

bronchial biopsies) should be 4 to 6 (from vital tissue without necrosis).

In the diagnosis of lung cancer, the determination of the clinical stage of the disease is important for determining further treatment and prognosis of NSCLC. The TNM classification (8th edition) issued by the American Cancer Committee-AJCC (American Joint Commission on Cancer TNM staging system for non-small cell lung cancer) is used to determine the stage of the disease [15], as well as the Union for International Control of Cancer cancer-UICC (Union for International Cancer Control) [16] and the International Association for the Study of Lung Cancer (IASLC) [17].

Establishing a histological diagnosis and determining the histological subtypes of lung cancer is crucial in determining the prognosis and further treatment of patients. Based on the knowledge of the significant effect of targeted therapy on the survival and quality of life of patients with lung cancer (LC), the American College of Pathologists, the International Association for the Study of Lung Cancer, the Association for Molecular Pathology of the Association of Pathologists of Macedonia updated their recommendations regarding the indications and the procedure for molecular testing of patients with LC [18-20]. A group of patients with non-small cell lung cancer have a tumor that contains an inversion of chromosome 2 consisting of a 5' end-located echinoderm microtubule associated protein 4 (EML4) gene with a 3' end lypmphoma kinase gene (ALK), resulting in the newly acquired fusion oncogene EML4-ALK. This mutation is found in about 5% of NSCLCs [21].

The arrangement/redistribution of ALK gene mutations with EGFR and KRAS [22] is highly incompatible. Screening for this fusion gene in NSCLC is particularly important because "ALK-positive" tumors are highly sensitive to targeted therapy with ALK inhibitors. For patients with locally advanced disease, treatment is aimed at adequately identifying the patients who will benefit most from resection in combination with chemotherapy and radiotherapy. The effects of chrysotinib (first-generation ALK inhibitor), alectinib and ceritinib (second generation) have already been established in the literature and in modern guidelines, enabling a significant improvement in survival and quality of life. Alectinib was approved for the treatment of ALK-positive LC by the European Medicines Agency in February 2017 [23]. The approvals were based mainly on two trials: in Japan, phase I-II trials, after approximately 2 years, 19.6% of patients achieved full response, and the 2-year survival rate without progression was 76% [24]. In February 2016, the J-Alex Phase III study comparing alectinib with chrysotinib was discontinued early because a provisional analysis showed that progression-free survival was longer with alectinib [25]. In November 2017, the FDA approved alectinib for first-line treatment of patients with ALK-positive metastatic lung cancer, NSCLC. This was based on the J-Alex Phase III study, comparing it to chrysotinib [26].

Objectives

1. To determine the distribution of non-small cell lung cancer (NSCLC) according to
- Gender and age
- Smoking status
- Histological subtype

2. To determine the stage of the disease determined by radiological TNM classification

3. To determine the frequency of ALK mutations in patients with NSCLC.

Material and methods

This was a prospective study conducted at the PHI University Clinic for Pulmonology and Allergology and the Institute of Pathology, Faculty of Medicine - Skopje over a one-year period.

Inclusion criteria for the study were:

- Signed informed consent
- Men and women over 18 years (presenting with a tumor mass detected on CT)
- Histopathologically confirmed diagnosis of NSCLC.

Exclusion criteria were:

- Previous diagnosis or therapy for another malignant disease
- Global respiratory failure, oxygen hyposaturation, recurrent pneumothorax and other comorbidities that may be a contraindication to bronchoscopy or transthoracic biopsy
- Pregnant and lactating women and people with special needs.

A total of 52 patients were included in the study. In all patients, tissue samples were obtained from lung biopsy (bronchobiopsy or transthoracic biopsy) at the PHI University Clinic for Pulmonology and Allergology.

The diagnosis and typing of NSCLC was confirmed by histopathological analysis of the obtained tissue. Genetic and histopathological analyses of the material were performed at the Institute of Pathology, Laboratory of Molecular Pathology and Experimental Gene Therapy. The microscopic description included defining the histological type of the tumor and the degree of histological differentiation.

Tissue samples from 52 histologically diagnosed NSCLC were included in the study.

To determine the mutation of the ALK gene, tissue samples were processed with FISH using an identical DNA probe. Commercial break apart probes (Abbott

Molecular Probes, Abbott Park, IL) consist of two different colored probes. In unarranged cells, the red and green test results in a yellow (fusion) signal. In the ALK redistribution, these samples are separated and the separation of the red and green signals is evident.
The study also processed clinical patient data and disease status by radiological TNM classification (determined by CT of the chest and contrast mediastinum).

Results

To date, the study has voluntarily included 52 patients, who met all inclusion and none of the exclusion criteria. The study protocol was explained in detail to the patients, as well as the possible complications of the planned intervention, their rights and responsibilities. Each patient voluntarily signed the information and consent form. Of the 52 patients included, the majority are men: 42, while the number of women involved is 10 (Figure 1).

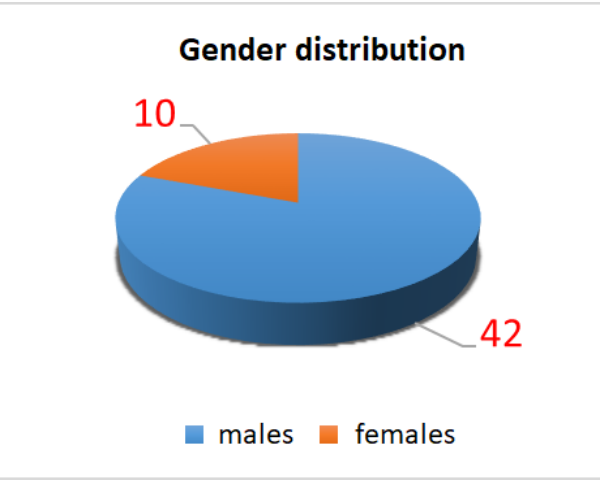

Fig. 1. Gender distribution of included patients

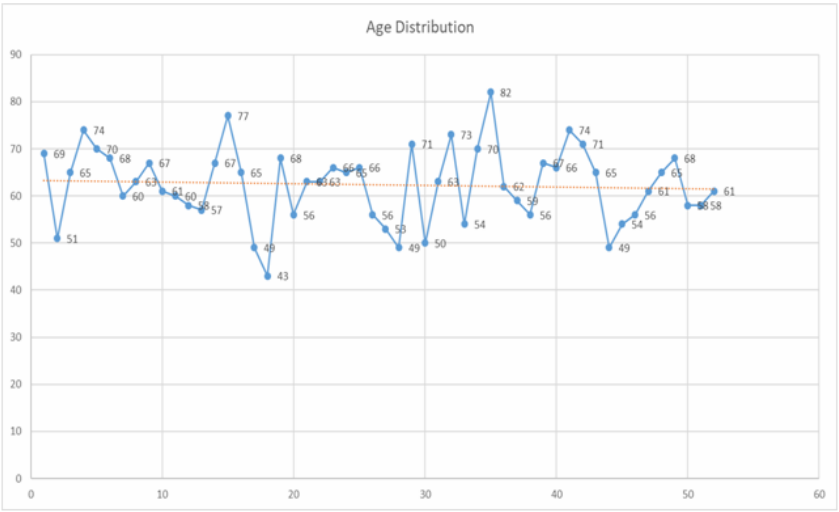

Fig. 2. Distribution according to the age of included patients

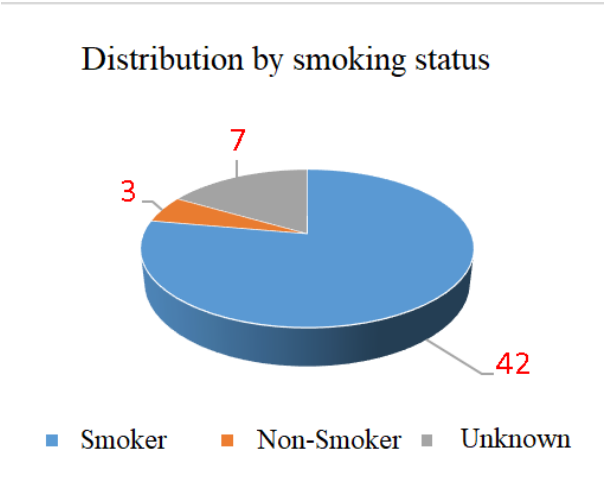

Fig. 3. Distribution by smoking status of patients

Patients are aged 43 to 82 years, with a mean age of 62.5 years. According to the average age distribution, the population falls into the group of working age population (Figure 2).
The breakdown by smoking status as a proven cause

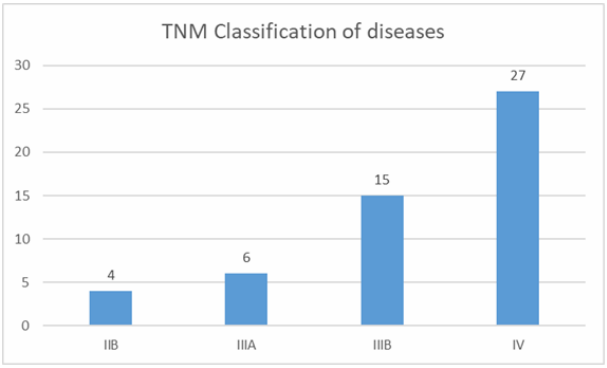

Fig. 4. TNM classification of the disease

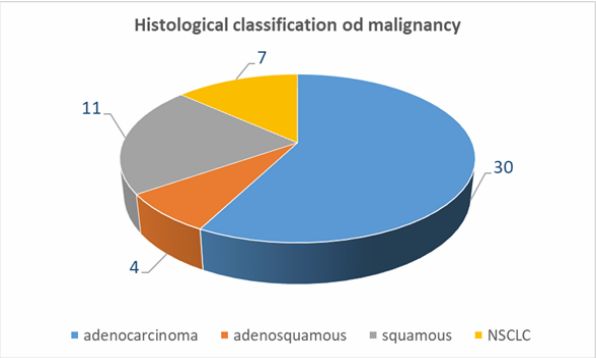

Fig. 5. Histological classification of malignancy

of lung malignancies: only 3 patients are non-smokers, 42 are smokers and 7 patients are of unknown status or refused to answer the question (Figure 3).

According to the TNM classification, stage IV was dominant with 27 (51.92%) patients, followed by stage IIIB with 15(28.85%) patients, stage IIIA with 6 (11.54%) patients and stage IIB with 4(7.72%) patients (Figure 4).

Adenocarcinoma was predominant, i.e., it was present in 30 (57.69%) patients, followed by squamous cell carcinoma in 11 (21.15%) patients, NSCLC in 7 (13.46%) patients and adenosquamous in 4 (7.69%) patients (Figure 5).

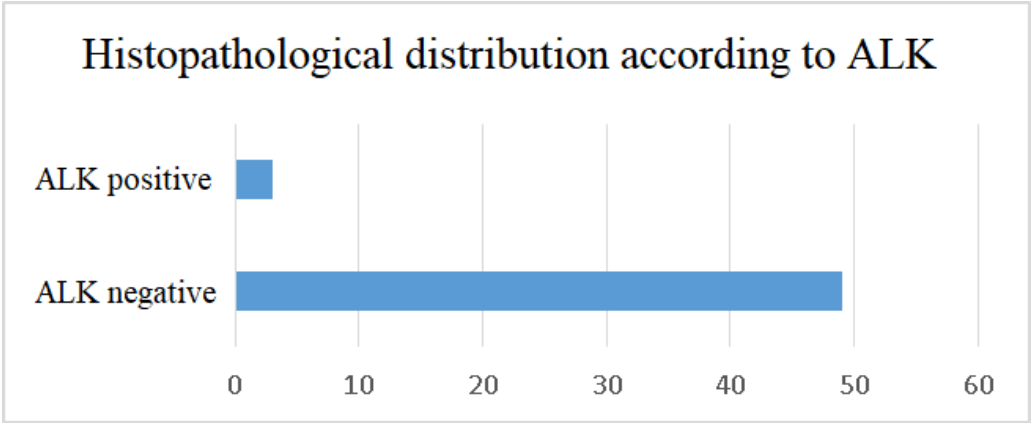

Fig. 6. Histopathological distribution according to ALK

Histopathologically, the included subjects dominanty had ALK-negative malignancies in 49 samples, while 3 (5.77%) samples of malignant tissue were ALK-positive (Figure 6).

Discussion

It is expected that the introduction of active screening will contribute to the detection of the disease in the early stages with consequent reduction of mortality. The results of the National Lung Screening Trial - NLST in the United States in 2011 [29] and the Dutch-Belgian Lung Cancer Screening trial (NELSON) [30] showed a 20-25% reduction in mortality in patients undergoing screening. According to these studies, screening with low-dose computed tomography of the lungs is recommended for all current heavy smokers or individuals who have smoked for 15 years or more, aged 55 to 80 years. All of this coincides with our study, where the majority of patients diagnosed with NSCLC were smokers over the age of 50.

Compared to ALK negative NSCLC, positive ALK mutations are mostly detected in young individuals, non-smokers and patients with tumors that show a special solid growth pattern and histology of adenocarcinoma [31]. These patients usually present in the late stages of the disease, are not amenable to surgical resection, and are therefore candidates for aggressive and new chemotherapy regimens targeting the mutated ALK protein. On the other hand, numerous studies confirm the significant benefit of the new targeted treatment, resulting in a prolonged survival and better quality of life [32,33]. It is, therefore, reasonable to think that ALK mutations should be analyzed in a large number, and possibly in all patients with NSCLC. Given the sheer number of cases of NSCLC and the relative rarity of this genetic change, any screening procedure will need to be highly sensitive, specific, reproductive, and effective.

It is important to note that in the last decade, highly developed countries, such as Japan, have reported a drastic increase in 5-year global survival rates of NSCLC, above 28%, probably due to early diagnosis, a multidisciplinary, holistic approach to the treatment of these patients, as well as new knowledge about the genetic basis of the pathogenesis of lung cancers and the progressive development of specific treatment of patients, especially targeted gene and immunotherapy.

Conclusion

According to this research, we can conclude that patients over 50 years of age, smokers, dominantly males with a histological subtype of adenocarcinoma are predominant. ALK mutations were noted in 3 of 52 patients, or 5.77%, which was very close to the global trend of mutations of about 5%. Despite the small sample of patients, it is interesting that the global po-

pulation of patients with NSCLC follows exactly the world trend. Since the vast majority of the patients present late for a medical examination, with symptoms that last for months and sometimes years, and are therefore diagnosed with stage four advanced disease, they miss the "golden" period (stage I and II of the disease) when they can be treated with a better success. In reality, a large number of patients become inoperable, and the radiological and chemotherapeutic treatment options are narrowed. The introduction of active lung cancer screening will help detect the disease at an early stage, when treatment is more likely to succeed, with consequent reduction in mortality, and their further development is seen as a prospect for the treatment of lung cancers with a higher survival threshold.

Conflict of interest statement. None declared.

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Case report

ISOLATED CASE OF PULMONARY THROMBOEMBOLISM AFTER RECEIVING THIRD BOOSTER DOSE OF SARS-COV-2 VACCINE IN A PATIENT WITH LUNG CANCER AND PARANEOPLASTIC SYNDROME

ИЗОЛИРАН СЛУЧАЈ НА ПУЛМОНАЛНА ТРОМБЕМБОЛИЈА ПО ПРИМАЊЕ НА ТРЕТА БУСТЕР ДОЗА НА ВАКЦИНА ПРОТИВ SARS-COV-2 КАЈ ПАЦИЕНТ СО БЕЛОДРОБЕН КАРЦИНОМ И ПАРАНЕОПЛАСТИЧЕН СИНДРОМ

Zlatana Petkovska, Dejan Kovachevikj, Branka Buntasheska, Magdalena Dimovska, Eva Palchevska and Jovana Topencharova

Clinical Hospital Acibadem Sistina, Skopje, Republic of North Macedonia

Abstract

Acute pulmonary embolism is a common cause of respiratory insufficiency and a case often to be found in emergency rooms worldwide. If untreated, 30% of the cases have lethal outcome [1]. During the SARS-CoV-2 pandemic, medical staff have been increasingly facing isolated cases of pulmonary thromboembolism, due to the effect this virus has on the blood coagulability and the damage it causes to the endothelium of the blood vessels. Only a few cases of pulmonary thromboembolism associated with SARS-CoV-2 vaccine have been reported in the literature, out of which we point out a case of a patient with paraneoplastic syndrome vaccinated with three doses (2 doses of Sinopharm and a booster dose of Pfizer). This patient was diagnosed with pulmonary thromboembolism. The aim of this study was to indicate the need for the patients to be cardiologically examined after a complete SARS-CoV-2 vaccination.

Keywords: COVID-19 infection, SARS-CoV-2 vaccination, echocardiography, pulmonary embolism, CT angiography, lung cancer

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Апстракт

Акутна белодробна емболија е честа причина за респираторна инсуфициенција (респираторна слабост) и случај кој се среќава низ ургентните центри во целиот свет, која доколку е нетретирана во 30% од случаите води кон летален исход. [1]. Во време на SARS-CoV-2 пандемија медицинскиот персонал се среќава со сè почести изолирани случаи на белодробна тромбемболија, која се должи на ефектот кој вирусот го има врз коагулабилноста на крвта и оштета на ендотелот на крвните садови. Во литературата се нотирани само неколку случаеви на белодробна тромемболија асоцирани со вакцина против SARS-CoV-2,од кои издвојуваме случај на пациент со паранеопластичен синдром вакциниран со три дози на вакцина (2 дози Sinopharm и една booster доза Pfizer еден месец пред да се јави во нашата установа), кај кој е дијагностицирана белодробна тромбемболија, со цел да укажеме на потребата пациентите кардиолошки да се иследуваат по комплетна вакцинација против SARS-CoV-2.

Клучни зборови: COVID-19 инфекција, SARS-CoV-2 вакцинација, ехокардиографија, белодробнаемболија, CT ангиографија, белодробенкарцином

Introduction

Pulmonary embolism is a blockage in one of the pulmonary arteries in the lungs (main pulmonary artery, big branch or small branches of pulmonary artery). In most cases, pulmonary embolism is caused by blood clots from deep veins in the legs or rarely from veins in other parts of the body reaching to the lungs through circulation. As a result, the oxygen supply to the lungs is insufficient. The clinical picture also depends on the severity of the thromboembolism. The most common symptoms are dyspnea, chest pain, hemoptysis, arrhythmia, presyncope and syncope. [2].

SARS-CoV-2 infection is a hypercoagulable condition and is associated with the development of pulmonary thromboembolism, the incidence of which has been increasing since the beginning of the global pandemic. SARS-CoV-2 leads to damage to the endothelium of blood vessels, increased blood viscosity caused by hypoxia, and further disruption of the coagulation cascade leading to thrombus formation [3].

Pulmonary thromboembolism is also one of the common complications of malignant diseases, especially lung cancer and the hypercoagulable state is the direct

Correspondence to: Zlatana Petkovska, CH AcibademSistina 1000 Skopje, R. N. Macedonia; Phone: +398 78 28 86 94; E-mail: zlatana.petkovska@acibademsistina.mk

factor of thrombosis [4]. Several mechanisms may contribute to the development of pulmonary thromboembolism in cancer patients, such as immobilization, surgery, downregulation of anticoagulants and upregulation of procoagulant proteins, endothelial damage caused by chemotherapy or stimulation of endothelial cells to produce procoagulant materials, inflammation due to necrosis or release of acute-phase reactants and hemodynamic disorders such as stasis [5].

Over the past two years, numerous efforts have been made to find a cure and prevent the spread of the SARS-CoV-2 virus, which has led to the rapid development of vaccines against it, such as mRNA vaccines-Pfizer and Moderna and vaccines containing a dead or attenuated virus-Sinopharm and Sinovac [6]. Currently, 54.8% of the total population is vaccinated worldwide, i.e. about 5 billion people [7].

Since the start of SARS-CoV-2 vaccination, several cases of pulmonary thromboembolism have been isolated in the literature immediately after vaccine administration, of which we single out a case of a patient with malignancy vaccinated with two doses of Sinopharm and a booster dose of Pfizer one month before the onset of symptoms of pulmonary thromboembolism.

Case report

A 76-year-old patient was diagnosed with lung adenocarcinoma (EGFR positive) in October 2019; since then continuously examined by an oncologist, on tablet therapy with Erlotinib. The patient was immunized against SARS-CoV-2, two doses of Sinopharm and a third dose of Pfizer given one month before admission. On February 10, 2022, due to severe pain in the left hemithorax, shortness of breath accompanied by hunger for air was sent to the Emergency Centre. During the examination he was conscious, oriented in time, space and persons, peripheral saturation of ambient air 93%, TA 158/93 mmHg. After this, a CT angiography of pulmonary arteries (Table 3) was performed, which was consistent with massive pulmonary embolism, i.e., present hypodense defects in filling at the level of the left and right pulmonary arteries and their branches, and additionally secondary metastatic deposits in vertebral bodies. In addition, chest X-ray was performed, and it showed discrete ground glass opacities; differential-diagnostic condition after Covid infection. On admission, laboratory-biochemical tests were done: D-dimers 27.9 ug/FEU/ml, Plt 103 10x9/L, CRP 5.15 mg/dl, Troponin 0.108 ng/ml, Urea 11.6 mmol/L, Creatinine 165 umol/L, ECG finding: (Table 1) sinus rhythm, pulse 120 beats/min, S1Q3T3 pattern on ECG present. Findings from transthoracic echocardiography (Table 2) performed on February 11, 2022 revealed increased dimensions of the left atrium of mild degree and right atrium of moderate degree, increased dimensions with reduced

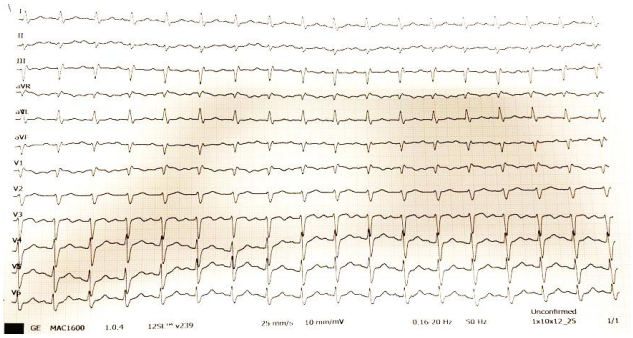

Fig. 1. ECG finding of the patient with S1Q3T3 form present

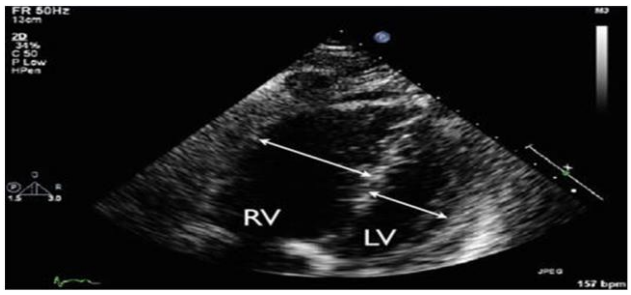

Fig. 2. Transthoracic echocardiography showing enlarged right heart cavities

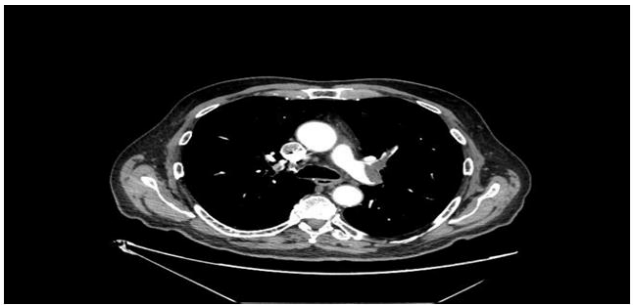

Fig. 3. Cross-sectional CT angiography

radial and longitudinal function of the right ventricle,TAPSE 17 mm, present McConelli sign (mid free wall akinesia and right ventricular apical wall hypercontractility) with left ventricular D-shape with disynchronous movement of the intraventricular septum, TR +1, SPAP 36-40 mmHg, PAH, EF 59%. During hospitalization the patient was clinically stable, treated with a low-molecular weight heparin (inj. Clexane a 40 mg, 2x2), antibiotic (vij. Ceftriaxone a 2 gr, 1x1), diuretic and hischronic medications**.** The patient was discharged from hospital on February 14, 2022 in improved clinical condition. Laboratory-biochemical tests at the day of discharge were as follows: D-dimers 3.6 ug/FEU/ml, PLT 124 10x9/L, urea 7.5 mmol/L, creatinine 118 umol/L, LDH 276 u/l, Troponin T 0.035, with a recommenddation at home to take: tbl. Rivaroxaban a 15 mg 1 x1, tbl. Eplerenone a 25 mg 1x1, tbl. Losartan/Hydrochlorothiazide 50/12.5 mg 1x1, tbl. Cefixime a 400 mg 1x1. ECHO control heart finding was similar to the initial but with improved right ventricular kinetics.

Discussion

Pulmonary thromboembolism may occur in isolated cases in COVID-19 patients without coexisting deep vein thrombosis. Studies to date have not shown a clear association between these two entities in patients with COVID-19 [8]. On the other hand, the number of cases of thrombosis associated with COVID-19 vaccines presented in the literature is increasing.

The time elapsed after vaccination to the development of thrombosis is nonspecific. It can occur in the first 7 to 10 days after immunization [9].In our case, the symptoms of pulmonary thromboembolism occurred one month after receiving a third booster dose against COVID-19.

Patients with lung cancer as well as patients with hematological malignancy are thought to be at the highest risk of COVID-19 mortality [10]. Above all, patients with lung cancer are highly vulnerable to Sars-CoV-2 infection due to frequent contacts with the healthcare system, immunocompromised state from cancer or its therapies, supportive medicines such as steroids, but also age and comorbidities. On the other hand, the mortality from COVID-19 is quite high due to the specific pathophysiological processes of the malignant disease, compromised pulmonary function, as well as oncological therapy [11].

As the number of COVID-19 cases increases worldwide, the incidence of COVID-19 in lung cancer patients is increasing. To date, immunization against COVID-19 is fully supported by the American Association for Cancer Research (AACR), indicating that all patients with malignancy, especially those with lung cancer, should be vaccinated, including those receiving chemotherapy, target therapy, and immunotherapy [12]. After vaccination, a complete cardiological examination is recommended in patients with malignant diseases.

Conclusion

During the SARS-CoV-2 pandemic, the medical staff has encountered an increased number of isolated cases of pulmonary thromboembolism due to the effect the virus has on blood coagulation and damage to the endothelium of blood vessels. With the rapid development of new methods for the treatment and prevention of COVID-19, it is necessary to evaluate their effectiveness and side effects, as well as their interaction with oncological therapies and pathophysiological mechanisms of cancer. This is extremely important when it comes to vaccines as a way of prevention, in order to continue effective care for the health of patients with lung cancer.

Conflict of interest statement. None declared.

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ММР

Case report

GENERALIZED TYPE OF EPILEPSY IN A PATIENT WITH CORNELIA DE LANGE SYNDROME TYPE 2

ГЕНЕРАЛИЗИРАНА ЕПИЛЕПСИЈА КАЈ ПАЦИЕНТ СО КОРНЕЛИЈА ДЕ ЛАНГЕ СИНДРОМ ТИП 2

Danilo Nonkulovski, Filip Duma, Ilija Kirovski¸ Lejla Muaremoska Kanzoska, Learta Adili Ademi, Ana Todoroska, Valentina Dukovska and Vankover Manchev

University Clinic for Pediatric Diseases, Department of Neurology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Cornelia de Lange syndrome is a rare genetic, developmental disorder that affects many parts of the body. Approximately 60% of people have a disease-causing variation (mutation) in the NIPBL gene, and about 10% of cases are caused by mutations in one of the four known genes: SMC1A, SMC3, HDAC8 and RAD21. In the remaining 30% of cases, the underlying genetic cause of the condition is unknown. The features of this disorder vary widely among affected individuals and range from relatively mild to severe [1,2].
Epilepsy in patients with Cornelia de Lange syndrome is presented in the range of 14% to 25% [4].
Epilepsy manifests between the ages of 0.6 and 16.3 years. The majority of patients (64.3%) presented with partial seizures and interictal EEGs mainly revealed focal epileptic paroxysms involving temporal and parietal areas [3-5].

Keywords: Cornelia de Lange syndrom (CdLs), genetics, epilepsy

Апстракт

Корнелија де Ланге синдром е ретко генегтско, развојно заболување кое зафаќа повеќе делови на телото. Кај околу 60 % од заболените настанува мутација во NPBL генот, кај 10 % од случаите е настанат како мутација на еден од познатите гени: SMC1A, SMC3, HDAC8 и RAD21. Во останатите 30% од случаевите, настанува нова de novo генетска мутација засега непозната. Сегашните истражувања покажуваат дека засегнатите пациенти можат да имаат различни клинички симптоми кои се манифестираат со лесна, средна или тешка клиничка слика. Епилепсијата кај пациентите со корнелија де ланге синдромот се манифестира кај 14-24 %.Таа се манифестира временски период помеѓу 0,6-16,3 години. Во најголем број на случаеви околу 64,3 % таа се презентира како парцијална, со почеток на парцијални напади и со промени на ЕЕГ кои се манифестираат во еден фокус најчесто во темпорална или париетална регија.

Клучни зборови: Конрнелија де ланге синдром, генетика, епилепсија

Introduction

Cornelia de Lange syndrome, also known by the name of ‘‘Brachmann-de Lange’’, is characterized by a wide range of characteristic clinical features: slow growth before and after birth leading to short stature, intellectual disability and abnormalities of bones in the arms, hands, and fingers and distinctive facial features. Many affected individuals also can have behavioral problems similar to autism, excessive body hair (hypertrichosis), an unusually small head (microcephaly), hearing loss, and problems with the digestive track, heart defects and eye problems. The estimated prevalence ranges from 1:45,000 to 1:62,000 as recently observed by a European working group [3,6].
For unexplained reasons, these patients have a higher incidence for the occurrence of seizures compared to the general population. It could be related to pathophysiological factors independent of those implicated in the characterization of main classical phenotypic features [5].
We present you a female patient with SMC1A associated CdLS and a moderately severe clinical presentation of the disease with a generalized type of epilepsy.

Case report

A five-month-old baby girl was referred to our Clinic due to several generalized convulsive seizures. Second child, from the third pregnancy, delivered in the 39th

Correspondence to: Danilo Nonkulovski, Department of Neurology, University Clinic for Children Diseases, 1000 Skopje, R. N. Macedonia; Phone: +389 70 62 92 49; E-mail: danilo_non@yahoo.com

week of gestation, spontaneously with a birth weight of 3400 grams, height 51 cm, APGAR score 9/10. A moderate facial dysmorphia was present (Figure 3). The neurological examination revealed normal findings. There was no family history of seizures.
MRI of the head also showed no presence of pathological lesions.
The implemented EEG revealed findings of slowed brain activity and the presence of a bi-hemispheric focus with generalization (Figure 1 and 2).

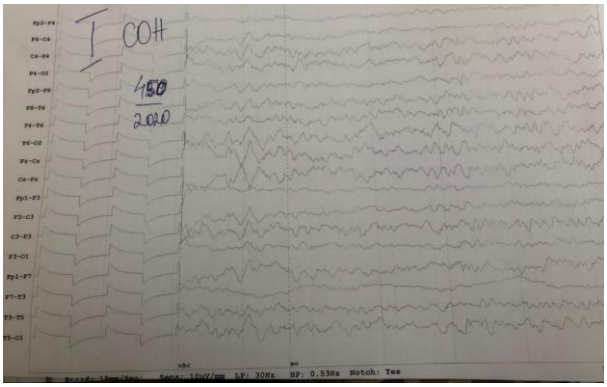

Fig. 1. EEG first page

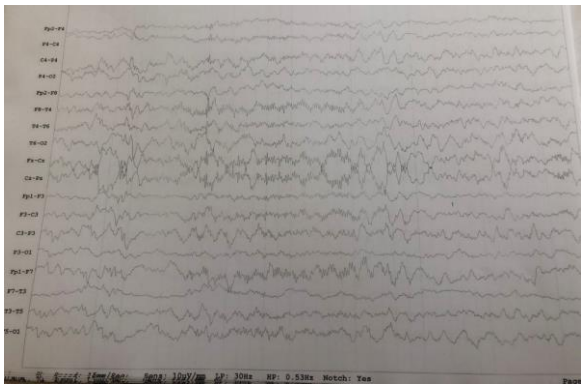

Fig. 2. EEG second page

Genetic analyses for epilepsy were performed, which showed the presence of a pathogenic change in SMC1A gene (c.1468G>A, p.Asp490Asn) in a heterozygous form. Pathogenic changes in the SMC1A gene are associated with Cornelia de Lange syndrome and developmental and epileptic encephalopathy or X-linked dominant inheritance.
Family examinations showed that the variant c.1468G>A in SMC1A gene was not present in the parents of the child. Additional analysis confirmed the biological connection with the parents of the child. These findings confirmed that the pathogenic variant c.1468G>A in the child had occurred *de novo*.
In our case, the echocardiogram did not reveal any heart defects; an ultrasound of the abdomen was performed which did not reveal anything abnormal.
Psycho-developmental tests were performed and the findings were appropriate for the age of our patient.
Examinations for possible progressive hearing loss and examination by an ophthalmologist due to myopia

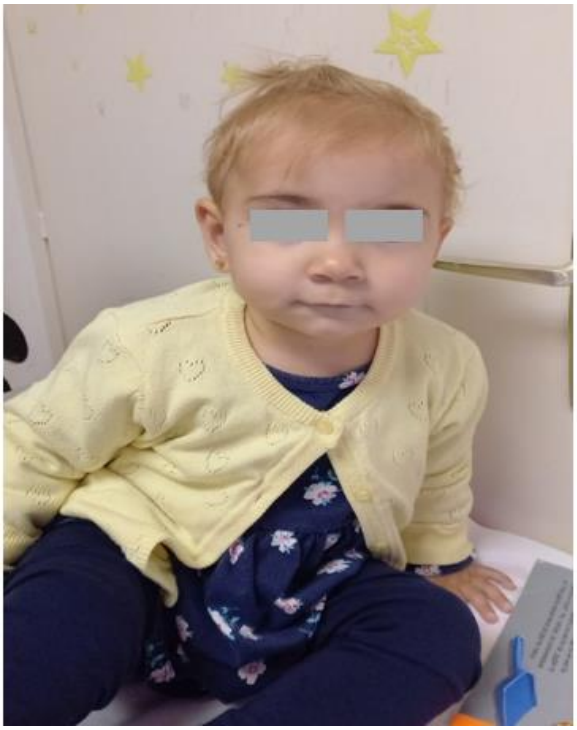

Fig. 3. Child with Cornelia de Lange syndrom

were also required.
Treatment with the first-line antiepileptic, valproic acid, was started. At the next check-up at the Department of Neurology after 2 weeks, due to repetitive seizures, change in the therapy with the second-choice drug (Levitracetam) was made.
At the next check-up after 6 months, the child did not experience a recurrence of seizures.

Discussion

Cornelia de Lange syndrome is a rare congenital disease that affects many organs and organ systems [1-3].
The diagnosis is established with the presence of the specific clinical features such as the appearance of the face and head, developmental delays, intellectual disabilities, limb abnormalities, excessive body hair (hirsutism) and/or by the genetic test showing a variation in any of the genes associated with the syndrome [1].
SMC1A gene mutations located at Xp11.22 account for approximately 5% of all cases of CdLS. A review of the literature revealed 60 cases of *SMC1A*-associated CdLS with a male-to-female ratio of 1:2. SMC1A and SMC3 mutations are responsible for a mild phenotype of the syndrome. Age at presentation with first epileptic seizures ranges from <1 month to 17 months [1,2].
About 4-6% of patients have mutations on the X-chromosome SMC1A gene, while 60% have mutations in the NIPBL gene located on chromosome 5. Most patients are female. The SMC1A gene usually avoids X-inactivation in females, which suggests that heterozygous mutations have a dominant-negative effect in manifestation in female carriers. In male patients, SMC1A

mutations are manifested with a more severe phenoltype than in female patients.
Congenital heart disease (CHD) is a commonly recognized feature of CdLS, hence the importance of early cardiac control. A variety of defects have been descrybed, with ventricular and atrial septal defects being the most common; complex heart defects such as tetralogy of Fallot and single ventricle anomalies [7].
There is likelihood of gastrointestinal disorders such as duodenal atresia, annular pancreas, imperforate anus, Meckel's diverticulum, congenital diaphragmatic hernia and pyloric stenosis [8].
Treatment is multidisciplinary and involves many teams of specialists who will systematically and comprehensively plan treatment.
The majority of patients presented with partial seizures and interictal EEGs mainly revealed focal epileptic paroxysms involving temporal and parietal areas. Most of the patients became seizures- free with treatment [4,9].
Treatment of choice is valproic acid as monotherapy followed by oxcarbazepine.
The second-line therapy is Levitiracetam and Topiramat [4,9].
In our case of generalized epilepsy, success was achieved with the second-line therapy (Levitiracetam).

Conclusion

Patients with Cornelia de Lange syndrome may have a normal lifespan, but this depends on the severity of the disease and expression of specific clinical features. The average life expectancy of patients with Cornelia de Lange syndrome is 17-24 years. A review of the literature has shown that only one patient lived 64 years. The biggest problem is recurrent pneumonia and heart defects, which are not detected early and treated properly [3,8].
In our case, we have had a child with CdLS syndrome with mild clinical manifestations. Partial epilepsy is the most common type of patients with CdLS, but in our case the child was with general epilepsy.
Prognosis of epilepsy associated with Cornelia de Lange in the majority of cases is favourable and therapy with antiepileptic drugs can be withdrawn after few years of complete seizure control [4,9].

Conflict of interest statement. None declared.

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Case report

ACCIDENTAL EPIDURAL MIGRATION INTO SUBARACHNOIDAL SPACE

СЛУЧАЈНА МИГРАЦИЈА НА ЕПИДУРАЛЕН КАТЕТЕР ВО СУБАРАХНОИДАЛЕН ПРОСТОР

Iskra Delova[1], Aleksandra Gavrilovska-Brzanov[1], Sasho Dohchev[2], Aleksandar Trifunovski[2], Marija Srceva Jovanovski[1], Ivana Panchevska[3], Antonio Georgiev[4] and Ognen Ivanovski[2]

[1]University Clinic for Traumatology, Orthopedic Disease, Anesthesiology, Reanimation and Intensive Care Medicine and Emergency Department, [2]University Clinic for Urology, [3]University Clinic for Radiology, [4]University Clinic for Cardiology, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

Abstract

Epidural anesthesia is commonly used to give pain relief, either for anesthesia, postoperative analgesia, chronic pain therapy, or to help with painless childbirth. However, epidural anesthesia has its own disadvantages such as inward migration of the catheter in the intravascular, subdural, subarachnoid and subcutaneous space.
We present a case report of 68-year-old male patient for open radical prostatectomy procedure, who was scored with American Society of Anesthesiology Physical Status Classification System (ASA) as II class. Anesthetic approach for this patient was planned general anesthesia (GA) combined with continuous epidural analgesia. The epidural space was easily detected on our first attempt using the loss of resistance technique, and the epidural catheter (EC) was placed through the needle following a negative aspiration test. After negative test for cerebrospinal fluid (CFS) and blood, we gave test dose of 2 ml of 0.5% Bupivacaine.
The patient was unable to lift his lower limbs 4 minutes after the insertion of the catheter as a consequence of a complete motor block. There were not any other adverse events despite the slight decrease in the blood pressure and pulse. We concluded subarachnoid migration of the catheter beside negative aspiration for CFS, so we decided to remove the epidural catheter.
Postoperatively, magnetic resonance imaging myelography (MRI myelography) on lumbar spine was made to determine the location of the EC and to exclude complications and to determinate any signs for EC migration.

Keywords: epidural analgesia, epidural catheter migration, MRI myelography

Апстракт

Епидуралната анестезија најчесто се користи за олеснување на болката како дел од интраоперативната аналгезија, постоперативната аналгезија, како терапија за хроничната болка или за помош при безболно породување. Сепак, епидуралната анестезија има свои недостатоци како што е миграцијата на катетерот во интраваскуларниот, субдуралниот, субарахноидалниот и поткожниот простор.
Нашиот приказ на случај е 68-годишен пациент со ASA II класификација, кај кого беше спроведена отворена радикална простатектомија. Анестетичкиот пристап за овој пациент беше планирана општа анестезија во комбинација со континуирана епидурална аналгезија. После добивањето на епидуралниот простор, кој лесно го лоцираме при првиот обид, со помош на техниката на губење на отпор, го пласираме епидуралниот катетер. Аспирациониот тест за цереброспинална течност и крв беше негативен. Последователно, аплицираме тест доза од 2ml, 0,5% бупивакаин.
Четири минути по вметнувањето на катетерот и аплицираната тест доза, пациентот не можеше да ги подигне долните екстремитети како последица на комплетен моторен блок. Интраоперативно, други несакани настани не беа регистрирани, освен мал пад на крвниот притисок и пулсот. И покрај негативниот аспирационен тест за цереброспинална течност и крв, решивме да го отстраниме епидуралниот катетер. Според клиничките знаци, заклучивме субарахноидална миграција на катетерот.
Миграцијата на епидуралниот катетер може да биде сериозна компликација ако не се открие на време, поради можност за администрирање на високи дози на лекови во несакан простор. Аплицирајќи мала тест доза, спречивме понатамошни компликации додека миграцијата на катетерот ја потврдивме со помош на клиничките знаци. Направивме миелографија со магнетна резонанца (МР миелографија) на лумбалниот 'рбет со цел да се

Correspondence to: Aleksandra Gavrilovska , University Clinic for Traumatology, Orthopedic Disease, Anesthesiology, Reanimation and Intensive Care Medicine and Emergency Department, 1000 Skopje, R. N. Macedonia; E-mail: d_rsani@yahoo.com

исклучат компликации како и индиректни знаци за миграција на катетерот.
Клучни зборови: епидурална аналгезија, миграција на епидурален катетер, МРИ миелографија

Introduction

The epidural nerve block has improved neuraxial anesthesia and analgesia. The technique is done routinely as a stand-alone anesthesia or in conjunction with spinal or general anesthesia. Over the last few decades, the clinical indications for epidural anesthesia and analgesia have grown dramatically [1]. Surgical anesthesia, postoperative analgesia, chronic pain therapy, and painless childbirth are all common uses of epidural anesthesia. The epidural catheter is frequently implanted blindly, and the site of the indwelling catheter is frequently unknown [2-5].

Epidural complications are grouped into four simple categories: psychogenic reactions, coincident complications, trauma from the technique, and untoward effects of the local anesthetic and adjuvant drugs [6].

The complications may be mild or severe. Pain at the injection site, accidental dural puncture, and vasovagal syncope are all mild adverse effects and consequences of epidural nerve block. Neural damage, epidural hematoma, and epidural abscess on the other side are all serious complications. These significant consequences are uncommon, but when they occur, they can be life-threatening [5].

Migration of an epidural catheter, which means entering the tip of the catheter from one space to another put for anesthesia or analgesia, is a known consequence of the technique. The migrations can be divided into inward or outward. Possible inward migrations of the catheter are intravascular, subdural, subarachnoid (intrathecal), and subcutaneous. Outward migration may result in analgesia loss and failed attempts to re-establish it. If the epidural catheter has migrated into the subarachnoid space, total spinal anesthesia usually develops within minutes of applying local anesthetic. It could occur at any moment after a previously functioning epidural catheter has migrated into the subarachnoid space due to changes in patient's placement or after a previously functioning epidural catheter has migrated into the subarachnoid space due to changes in patient's positioning [7,1].

Our case report is about a patient where we had an unwanted epidural catheter migration into the subarachnoid space that was detected in the early stages.

Case report

We present the case of a 68-year-old male patient (70 kg weight, 175 cm height, BMI 22.8) with prostate cancer for open radical prostatectomy procedure, ASA II classification. He had undergone aortobifemoral bypass surgery in the past. As chronic therapy he uses antiplatelet medication-Clopidogrel and alfa blocker-Tamsulosin. Chest X-ray, complete laboratory analysis, blood type and Rh factor analysis, coagulation tests (PT, aPTT, TT, INR) and D-dimer analysis were made before surgery. We decided to switch the patient on low molecular weight heparin (LMWH), enoxaparin 4000 I.U (40 mg)/day, 7 days before surgery.

The patient was educated about the anesthetic approach and informed high-risk consent was obtained. General anesthesia (GA) combined with continuous epidural analgesia was planned. On the day of the scheduled operation, we monitored the patient in the operating room with standard non-invasive monitoring (EKG, SaO2, NIBP). Invasive (EtCO2, urine output, IAP) monitoring was established after induction in GA. IV access was secured using an 18G Braun catheter and the patient was preloaded with normal saline solution at a dose of 10 ml/kg.

Under strict aseptic precautions in a sitting position, after infiltration of 2% lignocaine, an epidural needle was inserted in the L1/L2 interspace. The epidural space was easily detected on our first attempt using the loss of resistance technique, and the epidural catheter was placed through the needle following a negative aspiration test. The catheter was fixed with a sterile patch. We aspirated again for eventual CFS and blood, and after receiving a negative test we gave a test dose of 2 ml of 0.5% Bupivacaine. We waited for adverse events before induction in GA.

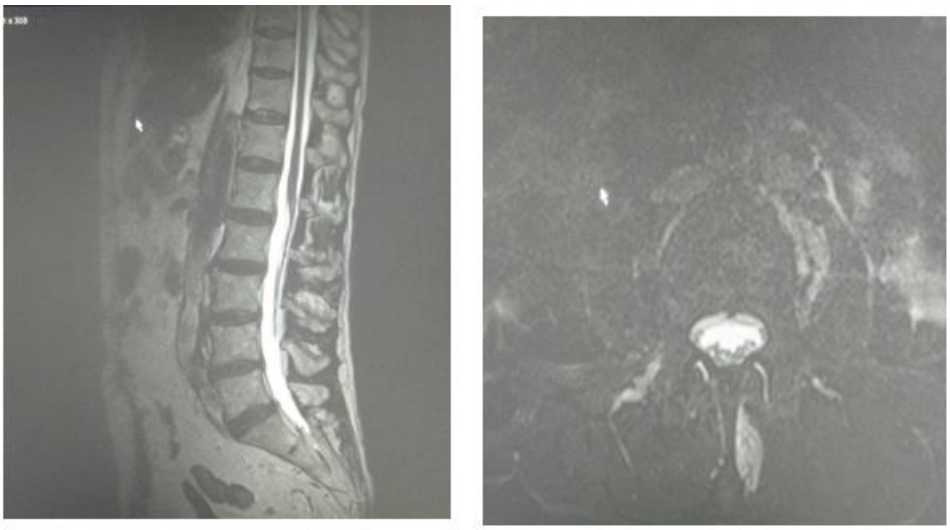
At our request, 4 minutes after the insertion of the catheter the patient was unable to lift his lower limbs as a consequence of a complete motor block. Also, there was a presence of sensory block at L1-L2 dermatome. He did not report any other adverse events despite the slight decrease in the blood pressure and pulse. We concluded subarachnoid (intrathecal) migration of the catheter based on the clinical signs beside negative aspiration for CFS, We removed the EC and immediately checked the tip, which was intact, undamaged and complete. After the removal of the catheter, we started the induction in GA.

Post-induction, an intra-arterial line was also inserted for monitoring. Intraoperatively, we had minimal opioid analgesia demand of 50 mcg Fentanyl. The patient was hemodynamically stable during the intra- and postoperative period. In the post- anesthesia care unit (PACU) the patient started to feel and move his toes and reported 0 out of 10 pain score on the visual analogue scale (VAS). One hour after discharging from the recovery room in the urology department, the patient started to move and feel his legs completely.

In order to exclude complications and to determinate any signs for EC migration, we decided to perform magnetic resonance imaging myelography (MRI myelography), as a noninvasive medical imaging

technique, providing anatomic information about the subarachnoid space.

MRI myelography on lumbar spine, from Th9 to the distal end of the coccygeal bone, in the first 60 minutes postoperatively. Besides the usual MR pulse sequences, T1 and T2 in sagital plane and T2 in axial plane, we proceeded with MR myelography using heavily T2-weighted fast spin-echo pulse sequences with fat presaturation for myelogram like images without use of a contrast medium. MRI finding was a discreet edema between the spinous processes and the muscle erector spine, from L2 to L4-L5, as a result of the applied anesthetic, so we excluded any serious event (Figure 1).



a)T2 sagital plane SPC myelo **b)** T2 axial plane

Fig. 1 a) and b); MRI myelography on the lumbar spine, showing discreet edema between the spinous processes and the muscle erector spine, sagital and axial plane views

We managed the postoperative pain with opioid and non-opioid analgesic, Paracetamol and Tramadol accordingly. The patient was discharged from the urology department on the 13th postoperative day in a good physical condition in collaboration with the surgeon.

Discussion

The migration of an epidural catheter into the subarachnoid space might result in major problems such as extensive block, including the cranial nerves and the respiratory muscle. When there is a negative aspiration test but an extensive block occurs in 15-20 minutes, an injection to the subarachnoid space should be investigated [8]. In our patient, the subarachnoid block was detected based on the clinical signs in the first 5 minutes after applying the test dose, so we removed the EC immediately.

According to Ravindran *et al*., EC migration into the subarachnoid or epidural venous space can occur even after one or two normal injection doses and may not be detected by aspiration alone; therefore, a small volume of test dosage should be injected before large volumes are injected [9].

In our clinical practice we prefer small test doses to be given before any larger doses. Accordingly, our test dose was 2 ml of 0.5% Bupivacaine.

In a study of 4107 patients, Kojiro Kuroda *et al.* found that accidental dural puncture occurred in 0.49% of all surgical patients receiving epidural anesthesia, and that the event was significantly related to those who received a puncture in the lower thoracic and lumbar intervertebral spaces, with age being an independent factor [10]. The epidural catheter in our patient was inserted in the L1/L2 interspace.

According to a North American survey on the management of dural puncture during labor epidural analgesia, accidental dural puncture happened in 0.4% to 6.0% of patients [11].

Radiological methods are very useful to confirm the placement of the epidural catheter, to confirm possible migration of the catheter and to exclude unwanted events such as epidural or spinal hematoma. Hoffman and Ferrante proposed a four-step algorithm for clinical presentation of unwanted subdural injection by analyzing radiographically proven cases [12].

Smith and Anderson used a CT myelogram with contrast through the epidural catheter to detect the placement of the EC after unwanted neurological symptoms in their case report [13]. We decided to make urgent

MRI myelography to exclude serious events. The MRI finding was discreet edema between the spinous processes and the muscle erector spine, as a result of the applied anesthetic, so we excluded any serious event. The edema cannot be consequence of multiple punctures, because we have had only one successful attempt.

In a prospective, randomized, double blind, clinical trial of 68 adult patients, Tripathi and Pandey suggested subcutaneous tunneling with a loop to prevent displacement. They found a higher incidence of inward migration of the catheter without subcutaneous tunneling. Burstal *et al*. in a randomized controlled study have also found that subcutaneous tunneling prevents clinically significant inward and outward displacement of epidural catheters. However, this technique, according to Kumar and Chambers, is effective in circumstances where the catheter is likely to remain in place for more than 48 hours [14-16]. We should consider subcutaneous tunneling in our clinical practice to prevent this kind of unwanted events, especially for long-term planned analgesia.

Conclusion

EC migration can be a serious complication if it is not detected on time. As a consequence, inadvertent administration of epidural drug dosages into the subdural space can be given. By giving a small test dose we prevented any further unwanted events. We managed to detect the migration of the catheter timely and confirm it with MRI myelography.

Conflict of interest statement. None declared.

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ММР

Case report

ПРИКАЗ НА СЛУЧАЈ: ТРЕТМАН СО ЕМБОЛИЗАЦИЈА НА ХЕМАТОМ ВО ПРАВИОТ СТОМАЧЕН МУСКУЛ КАЈ ПАЦИЕНТ СО КОВИД-19

A CASE REPORT: EMBOLIZATION TREATMENT OF RECTUS SHEATH HEMATOMA IN A COVID-19 PATIENT

Nikola Kuzmanovski[1], Zaklina Sopova[1], Arlinda L. Osmani[1], Menka Lazareska[2], Petar Janevski[2], Suncica Bogoeva-Tasevska[1] and Kosta Kapsarov[1]

[1]University Clinic for Infectious Diseases and Febrile Conditions, [2]University Institute for Radiology, Faculty of Medicine, Ss. Cyril and Methodius University, Republic of North Macedonia

Abstract

Introduction. In this case report we present a patient with COVID-19 pneumonia and rectus sheath hematoma (RSH) successfully treated with embolization.
Methods. A 63-year-old man positive for SARS-CoV-2 presented with cough, fever and dyspnea to our Clinic. The patient was admitted and treated with oxygen, antibiotics, corticosteroid, anticoagulant and oral antiplatelet therapy. Thirteen days after admission the patient had severe abdominal pain, the CT scan confirmed left rectus sheath hematoma and he underwent a CT angiography with embolization of the left inferior epigastric artery. Ten days after embolization the patient recovered completely and was discharged.
Result. SARS-CoV-2 infection is associated with coagulopathy, hence the anticoagulant therapy. The main side effect of anticoagulant therapy is an increased risk of bleeding. A rare complication of anticoagulant therapy is rectus sheath hematoma. The treatment is usually conservative with intravenous fluids, pain medication, discontinuation of anticoagulant therapy, and blood transfusion in cases of severe anemia. The computed tomography is the most common method to establish or confirm the diagnosis. Embolization of bleeding vessels can be performed in large RSH with hemodynamic instability and/or with evidence of active bleeding.
Conclusion. Inpatient treatment of COVID-19 pneumonia includes anticoagulant agents, but clinicians must carefully monitor their possible side effects and suspect a rectus sheath hematoma in patients with abdominal pain and palpable mass. Those with clinically relevant rectus sheath hematoma that do not respond to supportive care can be successfully treated using embolization, thus avoiding invasive surgical approach.

Keywords: anticoagulants, LMWH, COVID-19, rectus muscle of abdomen

Correspondence to: Nikola Kuzmanovski, University Clinic for Infectious Diseases and Febrile Conditions, 1000 Skopje, R. N. Macedonia. E-mail: nikolakuzmanovski@outlook.com

Апстракт

Вовед. Во овој приказ на случај претставуваме успешен третман со емболизација кај пациент со КОВИД-19 пневмонија и крварење во правиот стомачен мускул.
Методи. 63 годишен маж позитивен за SARS-CoV-2 кој се јави на нашата Клиника поради кашлица, покачена телесна температура и глад за воздух, беше примен и третиран со кислород, антибиотици, кортикостероид, антикоагулантна и антиагрегациона терапија. Тринаесет дена по приемот пациентот се пожали на силна стомачна болка, а компјутеризираната томографија покажа крварење во левиот прав стомачен мускул и пациентот подлегна на КТ водена емболизација на левата епигастрична артерија. Десет дена по емболизацијата пациентот се опорави и беше испишан.
Резултати. Инфекцијата со SARS-CoV-2 е асоцирана со коагулопатија, оттука и примената на антикоагулантна терапија. Главен несакан ефект на антикоагулантната терапија е зголемениот ризик за крварење, а нејзина ретка компликација е крварењето во правиот стомачен мускул. Третманот е обично конзервативен со инфузиони раствори, обезболување, прекин на антикоагулантната терапија, понекогаш и трансфузија на крв. Најчесто користена метода за поставување и потврдување на дијагнозата е компјутеризираната томографија. Емболизација на крваречки крвни садови се врши при големи крварења со хемодинамска нестабилност и/или знаци за активно крварење.
Заклучок. Хоспиталното лекување на КОВИД-19 пневмонија вклучува антикоагулантни агенси, но клиничарот мора внимателно да следи за можни несакани ефекти и да има сомнеж за крварење во правиот стомачен мускул кај пациенти со стомачна

болка и палпабилна маса. Пациентите со клинички значајно крварење во правиот стомачен мускул кои не реагираат на супортивна терапија може успешно да се третират со емболизација, а така се избегнува инвазивниот хируршки пристап.

Клучни зборови: антикоагулантна терапија; нискомолекуларни хепарини; КОВИД-19; прав стомачен мускул.

Introduction

Coronavirus disease 2019 (COVID-19) is an infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported in December 2019 in Wuhan, China. Two of the numerous complications that can occur in COVID-19 patients are coagulopathy (thrombosis) and vascular injury (hemorrhage). Anticoagulant therapy is used to prevent and treat abnormalities in coagulation, but may increase the risk of bleeding, especially in older patients with comorbidities [1]. One example for spontaneous bleeding is the rectus sheath hematoma (RSH). In this case report we present a patient with COVID-19 pneumonia, who developed a rectus sheath hematoma (RSH) during the hospital stay and was successfully treated with embolization.

Case presentation

A 63-year-old man presented to our Clinic after being tested positive for SARS-CoV-2 with COVID-19 rapid antigen test, because of persistent cough, fever and recently developed shortness of breath. His first symptoms dated 10 days prior admission. The patient was afebrile with mildly elevated pulse and dyspneic without oxygen therapy (pulse oxygen saturation=89% at ambient air). From his medical history, the patient has an essential hypertension that is regulated with enalapril once daily. The blood investigation results on admission are presented in Table 1.

Table 1. The trend of blood investigation on admission

	Reference values	Values on admission
HB[a]	115-180 g/l	117
RBC[b]	4000-5500×10^3/μL	3910
WBC[c]	4.0-11.0×10^3/μL	9.9
NLR[d]	<3	12.7
PLT[e]	150-400×10^3/μL	393
D-Dimer	<550 ng/ml	16760
LDH[f]	120-246 IU/ml	604
CRP[g]	0-10 mg/l	225
Urea	1.7-8.3 mmol/l	10.6

[a]HB: hemoglobin; [b]RBC: red blood cells; [c]WBC: white blood cells; [d]NLR: neutrophil-lymphocyte ratio; [e]PLT: platelets; [f]LDH: lactate dehydrogenase; [g]CRP: C-reactive protein

The patient was admitted in the COVID-19 ward and treated with oxygen therapy (simple face mask with oxygen flow 10 to 15 l/min), wide spectrum parenteral antibiotics (beta lactam and fluoroquinolones class), high-dose parenteral corticosteroid, therapeutic doses of enoxaparin as low molecular weight heparin (LMWH), as well as parenteral proton-pump inhibitor and oral antiplatelet therapy. Multifocal and bilateral consolidations were revealed on the chest X-ray. Similarly, the chest computed tomography (CT) with contrast registered peripheral bilateral progressive organizing consolidations without evidence of pleural effusion. All these findings suggested a COVID-19 bilateral pneumonia.

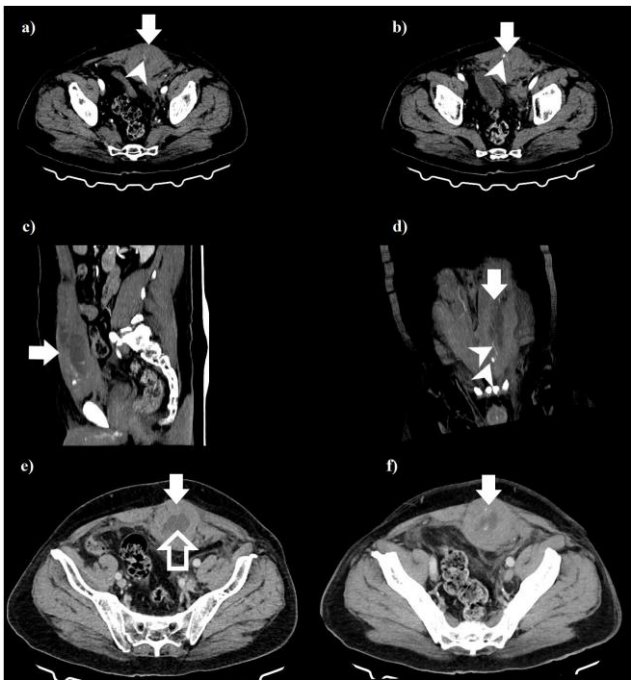

Fig. 1 Abdominal CT scan with contrast showing the hematoma (white arrows) in the left rectus abdominis muscle, extravasation of contrast (white arrowheads) and the colliquation (unfilled white arrow, the darker area inside the hematoma): a) to d) arterial phase; e) and f) venous phase

Thirteen days after admission the patient presented with severe lower abdominal pain radiating to the left inguinal region. Therefore, additional fluids and pain medications were administered that mildly relieved the pain. Later that day, due to reappearance of the pain, palpable tender mass located to the left of the umbilicus and a decrease in the blood pressure (100/70 mmHg), the patient underwent an abdominal CT scan with contrast. The CT scan confirmed 17 × 6 × 5 centimeter rectus sheath hematoma (RSH) with colliquation and extravasation of contrast, and an indication for embolization was set (Figure 1).
collaboration with the team from the Clinic for Radiology the patient underwent a CT angiography

Table 2. The trend of blood investigation on admission, during hospital stay, at discharge and at follow-up

	On admission	13th day	14th day	At discharge	At follow-up
HB[a]	117	87	91	92	114
RBC[b]	3910	2860	2970	3060	3910
PLT[c]	393	231	451	247	210
D-Dimer	16760	1492	/	2095	1132
LDH[d]	604	451	/	291	278
CRP[e]	225	6	/	48	8
Urea	10.6	10.6	15.7	4.1	4.8

[a]HB: hemoglobin; [b]RBC: red blood cells; [c]PLT: platelets; [d]LDH: lactate dehydrogenase; [e]CRP: C-reactive protein

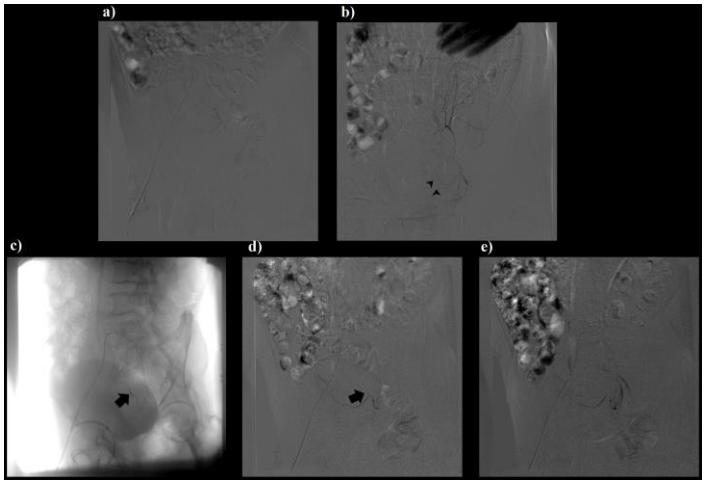

Fig. 2 CT angiography and superselective catheterization of the left inferior epigastric artery showing the points of bleeding (arrowheads), the inserted hydrophilic coils (black arrows) and the progressive occlusion; a) and b) are pre-embolization; c), d) and e) are after embolization

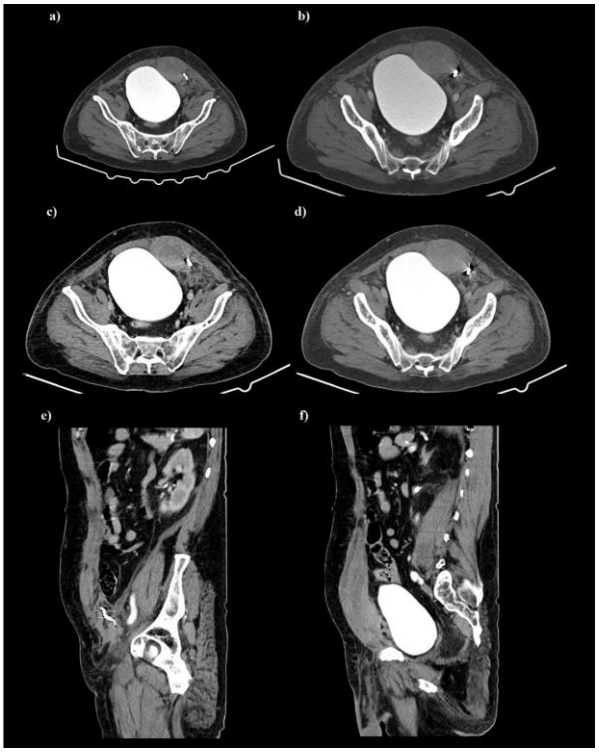

Fig. 3 The control abdominal CT with contrast demonstrates the placement of the coils, downsized hematoma, but also a distended bladder filled with contrast: a) and b) represent the arterial phase; c) and d) represent the venous phase; e) and f) represent the saggital reconstruction

The trend of blood investigation on admission, during the hospital stay, at discharge and at follow-up is shown in Table 2. During the evening hours, in with embolization of the left inferior epigastric artery (Figure 2).
Multiple hydrophilic coils were inserted, which led to a progressive occlusion of the possible points of bleeding. The next day, a control abdominal CT with contrast was performed which demonstrated the placement of the coils in the left inferior epigastric artery, no more active hemorrhage and extravasation of contrast in both the arterial and venous phase, as well as 1.5 cm downsized hematoma (Figure 3).
The anticoagulation therapy was ceased for two days and prophylactic doses of low molecular weight heaprin (LMWH) were used afterwards until the end of the hospitalization. Ten days after the embolization the patient recovered completely and was discharged. Due to high levels of D-dimer (2095 ng/ml) at discharge, we recommended direct-acting oral anticoagulant (DOAC) and oral proton-pump inhibitor for the next two weeks. At the follow-up, the patient was feeling well and the laboratory findings showed improvement in hemoglobin (HB) 114 g/l, red blood cell count (RBC) of $3910\times10^3/\mu L$ and still mildly elevated D-dimer values 1132 ng/ml.

Discussion

The infection with SARS-CoV-2 is associated with coagulopathy, hence the anticoagulant therapy in the COVID-19 protocols in order to decrease the risk for thromboembolism. The most used anticoagulant agents in inpatient settings are the different forms of low molecular weight heparin (LMWH) like enoxaparin or nadroparin. An increased risk of bleeding is their main side effect [2]. The rectus sheath hematoma (RSH) is a relatively rare complication of anticoagulant therapy. On the other side, the anticoagulant therapy is the most frequent cause for spontaneous rectus sheath hematoma. Even though the pathogenesis of anticoagulation-associated rectus sheath hematoma is not fully understood, it is assumed that heparin-induced microangiopathy or preexisting diffuse arteriosclerosis of the small vessels are one of the main reasons. Additional factors that can contribute to the pathogenesis of RSH are the older age, minor trauma, such as rapid change in position, twisting, Valsalva maneuvers, intraabdominal injections and especially sneezing or coughing [3-4]. A lot of muscles contract during cough, including the abdominal muscles that can cause tearing of the superior or inferior epigastric artery and their branches, and resulting in concomitant bleeding [5]. An interesting fact is that women are more susceptible to RSH, because of the smaller mass of the rectus muscle that provides less protection against trauma to the muscle and its associated blood vessels. Rectus sheath hematoma occurs after injury of either superior or inferior epigastric artery which leads to hemorrhage into the rectus sheath [6].
The clinical presentation of RSH is characterized with pain in the lower abdomen (usually sudden and can mimic acute abdomen) and abdominal wall tender mass on examination [4,6]. Obtaining information from the patient and examination of the patient are the first steps to diagnose rectus sheath hematoma. Carnett's sign is a finding on clinical examination that is used to differentiate the source of the abdominal pain, whether the pain is located inside the abdomen or in the abdominal wall. A positive Carnett's sign occurs when the pain increases as the muscles of the abdominal wall are tensed by raising and holding one's head from the pillow, and indicates that the pain very likely originates from the abdominal wall (as in rectus sheath hematoma). Contraction of rectus abdominis muscle compresses the hematoma and exacerbates the pain and tenderness [5,7]. Another important sign on physical examination is the positive Fothergill sign, which represents a fixed and palpable mass in the rectus abdominis muscle when the muscle contracts, and usually that mass is a rectus sheath hematoma. Both signs help in the rapid and accurate bedside diagnosis [7]. Laboratory findings are of more importance for deciding whether the patient needs blood transfusion or not.
The treatment is usually conservative with intravenous fluids, pain medication, and discontinuation of anticoagulant therapy. Blood transfusions are necessary in cases of severe anemia. Surgical ligation of the vessels is another option for treatment of rectus sheath hematoma, especially when the hematoma is large enough to make compression of internal organs and/or vessels. A case report published in 2020 in the *International Journal of Surgery Case Reports* showed a urinary obstruction by rectus sheath hematoma and compression of the left external iliac artery [8].
A study at Mayo Clinic in 2006 found that the computed tomography (CT) of abdomen and pelvis is by far the most common method to establish or confirm the diagnosis of RSH (in 76% of 126 patients). The CT of abdomen and pelvis also reduces the need of unnecessary laparotomy, as RSH can present with strong abdominal pain like in acute abdomen [6]. The injury of vessels is presented on CT with contrast as an active extravasation of contrast, which means escaping of contrast from the injured vessel to the surrounding tissue. The active extravasation is better differentiated with dual phase CT protocol compared to single phase CT. Dual phase CT reduces the time between the diagnosis and treatment. The interventional radiologist can address the suspected bleeding vessel and then perform non-selective angiography to look for additional injuries [9]. Endovascular embolization of suspected bleeding vessels can be performed in large rectus sheath hematoma with hemodynamic instability and/or with evidence of active bleeding, as in our case report. Embolization is a minimal invasive radiologic procedure with a great success in ceasing the active bleeding. Accor-

ding to a study from 2007 by Jordi Rimola published in the *American Journal of Roentgenology*, the rate of hemostasis after embolization is 100% and suggests that the procedure should be considered as first-option treatment for rectus sheath hematoma that cannot be controlled with supportive care [3].

Conclusion

The protocols for inpatient treatment of SARS-CoV-2 infection and COVID-19 pneumonia include anticoagulant agents such as low molecular weight heparin. Clinicians must carefully monitor possible side effects of the anticoagulant therapy, suspect a rectus sheath hematoma in patients with acute abdominal pain and a palpable mass in the abdominal wall and order a CT scan to evaluate and confirm the suspicion. Patients with clinically relevant rectus sheath hematoma can be successfully treated using embolization as an interventional radiology procedure, thus avoiding invasive surgical approach.

Conflict of interest statement. None declared.

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In memoriam

Полковник прим. д-р Стеван Грданоски
(1934-2021)

На 23.04.2021 г. почина полковник прим. д-р Стеван С. Грданоски. Роден е на 14.11.1934 година во Прилеп. Основно училиште и гимназија завршил во Прилеп. Дипломирал на Медицинскиот факултет во Скопје 1961 година. Стажирал на ВМА во Белград и продожил во Скопје. Во 1962 година, е примен како лекар со чин поручник во воена пошта 2678 Скопје. Во таа година продолжува да работи во Армиската болница во Скопје, ВП 2477 па ВП 3088. Обавувал повеќе значајни должности. Како лекар во ЈНА, бил одреден да замине со „плавите беретки“, во зимската смена во 1965 г., во Мировната мисија на Обединетите Нации во Египет (Синај), во рамките на УНЕФ за медицинско обезбедување на југословенскиот баталјон.

По специјализацијата по епидемиологија на Воено-медицинската академија во Белград 1968 година, работи како епидеомолог во воениот Хигиенско-епидемилошки одред (подоцна преименуван во Завод за превентивна медицинска заштита), кај што постанува и началник на Епидемиолошкото одделение и управник на Заводот. Во 1972 година учествува во сузбивањето на епидемијата на вариола во тогашна Југославија, за што добива и одликување од тогашниот претседател на СФРЈ. По формирањето Центар на военоздравствени установи назначен е за заменик управник на Центарот. Пензиониран е во 1995 година во чин полковник на АРМ.

Бил член на претседателството на Сојузот на лекарските здруженија на Македонија (1981-1984 година), а во 1983-1984 година, и негов претседател. Тогаш, по функција бил и член на претседателството на Сојузот на лекарските друштва на Југославија. Во периодот од 1985-1990 година, бил еден од двата македонски члена на Сојузната епидемиолошка комисија на Сојузниот извршен совет на СФРЈ. Бил секретар на Секцијата за превентивна медицина при СЛЗМ, како и генерален секретар на X и XI конгрес на лекарите на РМ (во 1978 и 1982 година).

Д-р С. Грданоски учествувал во реализацијата на наставата по предметот „здравствена заштита во време на војна“ за студентите по медицина и стоматологија на Универзитетот во Скопје, како и настава за лекари на специјализација по епидемилогија. Подолго време бил предавач и виш предавач и на скопскиот Факултет за општонародна одбрана.

Стручната активност на д-р С. Грданоски била тесно поврзана со неговата специјализација и истражувачка ориентација и афинитет, при кое што особено бил истакнат во делот за статистички анализи на масовните заболувања во медицината. Бил ангажиран во реализацијата на десетина превентивномедицински научноистражувачки проекти од интерес на населението во Македонија и од интерес на ЈНА. За време на професионалната кариера учествувал со реферати на стручни собири и објавувал трудови во медицински списанија, првенствено издавани од армијата. Автор и коавтор е на преку 80 стручни публикации од областа на епидемиологијата и од областа на организацијата на здравствената служба. Бил член на уредувачкиот одбор на „Медицински билтен на Третата армија на ЈНА“.

Д-р С. Грданоски останува трајно во колективната меморија во нашата средина како посветен стручњак и едукатор, со одлични комуникациски вештини и организациски и раководни способности, со воjнички строги но праведни критериуми во односите со колегите и соработниците. Тој знаеше да процени и успешно да делегира, мотивира и координира индивидуални и групни активности и проекти. Тој ги мотивираше соработниците и колегите за спроведување стандарди и подигнување на успешноста во нивата кариера.

За своите севкупни професионални и стручно-општествени активности д-р С. Грданоски добил повеќе дипломи, благодарници, стручни и општест-

вени признанија. Од страна на Министерството за здравство му е доделено звање „примариус“ во 1975 година. Одликуван е со медали и ордени, помеѓу кои се и: Орден на Народната Армија со сребрена ѕвезда; Орден за воени заслуги со златни мечови; Орден за воени заслуги со сребрени мечови; Медал за воени заслуги; Орден на трудот со златен венец; Орден на трудот со сребрен венец; Одликување за учество во силите на ОН; Повеќе јубилејни воени и цивилни одликувања. За исклучителни резултати во унапредувањето на медицинската наука, практика и развојот на здравствената заштита, на 6.4.1990 година, Сојузот на здруженијата на лекарите на СР Македонија, додели ПОВЕЛБА „д-р Трифун Пановски“ на д-р Стеван Грданоски.

За неговата дејност, посветеност и придонес во здравствената, наставната, организациската и раководната работа, благодарно е нашето општество, соработниците и посебно генерациите студенти и лекари и наставници на кои бил учител и, секако, неговите потомци со нивните семејства.

Нека почива во мир и нека му е вечна слава!

Прим. д-р Илија Глигоров

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 Times New Roman 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.

УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

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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 MeSH (Medical Sibject Headings) listata na Index Medicus.

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.

3. 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.

4. 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. [3-6]).
Литературата се цитира на следниов начин (кратенките за насловите на списанијата треба да се според листата прифатени во Index Medicus):
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*.) Neglia JP Meadows AT, Robison LL *et al*. Second neoplasms after acute lymphoblastic leukemia in childhood. N Engl J Med 1991; 325:1330-6.
б) заеднички автор
GIVIO (Interdisciplinary group for cancer care evaluation). Reducing diagnostic delay in breast cancer. Possible therapeutic implications. *Cancer* 1986; 58: 1756-61.
в) ***без автор*** **-** анонимно. Breast screening: new evidence. (*Editoriall Lancet* 1984; i :1217-8).
г) ***поглавје во книга или монографија***
Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. Vo: Sodeman WA Jr, Sodeman WA, Ed. Pathogenic physiology: mechanisms of disease. Philadelphia; W B Saunders, 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 – Комерцијална банка
со цел на дознака : уплата за стучен труд

Адресата на Редакцијата

Даме Груев бр. 3
Градски ѕид блок II,
1000 Скопје,
Тел.: ++ 389 02 3162 577
Електронска адреса (Е-маил): mld@unet.com.mk

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 до рецензентите на списанието Македонски медицински преглед

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 – 02/ 3162 557.