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Review article

LAPAROSCOPIC VS. ROBOTIC SURGERY IN GYNECOLOGY

ЛАПАРОСКОПСКА НАСПРОТИ РОБОТСКА ХИРУРГИЈА ВО ГИНЕКОЛОГИЈАТА

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Abstract

Gynecologic surgery has undergone a significant evolution over the past few decades. The techniques of gynecologic surgery have been significantly enhanced and the complications reduced.

We aimed to review randomized and observational, retrospective and prospective studies to compare robotic surgery as opposed mainly to laparoscopic surgery and also to abdominal surgery for treatment of both benign and malignant gyneciologic indications and to compare advantages and disadvantages between these surgical techniques. The comparison focused on operative times, surgical outcomes, and surgical complications.

A systematic review of literature was performed. Randomized controlled trials comparing robotic and laparoscopic techniques in gynecologic surgery were included. MEDLINE, Evidence-Based Medicine Reviews, PubMed, ACOG and others were the main search sources utilized in search of study data.

Laparoscopy (LPS) is characterized by two-dimensional visualization, a limited range of motions, difficulty with hand-eye coordination, and enhanced physiologic tremors. Robotic surgery (RS) has the same characteristics, with additional improvements in ergonomics and visualization. However, due to a specific equipment, RS has higher costs and longer operative times compared to open and LPS approaches.

In light of these data, there were no significant differencies between robotic and laparoscopic surgery with regard to mortality and morbidity outcomes in the largest number of studies. RS was frequently associated with longer operative times and higher overall costs, but many studies found potential benefits in postoperative recovery time. However, the decision whether robotic surgery should become mainstream in gynecologic surgery or remain another surgical technique requires a few more randomized controlled clinical trials.

Keywords: gynecology, robotic surgery, laparoscopic surgery, advantages, disadvantage.

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Апстракт

Во последните неколку декади гинеколошката хирургија доживеа значајна еволуција. Техниките на гинеколошката хирургија се значајно напреднати а компликациите се редуцирани.

Нашата цел е да разгледаме бројни рандомизирани, ретроспективни и проспективни студии со цел да ја споредиме главно роботската наспроти лапароскопската хирургија за третман на бенигни и малигни гинеколошки заболувања и да ги споредиме предностите и недостатоците помеѓу овие хирушки техники. Споредбата се фокусира на оперативното време и хирушките компликации асоцирани со различни хирушки техники.

Беше изведен систематски преглед на податоци од рандомизирани испитувања споредувајќи ги роботската и лапароскопската техника во гинеколошката хирургија. MEDLINE, Evidence-Based medicine reviews, PubMed, ACOG, и други беа главните извори на податоци во оваа студија.

Лапароскопијата се карактеризира со дводимензионална визуелизација, ограничени движења, потешкотии во координацијата раце-очи и зголемен физиолошки тремор. Роботската хирургија ги обезбедува истите предности но има дополнителни подобрувања во однос на визуелизација и подобрени ергономски карактеристики. Во секој случај поради специфичната опрема, роботската хирургија има повисока цена и подолго оперативно време во споредба со лапароскопската хирургија и отворениот пристап.

Според добиените податоци, не е забележана сигнификантна разлика помеѓу роботската и лапароскопската хирургија во однос на морталитетот и постоперативниот морбидитет. РХ беше почесто асоцирана со подолго оперативно време и повисока цена, но многу студии го потврдуваат потенцијалниот бенефит во постоперативното време на рехабилитација. Како и да е одлуката дали роботската хирургија треба да биде главна гинеколошка хирушка техника или да останат другите хирушки тех-

ници бара многу повеќе рандомизирани клинички истражувања.

Клучни зборови: гинекологија, роботска хирургија, лапароскопска хирургија, предности, недостатоци

Introduction

The first published description of laparoscopy in humans was made by the Swedish surgeon Dr. Hans Christian Jacobaeus in 1910. He used air pneumoperitoneum and a cystoscope to evaluate the peritoneal cavity of tuberculosis patients with ascites. Shortly thereafter, Dr. Bertram M. Bernheim of the Johns Hopkins Hospital reported a series of the first human laparoscopy performed in the United States, which he called *organoscopy*. A major breakthrough came with the introduction of the solid state video camera for laparoscopy in 1982. Today, operative laparoscopy is routinely used by gynecologists to perform a multitude of procedures, including hysterectomies, and for the diagnosis and treatment of gynecologic malignancies. In recent years, 3 innovations have been introduced or reintroduced into the field of laparoscopy:

1) robotic surgery [1],
2) natural orifice transluminal surgery [6],
3) single incision laparoscopic surgery (SILS). All 3 have their advantages and disadvantages compared to traditional laparoscopy. Of these 3 developing technologies, robotic surgery is having the largest impact on clinical care [1].

1. LPSC *vs*. robotic surgery - description

The adoption of conventional laparoscopy (CL) in gynaecological surgery has resulted in significant benefits for women and health providers in terms of fewer hospital admissions and a more rapid recovery and return to normal activity.

However, in complex pelvic procedures where greater precision is needed, CL has seen limited applications because of the restricted space and complex anatomy of the pelvis.

The introduction of a robot as an additional tool in laparoscopic procedures has overcome many of these limitations by providing superior dexterity, intuitive movement, 3D vision, improved ergonomics, autonomy of camera control and a shorter learning curve.

Compared to CL, robotic movements make possible to filter out tremor allowing for precise surgery. The 3D camera is secured and controlled by the surgeon providing a stable view and allowing for more exact dissection by enabling depth appreciation. To overcome lack of depth perception in 2D cameras, 3D laparoscopic cameras have been developed, but published data comparing 3D cameras and robotic-assisted laparoscopy (RAL) are sparse. Although the benefits of 3D cameras are well documented,

the surgeons' console vision in RAL might make the operative strains reported when using a 3D camera in CL, such as headache, dizziness and eye strain, less severe [2,3]. Another advantage of RAL is that instrument movement replicates movements of the surgeon's hands; this is in contrast to CL where hand and instrument movements are counterintuitive. The use of a third arm allows surgeon control in placing an instrument fixed in a secured place for safe assistance instead of requiring an assistant. The ergonomic advantages of RAL over CL have been reported using abdominal models representing a healthy weight range (BMI 18.5-24.9kg/m2) and those with obesity (WHO classification BMI 30 kg/m2 or over) [4]. The availability of a dual console enables collaboration and facilitates teaching. The disadvantages of RAL include lack of haptic feedback, the position of the surgeon away from the patient, and higher costs compared to CL. A structured training programme for robotic surgery is essential. Robotic surgical training programmes consisting of two components - generic robotic skills and specialty specific skills-have been developed to introduce RAL programmes safely [5].

A US study [6] of RAL for uterine cancer conducted in the period of 2008-15 in more than 35,000 women showed that RAL facilitated widespread adoption of minimal invasive surgery (MIS) and reduction of perioperative mortality without an increase in treatment costs. Future cost-effectiveness studies are needed to assess these findings in more high-volume robotic centres and using newly developed robotic platforms.

The higher costs of RAL remain a significant disadvantage, although it is important to consider that costs should decrease with time once surgical expertise increases (shortening operative time), high-volume robotic centres are introduced, hospital stays reduce and MIS increases, and the cost and time implications associated with laparotomy complications should also be considered [7]. A diverse range of robotic platforms are emerging with differing costs and set-up. Previous disadvantages of RAL, such as the need for de-docking if operating in the pelvis and upper abdomen, have been eliminated from newly developed platforms.

Patients expect an active and informed role in treatment decision-making, and healthcare providers have a duty to present choices without personal or institutional bias. A study of 18 examining patient perceptions of RAL acknowledged a general lack of understanding and the need for improved lay information [8].

Table 1. Recommendations and Conclusions for Robot-assisted surgery in Gynecology according to ACOG and SGS

The American College of Obstetricians and Gynecologists (ACOG) and Society of Gynecologic Surgeons (SGS) make the following recommendations and conclusions: - Studies suggest that robot-assisted gynecologic surgery can be performed safely in centers with experienced surgeons and has perioperative outcomes equivalent to laparoscopy and improved outcomes compared to laparotomy. - Robot-assisted cases should be selected based on the likelihood of improved outcomes compared to other surgical approaches due to the complexity of the case or patient factors, with appropriate consideration of costs.	- Robot-assisted surgery provides an alternative surgical tool for minimally invasive gynecologic surgery. Further comparative studies are needed to assess long-term outcomes and patient safety, and to identify specific subgroups of patients who would benefit from a robot-assisted approach. - As with any procedure, informed consent should be obtained from patients before surgery with discussion of the surgeon's experience with robot-assisted surgery, indications for surgery, and potential risks and benefits associated with the robot-assisted technique [8].

Table 2. Advantages of Laparoscopic and Robotic Surgery

Advantages of Laparoscopic Surgery	**Advantages of Robotic Surgery**
Reduces risk of injury during the procedure	Speed, accuracy, and control during the procedure.
Reduces pain during recovery	Enhanced range of motion
A shorter hospital stay	Greater precision as compared to laparoscopic surgery
Reduced use of anaesthetic drugs	Less expensive than traditional surgery
Reduced wound infections and complications	Minimizes postoperative complications
Less blood loss and faster recovery time	
Minimized pain and discomfort	
Helps patients to return to normal activities soon	
Better cosmetic results	

2. Robotic surgery *vs.* LPSC in gynecologic oncology

Role of robotic surgery in gynecologic cancer

In gynaecologic oncology, RAL hysterectomy is most commonly employed for endometrial cancer but also for cervical cancer and occasionally for restaging in early ovarian cancer.Women with endometrial cancer often present with a comorbidity, such as severe obesity, diabetes or hypertension.Other cancers have different challenges, mainly because of the technical difficulties associated with radical surgery. Robotic techniques have been developed for surgical procedures not regarded as readily amenable for CL, such as exenterative procedures for recurrent cancer.

Endometrial cancer

There is evidence from RCTs and a meta-analysis of RCTs that for endometrial cancer hysterectomy by CL is associated with a shorter hospital stay than laparotomy, reduced blood loss and fewer complications, with no apparent adverse effect on survival. However, CL can be more challenging in women with endometrial cancer who have a significant comorbidity, such as obesity or pulmonary problems. Several studies have shown that the introduction of robotics significantly reduced the number of open operations required by women with endometrial cancer and, therefore, the number of complications and overall costs were also reduced.

There are significant costs associated with the implementation of robotic surgery, which have been identified in two studies. However, it is vital to emphasize the benefits presented in the same papers, such as fewer repeat operations, medical complications and bladder injuries, and conversion to laparotomy. Subsequently, other papers examining the treatment of endometrial cancer have reported fewer complications and smaller conversion rates in RAL, and reduced blood loss compared to CL. A Danish study, including 5654 patients, evaluated the association between a nationwide introduction of RAL and survival in women with early-stage endometrial cancer. Improved survival and reduced risk of severe complications.

Cervical cancer

Over the past two decades, MIS techniques have gained widespread acceptance as an approach to radical hysterectomy for cervical cancer. A meta-analysis assessing RAL for radical hysterectomy in cervical cancer found fewer complications in RAL compared to open surgery. One study demonstrated that the introduction of robotics in the UK resulted in a reduced number of open radical hysterectomies. As a result, the introduction of robotics for cervical cancer surgery has become widely accepted, not just for hysterectomy but also for trachelectomy with a large meta-analysis including nine studies. In 2018, the publication of two studies resulted in the efficacy of minimally invasive radical hysterectomy for early stage cervical cancer to be questioned. They showed that in women who had MIS had lower disease-free and overall survival compared to those who underwent an open surgery. This was confirmed by the UK National Cancer Registration and Analysis Service and other studies. In light of these data, the European Society of Gynaecological Oncology (ESGO) recommen-

ded open surgery to be the gold standard for radical hysterectomy; also suggesting that all MIS procedures for cervical cancer should be prospectively recorded and only performed in a subgroup of women in highly specialised centres by appropriately trained surgeons. The National Institute for Health and Care Excellence guidance on minimally invasive radical hysterectomy recommends the evidence of efficacy for tumors smaller than 2 cm as inconclusive for disease-free and overall survival compared to open surgery, and therefore RAL for these patients should only be performed in the context of research. The LACC trial mainly explored the difference in overall survival and disease-free interval between CL and open radical hysterectomy with only a small percentage (16%) of RAL cases. A prospective multi-centre RCT comparing oncological safety of RAL and laparotomy for early-stage cervical cancer has been developed [9].

Robotic Surgery for Treatment of Cervical Cancer

A total of seven studies compared robotic radical hysterectomy with either laparoscopic hysterectomy or open radical hysterectomy [10,11]. In addition, we chose to include two more studies: one summary of 3 years of robotic radical hysterectomy experience and one prospective non-randomized phase II study [12].
Seven studies evaluated and compared operative times, length of post-surgical hospitalization, and estimated blood loss between robotic and open radical hysterectomy [10,11]. All studies presented similar results regarding significantly shorter length of post-surgical hospitalization after robotic surgery, ranging from 1 to 3.7 days for robotic-assisted procedures and 2.8 to 5 days for open surgery.
Three studies [11] found that robotic surgery required longer operative times (P<0.001). The two remaining studies [13] showed the exact opposite results regarding operative times, reporting that robotic surgery had shorter operative times (P=0.002). These inconsistencies are probably but not solely the result of surgeon's experience.
Cantrell *et al*. [13] evaluated the 3-year survival of patients who had undergone radical hysterectomy, whether robotic, laparoscopic, or open. No difference in overall survival was observed between the different groups. Recurrence was rare and similar between groups. Pelvic lymph nodes were dissected in 98% of patients, and were found to be positive for disease in 8.5%–10.9% of patients. The mean number of pelvic lymph nodes retrieved was higher in the minimally invasive group (19.4 *versus* 16.0, P<0.001). There was no difference in the rate of postoperative chemotherapy (P=0.32) or radiation therapy (P=0.28).
Gallotta *et al*. conducted a prospective non-randomized controlled trial (Canadian Task Force classification level 2) enrolling patients with stage IB2–III cervical cancer who underwent robotic radical hysterectomy plus pelvic and/or aortic lymph node dissection within 6 weeks after chemotherapy/ radiation therapy. Surgery feasibility and complications were analyzed. Pelvic lymph node dissection was performed in all cases. Robotic surgery was successful in 97.5% of cases. Median operative time was 185 min (range 100-330 min), and median estimated blood loss was 100 mL (range 50-300 mL). Median length of post-surgical hospitalization was 2 days (range 1-4 days). No intraoperative complications were recorded. During the observation period, 30% of patients had complications. Recurrence was documented in 12.5% of patients.

Robotic Surgery for Staging of Ovarian Cancer

Only one study published to date comparing robotic and laparoscopic approaches found no statistically significant difference between the two approaches with regard to final FIGO stage, histology, and tumor grade. In addition, 15.6% of patients were upstaged, with no statistically significant difference between the two groups. Median number of pelvic lymph nodes retrieved was 14 (range 3-42) and 11 (range 2-29) in the robotic and the laparoscopic groups (P=0.235), respectively. Median number of aortic lymph nodes retrieved was 11 (range 3-26) and 12 (range 1-39) in the robotic and the laparoscopic groups (P=0.263), respectively. Operative time was significantly shorter in the robotic group (P=0.043). Estimated blood loss was similar (P=0.691). No difference was found in terms of surgical complications [14].

OPINION (RCOG)

- Minimally invasive surgery in gynaecology should be promoted to decrease the large number of procedures still being completed via laparotomy.
- Evidence demonstrates the feasibility and safety of robotic technology in gynecology, and surgeons' preference would be to perform more complex operations with robotic assistance.
- Limitations of RAL remain to be higher costs; development of new platforms might change this.
- Surgical ergonomics are improved using RAL compared to CL, with fewer acute and long-term.
- WMS reported compared to CL or laparotomy.
- Introduction of RAL in gynaecologic surgery has resulted in a decreased conversion rate and laparotomy.
- For endometriosis, data indicate RAL is at least as good as CL, with a trend towards fewer complications associated with RAL.
- In urogynecologic surgery, RAL has shown at least the same or better postoperative results than CL.
- In endometrial cancer surgery, there is good evidence that introducing robotics into a service im-

proves operative outcomes for women and may provide cost savings.

- Studies looking at long term cost-effectiveness of RAL in gynecological surgery need to consider the complexity of the surgical procedure and women's comorbidities.
- Future research into patient perspectives and attitudes about robotic surgery as the field develops is recommended [14].

Conclusion

In light of these data, there were no significant differencies between robotic and laparoscopic surgery with regard to mortality and morbidity outcomes in the majority od studies. RS was frequently associated with longer operative times and higher overall costs, but many studies found potential benefits in postoperative recovery time. However, the decision whether robotic surgery should become a mainstream in gynecologic surgery or remain another surgical technique requires a few more randomized controlled clinical trials.

Conflict of interest statement. None declared.

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Review article

BRIEF REVIEW OF THE THROMBOTIC CEREBROVASCULAR COMPLICATIONS ASSOCIATED WITH NONSTEROID ANTI-INFLAMMATORY DRUGS IN PATIENTS WITH CEREBROVASCULAR DISEASES

КРАТОК ПРЕГЛЕД НА ТРОМБОТИЧНИ КОМПЛИКАЦИИ АСОЦИРАНИ СО УПОТРЕБАТА НА НЕСТЕРОИДНИТЕ АНТИ-ИНФЛАМАТОРНИ ЛЕКОВИ КАЈ ПАЦИЕНТИ СО ЦЕРЕБРОВАСКУЛАРНИ СОСТОЈБИ

Coskun Kerala[1], Anita Arsovska[1], Dragana Petrovska-Cvetkovska[1], Natalija Dolnenec-Baneva[1], Melda Emin[2], Katerina Tosheska Trajkovska[2] and Dragan Cibrev[1]

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Abstract

Introduction. The use of the commonly used nonsteroid anti-inflammatory medications can lead to specific complications in patients with cerebrovascular conditions. Their safety profile can be associated with both haemorrhagic and thrombotic complications, which can exert untoward influence in these patients. The aim of the present text is to 1) meaningfully recapture the main literature findings on the thrombotic complications due to use of NSAIDs in these patients and to 2) provide aid for physicians that encounter such decisions.
Inhibition of the cyclo-oxygenase as main effect. The use of these medications rely on a common mechanism that inhibits a specific set of enzymes called cyclooxygenase inhibitors. Here, we revisit the specific cascade that enables their effect and the important interplay that may occur in patients with cerebrovascular diseases, including medications that exert unspecific vs targeted inhibition of those specific enzymes.
Conclusion. The use of particular NSAIDs have been evaluated in patients with cerebrovascular diseases, and their specific risk for thrombotic complications in these patients have been examined in wide collection of patients. So far, these evaluations emphasize the association between the duration of use and type of selectivity of a particular NSAID and its risk for further thrombotic complications.

Keywords: cerebrovascular diseases, nonsteroid anti-inflammatory medications, haemorrhagic and thrombotic complications

Абстракт

Вовед. Употребата на нестероидни анти-инфламаторни лекови (НСАИЛи) кај пациенти со цереброваскуларни состојби може да произведе специфични компликации. Нивниот безбедносен профил се асоцира со можни хеморагички и тромботични компликации, кои предизвикуваат несакана еволуција на клиничкиот тек кај овие пациенти. Целта на актуелниот текст е да 1) ги заокружи последните сознанија од литературата на оваа конкретна тема и 2) да им помогне на лекарите кои се соочуваат со такви одлуки.
Механизми на остварување на ефектите. Употребата на овие медикации се потпира на заеднички механизам преку инхибиција на специфична класа на ензими наречена "инхибитори на цикло-оксигеназата". Тука, се навраќаме на специфичната каскада која го дозволува ефектот на овие лекарства и можните интеракции кои може да настанат кај пациентите со цереброваскуларни болести.
Заклучок. Употребата на конкретни НСАИЛи е евалуирана кај пациенти со цереброваскуларни болести во повеќе кохортни студии. Литературата посочува дека должината на користење на овие лекови и нивната селективност се асоцирани со одреден ризик за дополнителни тромботични компликации кај овие пациенти.

Клучни зборови: цереброваскуларни состојби, нестероидни анти-инфламаторни лекови, хеморагички и тромботични компликации

Introduction

Nonsteroid anti-inflammatory drugs (NSAIDs) are one of the most prevalent medications that can be given without prescription. Their pharmacokinetics properties

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are well described, and their wide range of use is associated with specific wanted and unwanted effects. One early description is the effects of the acetylsalicylic acid towards the renal function [1]. In this group of medications there are different drugs that exhibit effects over the cyclo-oxygenase (COX), specific enzymes that release important mediators for pain, inflammation, and maintenance of hemostasis. Although paracetamol and acetylsalicylic acid are often classified separately from the NSAIDs, both exhibit similar effects over this enzyme [2].

Gastrointestinal unwanted effects, such as gastric erosions upon use, motivated inquiry for developing similar medications with higher selectivity. After the development of the cyclo-oxygenase-2 (COX-2) inhibitors, it was indeed observed that these medications were associated with reduced rate for this specific unwanted effect, when compared to patients that used nonselective COX inhibitors [3]. Regardless, it became apparent that this specific class can be associated with other side effects, such as myocardial infarction. A meta-analysis from 2006 [4] was the first systematic review that demonstrated this association and motivated further study. The aim of our review is to summarize the findings from the latest literature, regarding the risks for cerebrovascular complications in patients with risks for cerebrovascular diseases, upon use of different NSAIDs.

Inhibition of the cyclo-oxygenase as main effect

COX are class of enzymes, also known as prostaglandin endoperoxide synthetase. These enzymes are present in many tissues, and the products of their activity are commonly labeled as prostanoids, a namesake that comes from their isolation from prostate of different animals. These enzymes have dimeric structure, and there are two iso-enzymes, cyclo-oxygenase-1 and cyclo-oxygenase-2. The first form is expressed in many tissues, including the thrombocytes, and it remains constitutionally active. The second form differs in that it requires specific stimulus to be activated. The function of these enzymes is related to the conversion of the arachidonic acid to prostaglandin H2. Prostaglandin H2 can be further converted to prostacyclines, which exhibit vasodilatation and it can inhibit the aggregation of the thrombocytes, or to thromboxane A2, which can maintain vasoconstriction and elicit the aggregation of the thrombocytes [5]. Although ubiquitous, these enzymes exhibit their activity mostly in the vascular walls, the gastrointestinal tract and in the kidneys. The NSAIDs can be classified further according to their selectivity for the two forms of the enzyme, and this classification is shown on figure 1 [6].

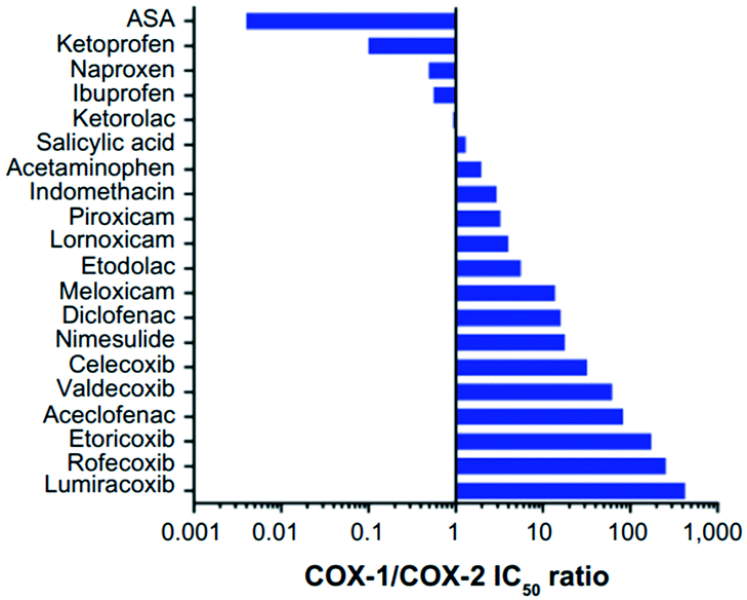

Fig. 1. Selectivity of different NSAIDs for COX-1/COX-2. Lower COX-1/COX-2 IC_{50} ratio would mean higher selectivity for COX-1 isoenzyme. Reproduced from [6] (https://creativecommons.org/licenses/by-nc/3.0/)

Patient groups of interest

The use of NSAIDs is not restricted, as many of them are over-the-counter medications. This group of medications is the most used for management of pain and inflammation in different clinical scenarios, but also in a situations which do not escalate to clinical scenarios, without the supervision of a physician [7]. For instance, a study from 2015 [8] which observed the use of these drugs in patients with chronic pain, shows that the use of these medications is with duration of 21 days in 97% of the patients. The use of these drugs is

separately examined in the elderly [9] in a study that exhibits its patterns of use.

Possible mechanisms of elevated risks for thrombotic events with the use of NSAIDs

There are multitude of mechanisms that can plausibly explain the associated risk of use of some NSAIDs with thrombotic complications. Given the fact that patients under risk for cerebrovascular diseases are already using some kind of therapy, are more likely to have additional comorbidities that require additional treatment, would mean that from the start there might be both pharmacokinetic and pharmacodynamic interactions that could lead to such unwanted effects.

Most highlighted mechanisms are those that come with the inhibition of the COX enzymes. The antiagreggation effect of the acetylsalicylic acid is exerted specifically by its inhibition of the COX enzymes. It inhibits the COX-1 completely, while its effects over COX-2 is shift in conversion of the arachidonic acid to 15-R-hydroxyeicosatetraenoic acid (15-R-HETE), instead to prostaglandin-2 (PHG2), leaving disabled pathways for the synthesis of thromboxane A2 [10]. The use of other NSAIDs might lead to imbalanced reduction in the generation of thromboxane A2 and the prostacyclines, where the latter opposes the vasoconstrictive effects of the first [11].

Another set of possible mechanisms is via their effect over the kidneys, especially in patients with arterial hypertension and alterations in their renin-angiotensin-aldosterone axis [12].

The use of acetylsalycilic acid has been investigated thoroughly for its effects over the kidneys and arterial blood pressure. The first published review on this topic [13] revealed that hypertensive patients that use NSAIDs have higher supine arterial blood pressure, when compared to patients that did not use those medications. Namely, inhibition of the COX enzymes is associated with reduced natriuresis and retainment of fluid, leading to elevated blood pressure. One such observation was made in a study that compared the effects of different doses of aspirin [14] in hypertensive patients treated with both aspirin (81 mg vs 300 mg) and enalapril, where it was found that patients that received the lesser dose of aspirin showed better outcome regarding their arterial blood pressure, an effect that at that time was demonstrated in animal studies as well. The effects of different NSAIDs have been confirmed, albeit with smaller estimates of effect, in participants that were substantially younger, in a prospective cohort from Spain [15].

The association between the use of these medications and the risk for thrombotic events has been investigated with regards to their duration of use, with some interesting results. For instance, it has been demonstrated that the use of rofecoxib is associated with elevated risk for thrombotic events during the first 60 days of use, while afterwards it slightly diminishes [16]. Oppositely, the longer use or the higher dose of etoricoxib or rofecoxib were indeed associated with elevation of the risks for thrombotic events [17], pointing out that although the mechanisms at play can be elucidated, each medication and patient profile warrants specific study.

Summaries from large investigations and reviews

First meta-analysis that documented the associations between use of different NSAIDs and the occurrence of acute myocardial or brain infarction or death associated with those conditions, from 2006 [4] used data from 145373 patients and 121 randomized controlled studies. It revealed that for 100 patients that were treated with COX-2 inhibitor (instead with unspecific COX inhibitor), there were 3 excess vascular events per year.

A study that examined the effects of different COX-inhibitors, based on the REACH registry [18], showed that the use of this medications in stable patients with known atherosclerotic condition, was associated with further risk of cerebrovascular pathology, even after adjustment for important confounders. Data from 22098 patients with mean age of 67.2 years, with duration of observation up to 2 years, showed that the risk for such complications is higher for 63%, when compared to patients that did not use these medications.

The largest investigation on this topic comes from the SOS project [19], a metanalysis of data from 4.5 million patients that used such medications. The study was looking for incident ischemic brain strokes, with almost 50000 patients that had these complications, and it reveals that the use of any NSAID increases the risk of such complication by 5%, when compared to patients that did not use NSAIDs, after adjusting for the effects of important confounders. From the group of COX-2 inhibitors, rofecoxib showed strongest association (OR 1.10 - 1.34). From the group of COX-1 inhibitors, the use of ketolorac was associated with 46% increase in risk. Other important mentions are the use of diclofenac (26% increase), indomethacin (24%), ibuprofen (15%), nimesulide (14%), piroxicam (14%). The same study revealed that these associations were slightly increased in younger patients, as well as in male patients. Additionally, the study did not find any association between the present of some rheumatological condition and the outcome, suggesting that the underlying inflammation cannot explain this excess in thrombotic events. The findings of this investigation are present in table 1 [19].

Table 1. Results from the SOS study for the associated OR for use of particular NSAID and the occurrence of ischemic brain stroke, with different duration of use of the investigated medication. Reproduced from [19] (https://creativecommons.org/licenses/by-nc/3.0/)

	Duration					
	Very short (0-6 days)		Medium (30-89 days)		Long (≥ 90 days)	
	Cases/Controls	OR (95% CI)	Cases/Controls	OR (95% CI)	Cases/Controls	OR (95% CI)
Diclofenac	419/24.412	1.41 (1.21-1.63)	435/34.922	1.03 (0.89-1.19)	224/15.645	1.24 (1.02-1.52)
Piroxicam	99/6.874	1.32 (0.89-1.97)	60/6.138	0.83 (0.50-1.39)	13/1.189	1.93 (0.54-6.97)
Meloxicam	29/2.597	0.90 (0.36-2.26)	93/8.578	1.03 (0.59-1.82)	67/6.411	2.16 (0.97-4.80)
Ibuprofen	231/14.924	1.31 (1.08-1.59)	228/18.178	0.97 (0.79-1.18)	152/8.500	1.17 (0.89-1.54)
Naproxen	23/2.269	1.30 (0.55-3.11)	87/7.206	1.67 (0.91-3.08)	53/4.406	1.00 (0.46-2.18)
Ketoprofen	80/6.762	1.23 (0.79-1.94)	40/3.678	0.91 (0.49-1.68)	13/1.413	1.30 (0.47-3.64)
Celecoxib	52/4.648	1.19 (0.77-1.83)	163/15.988	1.08 (0.80-1.44)	123/10,398	1.04 (0.72-1.51)
Rofecoxib	44/3.003	1.47 (0.97-2.23)	128/10.362	0.99 (0.73-1.34)	91/6.536	1.03 (0.70-1.50)
Etoricoxib	32/1.855	2.33 (1.04-5.24)	73/6.715	1.94 (1.01-3.74)	40/3.230	1.89 (0.78-4.56)
Nimesulide	143/11.231	1.08 (0.86-1.35)	176/12.050	1.21 (0.98-1.50)	16/776	1.30 (0.71-2.40)

Conclusion

The use of NSAIDs is common, and many patients with pain management issues use it for a duration beyond 30 days. The use of some specific drugs can be associated with elevated risk for thrombotic cerebrovascular complications, while others elevate the risk for hemorrhagic consequences. Although the selectivity of these drugs suggests that the drugs that are selective for COX-2 are associated with less hemorrhagic complications, while those that are less selective, are associated with less thrombotic complications, each combination of drug - patient - comorbid conditions warrant careful consideration. The latest metanalysis on this subject suggests that use of naproxene was not associated with excess thrombotic events after 90 days use, when compared to other medications of this type. Patients that are younger or males have slightly increased risk for this unwanted association. The choice of NSAIDs in these scenarios also depends on its intended duration of use, and it requires an approach that is tailored to the patient's needs.

Conflict of interest statement. None declared.

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Review article

GLAUCOMA AND OCULAR VASCULAR PATHOLOGY

ГЛАУКОМ И ОКУЛАРНА ВАСКУЛАРНА ПАТОЛОГИЈА

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Abstract

Introduction. There is already postulated evidence of the role of vascular factors in the pathogenesis of glaucomatous neurodegeneration, while it is indisputable that glaucoma induces structural and functional changes in retinal blood vessels, indicating a bidirectional relationship between vascular disorders and glaucoma.
The aim of this study was to explore published evidence in order to clarify the relationship between glaucoma and vascular disorders and to provide an overview of the "chicken or egg" dilemma of these seemingly unrelated pathologies.
Methods. A systematic search of the Medline and PubMed medical databases was made using the key words glaucoma, and vascular abnormalities. We found more than 60 articles related to our search in the last 10 years, of which 33 were analyzed in this review. We present the most important findings from the literature, as well as our opinions and considerations on the topic.
Conclusion. Glaucoma, as a pathology leading to a progressive visual loss, is still inadequately understood, and thus treatment options are limited. Evidence of bidirectional association between vascular diseases and glaucoma further complicates treatment and expectations, given the age of patients and the percentage of comorbidities they carry. However, by investigating new biomarkers and the possibility of multidisciplinary approaches to this disease, there is hope for new therapeutic options in the future. In any case, the treatment of glaucoma should not be only ocular, but systemic, aimed at treating these systemic comorbidities as well.

Keywords: glaucoma, vascular abnormalities, vascular occlusions, glaucoma pathogenesis

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Абстракт

Вовед. Веќе се постулирани докази за улогата на васкуларните фактори во патогенезата на глаукоматозната невродегенерација, а од друга страна непоборлив е фактот дека глаукомот предизвикува и структурни и функционални промени на крвните садови на мрежницата, односно дека врската помеѓу васкуларните заболувања и глаукомот е двонасочна.
Целта на овој труд е да ги истражи објавените докази за да се разјасни поврзаноста помеѓу глаукомот и васкуларните заболувања и да направи преглед на дилемата „кокошка или јајце“ на овие две навидум неповрзани патологии.
Методи. Ги пребаравме главните бази на податоци на медицинските публикации Medline и Pubmed, внесувајќи клучни зборови: глауком, васкуларни абнормалности. Добивме повеќе од 60 статии поврзани со нашето пребарување во последните 10 години, од кои 33 се искористени за овој преглед. Во овој прегледен труд ги презентиравме најважните сознанија од литературата, како и нашите мислења и согледувања на темата.
Заклучок. Глаукомот како патологија која води до прогресивно намалување на видот за жал е сеуште недоволно разјаснета, а со тоа и можностите за третман се ограничени. Доказите дека васкуларните болести и глаукомот се поврзани двонасочно, од една страна дополнително го отежнува третманот и очекувањата од истиот, со оглед на возраста на пациентите и процентот на коморбидитети кои го носат, но од друга страна, преку истражување на новите биомаркери и можноста мултидисциплинарно да се пристапи на оваа болест, остава простор за надеж дека во иднина ќе имаме нови терапевтски можности. Во секој случај, лекувањето на глаукомот не треба да биде само окуларен пристап, туку системски, насочен кон лекување и на овие системски коморбидитети.

Клучни зборови: глауком, васкуларни абнормалности, васкуларни оклузии, патогенеза на глауком

Introduction

Glaucoma is a leading cause of irreversible blindness worldwide. While intraocular pressure (IOP) represents a major risk factor, the underlying pathophysiology remains largely unclear. It is characterized by progressive death of retinal ganglion cells and subsequent functional damage to the visual field. However, despite extensive research efforts, the precise pathogenesis of glaucoma remains insufficiently understood [1].

The correlation between vascular abnormalities and glaucoma has been deliberated for decades. The vascular theory in glaucomatous neurodegeneration postulates that decreased perfusion pressure, impaired vascular autoregulation, and disrupted neurovascular coupling serve as fundamental mechanisms. These factors collectively contribute to a progressive degeneration of the optic nerve and retinal ganglion cells in glaucoma [1,2].

Regarding hemodynamics, a notable association between glaucoma and systemic cardiovascular diseases such as diabetes mellitus, high blood pressure, and low blood pressure has been documented, indicating an increased incidence of glaucoma in affected individuals. These observations highlight the potential influence of hemodynamic factors on the development and progression of glaucoma. Abnormal vascular function may also affect the secretion and drainage of aqueous humor, thereby influencing IOP regulation. Elevated IOP resulting from vascular abnormalities may compress retinal ganglion cells and their axons, as well as reduce retinal blood supply. Consequently, these factors contribute to the pathogenesis and progression of glaucoma [3-5].

On the other hand, retinal vascular dysfunction is reported as secondary to glaucoma, indicating a bidirectional relationship between glaucoma and retinal vascular pathology. These secondary manifestations of glaucoma include reduced diameter of retinal blood vessels, decreased number of blood vessels, changes in retinal vascular molecules, impaired autoregulation, arteriolar dysfunction in the retina, and even occurrences of retinal vein occlusions [6,7].

It is believed that the high oxygen demands of the retina and the relatively sparse nature of the retinal vasculature are responsible for the retina's particular vulnerability to vascular diseases. This suggests that neurons in the inner retina, such as retinal ganglion cells, are susceptible to limited internal blood supply in retinal diseases such as glaucoma and diabetic retinopathy. Animal studies have shown that bilateral occlusion of the common carotid arteries, leading to retinal hypoperfusion, significantly reduces the density of retinal ganglion cells in elderly individuals due to apoptosis [6-8].

How do vascular abnormalities affect the development and progression of glaucoma?

The fact that there is an increased incidence of glaucoma in patients with systemic cardiovascular diseases, such as diabetes mellitus and high blood pressure, indicates that there is a correlation between these conditions.

In terms of hemodynamics, an increased incidence of glaucoma has been observed in patients with systemic cardiovascular diseases, such as diabetes mellitus and high blood pressure [3].

On one hand, clinical studies show that individuals with hypertension have a greater risk of developing glaucoma, with more pronounced damage to the optic nerve in patients with hypertension compared to those with normal blood pressure. On the other hand, systemic hypotension and nocturnal drops in blood pressure are also associated with an increased risk of glaucoma. The association between blood pressure and nerve fiber damage is mediated by reduced blood flow, increased vascular resistance, or lack of autoregulatory reserve. Therefore, controlling blood pressure in patients with concomitant systemic hypertension and glaucoma is important. It is necessary to avoid excessive nocturnal blood pressure reduction and to monitor potential damage to retinal neurons due to nocturnal hypotension induced by hypertension treatment [3,4].

In the Barbados Eye Study, individuals with low average ocular perfusion pressure (OPP-the difference between systemic blood pressure and intraocular pressure) showed a 2.6-fold risk ratio for incident open-angle glaucoma (OAG) over a 9-year follow-up period. In two studies (Singapore Malay Eye Study, Los Angeles Latino Eye Study), a direct relationship was found between reduced OPP and increased prevalence of OAG. It has also been demonstrated that even healthy optic nerves with normal autoregulation are prone to hypoperfusion with minor fluctuations in blood pressure or intraocular pressure. However, in pathological conditions such as diabetes mellitus and hypercholesterolemia, where autoregulation in the optic nerve head is significantly impaired, there is significant impaired perfusion [9,10].

It has been found that endothelin-1 (ET-1), a key regulator of vascular autoregulation, is overexpressed in the retina of diabetics, and increased expression of ET-1 has also been observed in the plasma of patients with high blood pressure. Therefore, excessive expression of ET-1 may play a role in disrupted autoregulation of retinal blood vessels. Furthermore, increased expression of ET-1 is a biomarker indicating retinal vascular dysfunction, which is another potential mechanism contributing to reduced retinal blood flow. This hypothesis is supported by the finding that an ET-1 blocker can increase blood flow in the retina. Clinical studies have reported that glaucoma patients have higher le-

vels of plasma ET-1 compared to healthy controls, and patients with advanced visual field changes have higher levels of ET-1 in plasma than those with normal visual fields [9,10].

Nitric oxide is generated by neuronal nitric oxide synthase and endothelial nitric oxide synthase in a Ca2+/calmodulin-dependent manner. Neuronal nitric oxide synthase is predominantly expressed in the ciliary processes and nerve terminals, with high expression in the anterior segment of the eye, such as the ciliary muscle, trabecular meshwork, Schlemm's canal, and collector channels. These structures are crucial for regulating aqueous humor dynamics and IOP homeostasis in both physiological and pathological conditions. Ocular hypertensive patients have decreased nitric oxide formation compared to healthy individuals, and it has been shown that administration of exogenous nitric oxide reduces IOP in these patients. On the other hand, there is an imbalance between the vasoconstrictor ET-1 and the vasodilator nitric oxide in glaucoma patients, leading to endothelial dysfunction and reduced ocular blood flow. Nitric oxide is a molecule that dilates blood vessels and is believed to be involved in regulating ocular blood flow in glaucoma. Several studies have shown that the availability of nitric oxide is decreased in glaucoma, leading to a shift in the balance between vasoconstriction and vasodilation and resulting in reduced blood flow to the optic nerve head. Collectively, ET-1 and nitric oxide may serve as reliable biomarkers in predicting the progression of glaucoma and/or targeting pharmacological intervention from a vascular perspective [9,10].

It has been proven that glaucoma involves disrupted neurovascular coupling (also called functional hyperemia), especially in patients with systemic circulatory disorders, such as diabetes mellitus. Specifically, under conditions of increased demand from nerve cells, blood flow in that area increases, a phenomenon known as neurovascular coupling (NVC). Experiments with flickering light at the level of ONH blood flow have shown that vasodilation in response to flickering light and vasoconstriction in response to hyperoxia are attenuated. Ischemia/hypoxia/perfusion instability affects astrocytes in ONH and/or mitochondria in RGC axons, resulting in neurotoxic effects on RGCs [10].

Abnormal vascular function can also affect the secretion and drainage of aqueous humor, leading to increased IOP and contributing to the pathogenesis and progression of glaucoma [8].

On the other hand, these systemic diseases also lead to changes in the trabecular meshwork, where under conditions of high glucose, synthesis of extracellular matrix components including fibronectin is significantly increased, resulting in increased resistance to aqueous humor outflow through the trabeculum, thus increasing IOP [11].

Arterial hypertension is another vascular disease that can increase IOP by raising blood pressure in ciliary capillaries, leading to an increased aqueous humor secretion. High blood pressure can also increase episcleral venous pressure (EVP), which can increase resistance to aqueous humor drainage and further contribute to increased IOP [12].

Another vascular abnormality that can lead to increased IOP is hyperlipidemia. Studies have shown that hyperlipidemic patients are at increased risk of glaucoma, potentially due to increased blood viscosity, which in turn increases EVP. Additionally, a lower incidence of glaucoma has been observed in patients receiving therapy for hyperlipidemia [11,12].

Matrix metalloproteinases (MMPs) are enzymes that play a role in degrading proteins from the extracellular matrix and are believed to contribute to changes in IOP in glaucoma. The trabecular meshwork generates the main resistance to aqueous humor outflow, and its extracellular matrix is constantly remodeled by MMPs. Increasing MMP activity has been shown to increase the rate of outflow, while inhibiting MMP activity leads to a decrease in the rate of aqueous humor outflow. A recent study using a porcine model reported similar results, showing that decreased activity of MMP-2 and -9 is associated with elevated IOP [12,13].

Together, biomolecules such as VEGF, nitric oxide, and MMPs hold promise as potential biomarkers for predicting the progression of glaucoma and as targets for pharmacological intervention [12,13].

How does glaucoma affect the occurrence of retinal vascular abnormalities?

Several studies have already shown that glaucoma poses a risk for the development of retinal vein occlusions, and the reasons seem to be changes occurring at the level of the lamina cribrosa, which subsequently influence on the flow of veins passing through the optic nerve head [14-16].

Retinal vascular dysfunctions are observed as secondary manifestations of glaucoma. These secondary manifestations include various changes, such as a significantly decreased vascular diameter of the retina, reduced vascular density of the retina, disturbances in retinal vascular molecules, impaired autoregulation of blood flow, dysfunction of retinal arterioles, and cases of retinal vein occlusion [17,18].

A study conducted on individuals with early normal-tension glaucoma showed no significant differences in the diameters of retinal arteries compared to control groups. This discovery suggests that the narrowing of retinal arterioles observed in glaucoma patients is a consequence of the disease (elevated IOP), rather than its underlying cause [19].

In another study involving mice, acute elevation of IOP resulted in decreased total retinal blood flow and average lumen of retinal blood vessels. One proposed mechanism underlying glaucomatous conditions suggests that compromised RGCs demand reduced blood supply, subsequently causing retinal arteriolar constriction through the autoregulation process. This mechanism appears plausible, as constriction of retinal blood vessels has been observed not only in glaucoma but also in other diseases involving optic nerve damage [20].

In another experiment with induced high IOP in the anterior chamber, increased expression of beta-3 tubulin (a neuro-specific marker) was observed in pericytes and endothelial cells of blood vessels, which was associated with vascular remodeling, as well as increased oxidative stress, leading to endothelial dysfunction and impaired autoregulation.

In a case series study, it was noticed that patients with POAG had a significant decrease in the density of parapapillary blood vessels, which increased after IOP normalization. A study was also conducted on mice, where sodium hyaluronate was injected into the anterior chamber to transiently increase IOP, resulting in a significant decrease in the number of capillary blood vessels in both the superficial and intermediate vascular plexuses of the retina. Patients with acute angle-closure glaucoma showed a reduction in the number of blood vessels, which increased after IOP normalization, but still remained significantly smaller compared to the eye that did not have elevated IOP. Beta-3 tubulin, a neuron-specific marker found in endothelial cells and pericytes, is considered the main factor in this vascular remodeling [21-23].

Discussion

Multiple studies have reported a significant association between glaucoma, specifically open-angle glaucoma, and increased incidence of retinal vascular occlusions. In a comprehensive Korean population-based retrospective study, individuals diagnosed with open-angle glaucoma showed a significantly higher incidence of retinal vascular occlusions compared to the general population. A case-control retrospective study conducted by Schreiber *et al.* (2018) found that glaucoma was a risk factor for incident retinal vascular occlusions with an odds ratio of 6.19 ($p < 0.001$). However, some studies have not found this association. A meta-analysis by Yin *et al.* (2019) reported that glaucoma was a risk factor for retinal vascular occlusions with an odds ratio of 4.01. Subgroup analysis within the study showed that glaucoma was significantly associated with various types of retinal vascular occlusions, including central retinal vein occlusion, branch retinal vein occlusion, and hemicentral retinal vein occlusion [24,25].

In a study tracking the frequency of glaucoma among patients with retinal vein occlusions (RVO), it was demonstrated that the frequency of glaucoma in patients with RVO (18.91%) was much higher than that in the general population (2.66%), indicating that glaucoma may be a risk factor for the occurrence of retinal vein occlusions [24,25].

In another study conducted from September 2020 to September 2022, 111 eyes of 111 patients with unilateral CRVO or BRVO were investigated, of which 21 patients had previously had glaucoma, and 12 developed neovascular glaucoma as a complication. Patients were followed for 12 months. In conclusion, it was found that elevated intraocular pressure, even within normal ranges, may be a trigger for venous occlusions, associated with reduced blood flow or poor circulation. A statistically significant increase in IOP was recorded in affected patients compared to those without RVO. The cup-to-disc ratio was higher in patients with RVO than in those without, indicating an association between these entities, although the exact mechanism still cannot be explained [25].

Regarding retinal occlusions, the pathogenic mechanism remains incompletely understood. Throughout history, this entity has been called "retinal apoplexy", "hemorrhagic retinitis", "venous stasis", "central retinal vein occlusion", and so on. Subsequently, the terms ischemic and non-ischemic retinal vein occlusion were introduced.

Furthermore, the question arises whether a thrombus exists in central retinal vein occlusion. In 1878, von Michel documented two cases of venous thrombosis, one showing proliferation of the vessel wall, and the other was with thrombus formation in the lumen. Verhoeff treated 39 cases of central retinal vein occlusion, of which only two showed evidence of thrombosis. His belief was that the blockage occurred due to dissection of the venous channel through thickening of the intima of the vessel wall, a finding he also detected in patients with glaucoma [26,27].

There is a popular theory that elevated intraocular pressure causes external compression on the central retinal vein as it passes through the lamina cribrosa. This results in turbulent blood flow distal to the vein constriction, subsequently leading to thrombus formation. Although an association between CRVO and elevated intraocular pressure has been demonstrated, turbulent flow has not been proven by Doppler [28].

CRVO is detected in 4 to 4.5% of eyes with open-angle glaucoma, while POAG or ocular hypertension is detected in 4 to 43% of patients with CRVO. Although initially there is a decrease in IOP after occlusion, increased IOP was measured compared to control groups after several weeks [29].

Pseudoexfoliative syndrome (PEX) is a common age-related disorder that affects both intraocular and extra-

ocular tissues. In patients with PEX, dysregulation of lysyl oxidase-like 1 (LOXL1) expression has been discovered to be significantly altered. This may negatively impact elastin metabolism and lead to elastic tissue changes such as lamina cribrosa. There is increasing evidence that cellular stress conditions and low-grade chronic inflammatory processes are involved in the pathogenesis of PEX. Although there is an increased risk of developing glaucoma in patients with PEX and ocular hypertension compared to patients without PEX with ocular hypertension, single-nucleotide polymorphisms in LOXL1 have not been associated with differences in intraocular pressure (IOP). Despite the high prevalence of LOXL1 variants in the general population, only a small portion of the population develops PEX, suggesting that besides LOXL1, other genetic, epigenetic, and environmental factors may contribute to the development of PEX. Additionally, LOXL1 cannot help identify those with PEX at increased risk of developing glaucoma. The increased risk of glaucoma development in PEX patients with increased IOP may be associated with factors outside of IOP, contributing to increased vulnerability of the optic nerve to glaucoma development in the presence of PEX [30].

Pseudoexfoliative syndrome is a common cause of open-angle glaucoma characterized by elastic microfibrilopathy induced by stress associated with matrix metalloproteinase accumulation. Accumulation of matrix metalloproteinases increases protein substance deposition in ocular structures and other organs including the heart. Many studies link the presence of cardiovascular diseases with pseudoexfoliation syndrome, but there is considerable debate among studies regarding significant associations. Associated diseases include myocardial infarction, ischemic heart disease, angina, congestive heart failure, cardiomyopathy, aortic aneurysm, hypertension, and homocystinuria. According to literature review, the association between ischemic heart disease and PEX was statistically significant ($p=0.045$). Myocardial infarction, chronic ischemic heart disease, angina, and hypertension did not show correlation with the presence of pseudoexfoliation. Patients with PEX are prone to ischemic heart disease, abdominal aortic aneurysms, and homocystinuria despite not showing correlation with myocardial infarction, chronic ischemic heart disease, angina, and hypertension [31].

PEX is a well-recognized disease with a gradual onset caused by generalized fibrilopathy. It is associated with a wide range of ocular complications, including glaucoma and perioperative problems during cataract surgery. Besides the well-known intraocular manifestations, deposits of PEX have been found in various extraocular locations and seem to represent a systemic process associated with an increased cardiovascular and cerebrovascular morbidity. However, because published results are inconsistent, the clinical significance of extraocular PEX deposits remains controversial. Identification of PEX deposits in the heart and blood vessel walls, epidemiological studies, as well as similarities in pathogenetic mechanisms, have led to a hypothesis of a possible association between fibrillar material and cardiovascular diseases. Recent studies suggest that PEX syndrome is often associated with impaired heart and blood vessel function. Systemic and ocular changes in blood flow, altered parasympathetic vascular control and baroreflex sensitivity, increased vascular resistance and decreased blood flow rate, arterial endothelial dysfunction, high plasma homocysteine levels, and arterial hypertension have been demonstrated in PEX subjects. Common characteristics in the pathogenesis of atherosclerosis and PEX, such as oxidative stress and inflammation, and possibly a higher frequency of abdominal aortic aneurysm in patients with PEX, may imply that these gray-white deposits and cardiovascular disturbances are related or reflect different manifestations of the same process [32].

In a study involving patients with central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO), a correlation between pseudoexfoliative syndrome (PEX) and vascular occlusions was demonstrated. It was also investigated in patients with pseudoexfoliative syndrome but normal intraocular pressure (IOP). Among CRVO patients, 29.17% had PEX, and among BRVO patients, 8.7% had PEX. The conclusion was that PEX may act as an independent factor from glaucoma in causing retinal vascular occlusions [33].

In another study, the association between PEX and pre-existing glaucoma in patients with CRVO was explored. The results showed that 20.8% of CRVO patients had PEX, and 18.9% had glaucoma. The conclusion was that there was a statistically significant difference in the frequency of PEX and glaucoma in CRVO patients compared to the control group [34].

Research on glaucoma is aimed at opening up new therapeutic possibilities, indicating that the approach must be multidisciplinary and directed towards these systemic comorbidities. Therefore, consideration is being given to introducing systemic therapy in glaucoma treatment [35].

Retinal ganglion cells (RGCs) are highly sensitive to metabolic fluctuations resulting from various stress factors, and thus their sustainability depends on healthy mitochondrial functioning. Metformin, known for its use in type 2 diabetes, has come to the forefront of medical research in multiple organ systems. Its recent use has been associated with a 25% reduced risk of glaucoma in a large population study. Its application in glaucoma therapy is being considered, highlighting its effects on fibrotic signaling pathways, mitochondrial

bioenergetics, and NAD (Nicotinamide Adenine Dinucleotide) oxidation [35].

Conclusion

Glaucoma, as a pathology leading to progressive vision loss, remains insufficiently understood, and therefore treatment options are limited. Evidence of bidirectional association between vascular diseases and glaucoma further complicates treatment and expectations, considering the age of patients and the percentage of comorbidities they carry. However, by investigating new biomarkers and the possibility of multidisciplinary approaches to this disease, there is hope for future new therapeutic options. In any case, glaucoma treatment should not be solely ocular-focused but systemic, targeting treatment of these systemic comorbidities as well.

Conflict of interest statement. None declared.

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Original article

CHEMICAL ABLATION AS SUCCESSFUL ALTERNATIVE FOR TREATMENT OF INCOMPETENT PERFORATORS

ХЕМИСКА АБЛАЦИЈА КАКО УСПЕШНА АЛТЕРНАТИВА ЗА ТРЕТИРАЊЕ НА ИНКОМПЕТЕНТНИ ПЕРФОРАНТНИ ВЕНИ

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Abstract

Introduction. Superficial venous incompetence (SVI) is the most common cause of lower extremity superficial venous reflux and varicose veins; nonetheless, incompetent perforator veins (PVs) are the most common cause of recurrent varicose veins after treatment, often unrecognized. Current minimally invasive treatment options include ultrasound-guided sclerotherapy (USGS), endovascular thermal ablation (EVTA) with either laser or radiofrequency energy sources, subfascial endoscopic perforator surgery (SEPS) and the relatively new chemical ablation procedure using cyanoacrylate adhesive, which we chose as our primary treatment option for this study, using and comparing the results of two different adhesives.

Methods. A retrospective review of a prospectively managed database of chemical ablation as perforator vein treatment performed at a single institution from September 2023 to March 2024 was conducted. The indications for PV treatment were >4 mm in diameter and reflux of >500 milliseconds upon leg compression.

Results. A total of 32 patients and 49 limbs presenting PV insufficiency (coexisting with GSV insufficiency in 19 patients) were divided into 2 groups of 16 patients, each group based on the chosen chemical ablation adhesive - VenaBlock and VenaSeal. The VenaBlock group had PV closure rate of 100% immediately intraoperative, at 3 days, 2 weeks, 3 weeks and 1 month from the procedure for each treater perforator. From the VenaSeal group, 13 patients had immediate and continuous treatment success during the follow-up, while in 3 patients there was intraoperatively registered treatment failure (P=0.0127).

Conclusion. We find the chemical ablation procedure to be safe and effective for PVs, specifically in the case of using rapid polymerization adhesive. Due to its simplicity and short procedural time, we consider this to be the procedure of choice in case of multiple incompetent PVs present, as well as in significant PV tortuosity.

Keywords: superficial venous incompetence, perforating veins, chemical ablation, VenaSeal, VenaBlock

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Апстракт

Вовед. Површната венска инсуфициенција е најчестата причина за површниот венски рефлукс на долните екстремитети и проширените вени, додека некомпетентните перфораторни вени се најчестата причина за рецидивни проширени вени по третманот, коишто често остануваат неидентификувани. Современите минимално инвазивни опции за третман вклучуваат ултразвучно водена склеротерапија (USGS), ендоваскуларна термална аблација (EVTA) со ласерски или радиофреквентни енергетски извори, ендоскопска субфасциална перфораторна лигација (SEPS) и релативно новата процедура на хемиска аблација, којашто ја избравме како примарна опција на третман во оваа студија, како и употребата на две различни лепила (slow polymerizing vs fast polymeryzing) и споредбата на резултатите меѓу нив.

Методи. Во оваа ретроспективо-проспективна студија во нашата институција во периодот од септември 2023 до март 2024 беа третирани 32 пациенти со хемиска аблација, поради инкомпететни перфорантни вени. Индикациите за третманот вклучуваат: перфоратор со дијаметар над 4мм и рефлукс над >500 милисекунди.

Резултати. Вкупно 32 пациенти или 49 екстремитети кои се презентираат со инсуфициенција на перфоратори, беа поделени во 2 групи од по 16 пациенти врз основа на избраниот атхезив- брзоделувачки (Венаблок) или спороделувачки (Венасил). Кај 19 пациенти беше регистрирана коегзистирачка инсуфициенција на ВСМ. Групата Венаблок имаше стапка на затворање од 100% веднаш интраоперативно, по, 3 дена, 2 недели, 1 месец и 2 месеца од процедурата, за секој третиран перфоратор. Од групата Венасил, 13 пациенти беа успешно третирани, додека кај 3 пациента беше констатиран неуспех при првата апликација, поради што се наложи повторна апликација на лепило (P=0.0127)
Заклучок. Ние сметаме дека процедурата на хемиска аблација со цианоакрилат е безбедна и ефикасна за затварање на инсуфициентни перфорантни вени, посебно кога се користи асстхезив со брза полимеризација. Поради едноставноста и брзината на процедурата, сметаме дека ова е процедура на избор во случај на присутни повеќе некомпетентни перфоратори, како и при изразен тортуозитет.

Клучни зборови: површна венска инсуфициенција, перфорантни вени, хемиска аблација, Venoseal процедура, VenaBlock

Introduction

One of the most frequently reported health problems worldwide is the chronic venous insufficiency (CVI) and venous ulceration, resulting in a significant patient morbidity. Aside from the chronic physical disability caused to the patient and its subsequent psychological effect, it also generates a substantial economic impact to the health care administration. Global prevalence rates of CVI are variable but may be as high as 40% among females and 17% among males [1]. This variation in global prevalence is due to the wide variability in reporting, diagnosis and risk factors. Nevertheless, its morbidity and health care economic burden remain universal.

In his landmark publication, as early as 1917, Homan described the pathophysiology of CVI caused by superficial and deep venous incompetence along with the importance of perforator vein incompetence (PVI) in the development of venous ulcerations [2]. The importance of PVI in the manifestation of CVI and ulceration has since been well-acknowledged and widely studied. However, while the role of definitive management for junctional and truncal venous reflux in symptomatic CVI is well-established, the exact indications for management of PVI in isolation remains, in some part, unclear.

The current recommendation by the guidelines of the American Vascular Society is to treat the PV in cases of CEAP score 5 and 6, with treatment of the perforator at the level of previous or active venous ulceration [3]. Several authors also suggest treating incompetent perforator veins in cases of focal pain, focal swelling, associated varicose veins, focal skin irritation and/or discoloration in the area of the incompetent perforator vein [4,5]. Nevertheless, there is growing consensus that perforators which are >4 mm in diameter and show reflux of >500 milliseconds upon leg compression should be categorized as incompetent [6,7], and those are the parameters which we have adopted in our practice.

Minimally invasive treatments have replaced traditional surgical treatments for incompetent perforator veins. Current minimally invasive treatment options include ultrasound-guided sclerotherapy (USGS), endovascular thermal ablation (EVTA) with either laser or radiofrequency energy sources, subfascial endoscopic perforator surgery (SEPS) and the relatively new chemical ablation procedure using cyanoacrylate adhesives. Advantages and disadvantages of each modality and knowledge on these treatments are required to adequately address perforator venous disease.

Cyanoacrylate chemical ablation is a relatively new treatment for treating varicose veins. This procedure introduces a resilient glue into the large veins through a small catheter via the Seldinger technique or through small incision. Upon contact with blood, the adhesive begins to bond with the intima and compression is applied to close the vein. The adhesive is designed to remain permanently in the diseased vein and is encapsulated by chronic fibrosis.

While there are multiple articles reporting results of the VenaSeal procedure for truncal insufficiency, such literature remains deficient regarding the PVs.

Chemical ablation has the advantage of not requiring anesthesia before treatment and has been found to be very effective for closing the large saphenous veins. It delivers immediate and lasting vein closure with its proprietary medical adhesive formula, with a demonstrated 94.6% closure rate used for the GSV at 5 years [8-12].

In September 2023 we were presented with an alternative product: the short-chain obliterating agent named VenaBlock (Invamed Saglic Ilac A.S., Ankara, Turkey), which is characterized by its low viscosity and fast polymerization. Given these attributes, which would theoretically make it an excellent choice of treatment of PVS, we have decided to include it in our PV closure procedure and compare it against Venaseal - slow polymerizing long-chained cyanoacrylate, which has been in the market since 2011.

Materials and methods

A retrospective review of a prospectively managed database of chemical ablation of perforator vein performed at a single institution from September 2023 to March 2024 was conducted. The main inclusion criteria for PV treatment were >4 mm in diameter and reflux of >500 milliseconds upon calf compression. A Duplex scan was performed at 3 days, 2 weeks, 1 month and 2 months after the procedure. Standard statistical methods were used to compare subgroup characteristics.

Results

A total of 32 patients and 49 limbs presenting PV insufficiency coexisting with GSV insufficiency in 19 patients (which we treated concomitantly by RFA of the subfascial GSV and VenaSeal of the distal portion of GSV in the same act), were divided into 2 groups of 16 patients (25 limbs and 24 limbs respectively), each based on the chosen adhesive kit. Each group had 2 further subgroups, solely based on the PV diameter: subgroup A diameter 3.5-5 mm, subgroup B >5 mm.

Table 1. Patient population

Variable	VenaBlock (n=16)	VenaSeal (n=16)
Age	43±14.4	48±11.2
BMI	28.2±9.5	30.4±11.8 kg/m^2
Comorbidities		
Mild hypertension	4(25%)	3(18.75%)
Hashimoto disease	2(12.5)	1(6.25%)
Clinical stage CEAP		
2-4	12(75%)	16(100%)
5	3(18.75%)	
6	1(6.25%)	
Concomitant GSV insufficiency	10 (62.5%)	9(56.25%)
Diameter of the treated PV		
Subgroup A 3.5-5 mm	9(56.25%)	10(62.5%)
Subgroup B >5 mm (5-7 mm)	7(43.75%)	6(37.5%)
Length of the treated PV	1.9±0.56 mm	1.8±0.47 mm

Each of the 16 patients in the VenaSeal group were in CEAP stage 2-4, while 12 patients in the VenoBlock group were in CEAP stage 2-4, 3 in CEAP stage 5 and 1 in CEAP 6. The average age of the VenaSeal group and the VenaBlock group were 43±14.4 and 48±11.2, respectively (P=not significant [NS]). Body mass index was 28.2±9.5 and 30.4±11.8 kg/m^2, respectively (P=not significant [NS].

The VenaBlock group had PV closure rate of 100% immediately intraoperative, at 3 days, 2 weeks, 1 month and 2 months from the procedure for each treated perforator. From the VenaSeal group, 13 patients (81.25%) had immediate, as well as continuous treatment success during the follow-up period, while in 3 patients (18.75%, P=significant [S], P=0.0127) there was intraoperatively registered treatment failure, which we assigned to our hesitation to use adequate amount of this prolonged polymerization glue in this short length and relatively large diameter (6, 6.2 and 7 mm, respectively) perforator vein, due to the high estimated risk of adhesive leakage toward the deep venous system. We subsequently retreated the PV with the VenaBlock adhesive and achieved immediate and durable closure. The Duplex scanning revealed complete obliteration of the treated PVs in both groups (except in the aforementioned cases), with the PVs having dense, well rounded cross-section image in the VenaBlock group and flatter cross-section shape in the VenaSeal group. The single patient presenting active ulcer in the VenaBlock group showed progressive ulcer diameter reduction at each visit and the ulcer was healed by the 3rd week. There was no clinical or instrumental evidence of DVT in any patient. There were no infectious complications and/or hematomas of the puncture site. No extravasation of the glue was registered at duplex scanning. No foreign-body type reaction was observed during the follow-up period.

Discussion

Chemical ablation procedure is a relatively new treatment for treating varicose veins. It is the only FDA-approved procedure that uses an injection of medical adhesive to close varicose veins, and so far, has been reported as highly effective, according to several authors [8-12].

There is no risk of thermal nerve and skin injury, hence the hyperpigmentation is avoided and there is less pain and bruising than in thermal treatment. Tumescent anesthesia is not required. The application itself is very simple and the procedure time is short.

We perform the procedure in outpatient settings, without any anesthesia, under ultrasound guidance, via venepuncture with 21G needle, directly above the incompetent perforator, in its portion closest to the superficial vein. We use 2cc syringe for adhesive delivery, which we flush beforehand by 10% Dextrose solution. Gently, we apply just the necessary amount

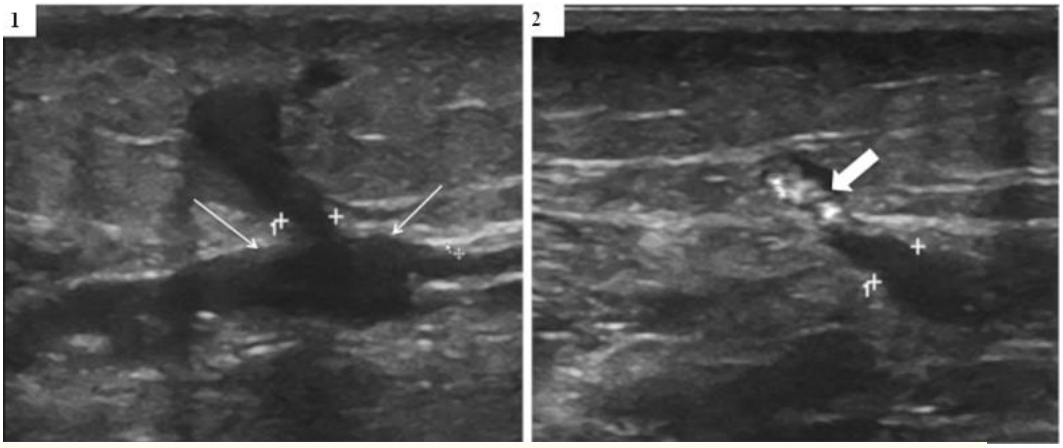

Fig. 1. (1) Ultrasound (US) imaging showing an abnormally dilated perforator vein connecting dilated superficial varicose veins to the deep venous system. Thin arrows show the deep fascia; (2) US image after treatment with VenoSeal, showing thrombosis and fibrosis of the perforator vein up to its connection with the deep system. Thick arrow shows bridging polymerized adhesive

to achieve visual confirmation of occlusion only in the segment above the deep fascia and we apply manual compression to the puncture site. Ultrasound confirmation of unobstructed flow in the contacting deep vein is mandatory (Figure 1).

It is our observation that the miniphlebectomy of the tributaries in the lower leg, following chemical ablation of the incompetent large veins and PVs, seems to have a significantly reduced intraoperative bleeding compared to other procedures, due to the immediate intraoperative occlusion of the lumen.

We apply thigh-level compressive stockings CCL 2, which are advised to be worn during the next 4 weeks. We prescribe a peroral broad-spectrum antibiotic for the first 5 days. The patient is discharged within 2 hours from the procedure.

The problem with this treatment is that the glue never fully dissolves, meaning it will become a permanent fixture in the vein, which can be felt under the skin, specifically in the mobile areas, like the knee joint. Additionally, according to the worldwide literature, about 5% of patients treated with VenaSeal have an allergic reaction to the glue that can cause pain and inflammation-especially problematic considering the glue cannot be removed.

There is also the high financial cost aspect to this procedure, considering the adhesive cannot be purchased separately and we are obligated to order the full kit, even when the application of long catheters and most of the other elements in the kit is redundant in cases of isolated PVs.

In our institution, we have used the fast-polymerizing VenaBlock and the slow polymerizing VenaSeal adhesives, both having similar features of their deployment catheters, with the important difference being the adhesive characteristics due to a difference in the chemical structure (short-chained *vs.* long-chained cyanoacrylate) resulting in different polymerization times and viscosity).

It is important to note that PV application is not included in any of the manufacturers' instruction manuals.

A polymerization time of 24-54 sec for the VenaSeal glue with significantly higher viscosity, with its final form being softer and more flexible, and less likely to be felt by the patient, as opposed to the extremely short polymerization time for the VenaBlock adhesive of 2-5 secs, with its much lower viscosity and high pushability, leading to rapid formation of a firm seal, which we find to be significantly safer to use near the deep veins, but has denser final structure, more likely to be felt under the skin. We find them both equally echo-positive (Figure 1).

In this trial, we have noticed a statistically important difference in treatment success between the 2 adhesives exclusively for the large diameter PVs (>6 mm), which we mainly attribute to our restraint while filling up relatively large and short perforators with the slow-polymerizing glue.

Its high viscosity formula makes it difficult to inject and the slow polymerization leads to a more difficult visual control of the precise occlusion point, which in the case of PVs is paramount.

Based on these differences, we prefer the VenaSeal set when treating the longer segments of the GSV, while for closing perforators we find the Venablock adhesive safer and easier to apply, whose extremely short polymerization time makes it less likely to penetrate and embolize the deep venous system.

Derived from our results, we find this closure procedure, regardless of the adhesive type, to be safe and highly effective for treatment of incompetent PVs.

Conclusion

Minimally invasive treatment of perforating veins will continue to improve. We find the chemical ablation procedure to be safe and effective for PVs, specifically when using a rapid polymerization adhesive. Due to its simplicity and short procedural time, we consider this to be the procedure of choice in case of multiple incompetent PVs present.

Conflict of interest statement. None declared.

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Original article

PRENATALLY DIAGNOSED SEVERE CONGENITAL ANOMALIES

ПРЕНАТАЛНО ДИЈАГНОСТИЦИРАНИ ТЕШКИ КОНГЕНИТАЛНИ АНОМАЛИИ

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Abstract

Introduction. The use of ultrasound in pregnancy is for screening and diagnostic purposes. Screening and diagnosis are two related but different practices. The purpose of screening is to determine risk in terms of chromosomal abnormalities, while diagnostic ultrasound is used to detect major structural abnormalities.
Aim. To determine the prevalence, incidence, distribution and to give an overview of the characteristics of severe fetal malformations detected prenatally in pregnant women at the Clinic for Gynecology and Obstetrics in "Remedika Hospital"-Skopje, North Macedonia.
Methods. Through a retrospective monocentric study, all collected database of pregnant patients and obstetric characteristics were analyzed using the electronic system (BIRPIS) of our hospital between January 2019 and December 2021, in which routine ultrasound prenatal screening was carried out in the first trimester (11+0-13+6 g.w) and in the second trimester (18-22 g.w.) in accordance with the recommendations given in the current guidelines of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). Detected anomalies were classified into minor and major categories, while major ones into severe and lethal anomalies. Congenital malformations were divided into subgroups according to the main organ systems involved according to the EUROCAT classification.
Results. The prevalence of severe fetal malformations prenatally diagnosed by ultrasound screening at the Clinic for Gynecology and Obstetrics in PZU "Remedika"-Skopje, in the examined period 2019-2021 in the first trimester is 1.8%, and in the second trimester 2.9%. 3954 antenatal screenings were performed in the first and second trimester of pregnancy, where 6(6.1%) chromosomal anomalies, 64(64.6%) isolated anomalies and 29(29.3%) multiple anomalies were represented. The average gestational week of detection in the first trimester is 12.7±0.8, while in the second trimester it is 21.1±2.2. The average age of the patients is 31.0±5.4 years, ranging from 20 to 46 years.
Conclusion. The highest percentage of congenital heart defects is registered, namely 37(37.4%). The percentage difference in relation to the representation of the other anomalies is statistically significant for $p<0.05$ (Difference test, p=.0000). Of them, 30 (81.1%) belong to the systemic anomaly Ventricular septal defect (VSD). In the group of ear, neck and face anomalies, 12(12.1%) are registered, all of them are Hygroma colli, NT>95th centile. Anomalies of the nervous system are registered 9(9.1%), of which one third (33.3%) belongs to Neural Tube Defects-Anencephalus and similar, and Spina Bifida.Comparisons with other studies in relation to European countries show a similar incidence, with a prevalence of either defects of the central nervous system or of the cardiovascular system.

Keywords: fetal malformations, congenital anomalies, prenatal diagnosis

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Апстракт

Вовед. Употребата на ултразвукот во бременоста е со скрининг и дијагностички цели. Скринингот и дијагнозата се две поврзани, но различни практики. Целта на скринингот е да се определи ризикот во однос на хромозомските аномалии, додека дијагностички ултразвук се користи за откривање на големи структурни аномалии.
Цел. Да се детерминира преваленцата, инциденцата, дистрибуцијата и да даде преглед на карактеристиката на тешките фетални малформации детектирани пренатално кај бремени жени на Клиниката за гинекологија и акушерство во ПЗУ “Ремедика”-Скопје.
Методи. Преку ретроспективна моноцентрична студија беа анализирани сите колектирани база податоци за бремени пациентки и акушерските карактеристики со помош на електронскиот систем (BIRPIS) на нашата болница помеѓу Јануари 2019 г. и декември 2021 г. кај кои е спроведен рутински ултразвучен пренатален скрининг во прв триместар (11+0-13+6 г.н) и во втор триместар (18-22 г.н) во согласност со препораките дадени во тековните упатства на International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). Детектираните аномалии

беа класифицирани во минорни и мајорни категории, додека пак мајорните во тешки и летални аномалии. Конгениталните малформации беа поделени во подгрупи според инволвираните главни органски системи според EUROCAT класификацијата.
Резултати. Преваленцата на тешките фетални малформации пренатално дијагностицирани со ултразвучен скрининг на Клиниката за гинекологија и акушерство во ПЗУ "Ремедика"-Скопје, во испитуваниот период 2019-2021 во првиот триместар изнесува 1.8%, а во вториот триместар 2.9%. Извршени се 3954 антенатални скринизи во прв и втор триместар на бременоста при што застапени се 6 (6.1%) хромозомски аномалии, 64(64.6%) изолирани аномалии и 29(29.3%) се мултипни аномалии. Просечната гестациска недела на детекција во првиот триместар изнесува 12.7±0.8,додека во вториот триместар изнесува 21.1±2.2.Просечната возраст на пациентките е 31.0±5.4г., со ранг од 20 до 46 години.
Заклучок. Најголем процент се регистрираат конгенитални срцеви дефекти и тоа 37(37.4%). Процентуалната разлика во однос на застапеноста на останатите аномалии е статистички сигнификантна за $p<0.05$ (Difference test, p=.0000) Од нив 30 (81.1%) припаѓаат на системска аномалија Ventricular septal defect (VSD). Во групата анаомалии на уши, врат и лице се регистрираат 12(12.1%) сите се Hygroma colli, NT >95th centile. Аномалии на нервниот систем се регистрираат 9(9.1%), од кои една третина (33.3%) припаѓа на Neural Tube Defects-Anencephalus and similar, и Spina Bifida. Компарациите со другите студии во однос на Европските земји покажуваат слична инциденца, со преваленца или на дефекти на централниот нервен систем или на кардио-васкуларниот систем.

Клучни зборови: фетални малформации, конгенитални аномалии, пренатална дијагноза

Introduction

Congenital malformations, otherwise known as birth defects, congenital disorders, or congenital malformations, are defined as structural abnormalities determined by factors that operate largely before conception or during pregnancy and can be identified prenatally, at birth, or later in life [14]. Prenatal detection and optimal management of these abnormalities are critical to ensure optimal health care for both mother and fetus.
Congenital malformations (CM) differ in terms of the effect on live births and are classified as major or minor congenital abnormalities. Major congenital malformations are defined as any birth defects associated with physical or cosmetic damage [1]. Major CMs show considerable variation worldwide with prevalence ranging from <1% to 8%, and they cause 20% and 30% of perinatal deaths [14]. However, the rate of congenital malformations is estimated at around 2-4% per live birth in current studies [1].
The introduction of prenatal ultrasound screening for anomalies, genetic testing, and biochemical testing in developed countries has profoundly improved the prenatal detection rate of CM. It contributes to the reduction of perinatal morbidity and mortality by enabling parents and clinicians to make informed decisions about pregnancy management such as a continuation of pregnancy, termination of pregnancy, effective planning in order to prevent complications that may arise during childbirth and after birth, as well as in identifying potential risk factors for future pregnancies. About 53% of CM can nowadays be detected earlier in gestation (up to 14g.w) by an experienced sonographer and between 60% and 90% of anomalies, depending on their nature, can also be detected during a detailed fetal anatomical scan between 18 and 22 g.w. With the introduction of fetal echocardiography in prenatal ultrasound screening, cardiac anomalies are now diagnosed in utero with high specificity and sensitivity [14]. The role of ultrasound (US) in daily practice is very important to make decisions during the monitoring of pregnancy and delivery. In many countries, second-trimester screening is routinely performed in low- and high-risk pregnancies to assess fetal anatomy [21]. Screening, diagnosis, and management of fetal anomalies in pregnancy allows parents to face the situation and make a decision for the future [16]. Fetal US screening is carried out with great accuracy in all weeks of pregnancy now, and the time spent on screening, the quality of the device used and the expertise of the specialist affects the level of anomaly detection.

Aim

Our goal is to determine the prevalence, incidence, and distribution of severe fetal malformations prenatally diagnosed with ultrasound screening at the Clinic for Gynecology and Obstetrics in the "Remedika Hospital"-Skopje, North Macedonia and to determine the characteristics of the detected severe fetal malformations.

Materials and methods

We retrospectively evaluated all the collected databases of pregnant patients and the obstetric characteristics in which routine ultrasound prenatal screening was performed in the first trimester (11+0-13+6 gestational weeks) and in the second trimester of pregnancy (18-22 g.w.) at the clinic for gynecology and obstetrics in Remedika Hospital-Skopje in the period from January 2019 - December 2021 using the electronic system

(BIRPIS) of our hospital. We implement an antenatal screening program according to international and national guidelines and the screenings are performed in accordance with the recommendations given in the current guidelines of the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG).

All pregnant patients followed in our institution are offered a screening test for fetal aneuploidy and determination of non-chromosomal anomalies, as well as screening for preeclampsia in high-risk pregnancies in the first trimester between the 11th and 13+6 weeks of gestation with US and combined dual screening test. A combined triple screening test with ultrasound examination is performed in the 16th-18th week of pregnancy for every pregnant woman who could not do a double screening test earlier and who would like to have a screening done. In the second trimester, fetal screening is done for anomalies with ultrasonography between the 18th and 22nd gestational weeks. Fetal echocardiography is performed as part of fetal screening in the second trimester. Fetal karyotyping such as chorionic villus sampling (CVS), amniocentesis, or prenatal non-invasive screening tests (NIPT) is offered in necessary cases and performed upon obtaining informed consent.

Three options for genetic screening for chromosomal abnormalities are possible. In the first, pregnant women undergo combined screening in the first trimester and those identified at high risk are offered invasive prenatal diagnosis (PD) for chromosomal abnormalities. In the second option, high-risk pregnant women identified by first-trimester screening could choose to undergo cell-free DNA noninvasive prenatal screening, and abnormal results would be confirmed by invasive PD for chromosomal abnormalities. In the third option, pregnant women who have had cell-free DNA screening and have positive results will be confirmed with PD for chromosomal anomalies.

Detailed prenatal ultrasound screening is performed transabdominally by three experienced specialists with specialized training in fetal anomaly scanning. US examination is performed using a General Electric Voluson E10 with a 1-6Mhz convex 4D probe.Cases such as soft only fetuses markers such as hyperechoic bowels or an intracardiac echoic focus without an identifiable anomaly were excluded from this study. Major congenital malformations were defined as any structural abnormalities, or birth defects related to physical or cosmetic damage to the fetus. Demographic characteristics, clinical characteristics, associated risk factors, and type and distribution of fetal anomalies by organ systems were analyzed. Age, pregnancy and parity, gestational week of pregnancy, method of pregnancy (spontaneous/assisted reproductive technologies), number of pregnancies (singleton/multiple), risk factors in the mother, and if there is a karyotype assessment of the pregnancy were analyzed. The obtained results of the cases with congenital anomalies were grouped systematically, and their incidence and distribution were compared with the literature. The criteria for the inclusion of CM, their severity, and their classification, are according to the guidelines of EUROCAT, a computer algorithm implemented by EUROCAT for the classification of cases of congenital anomalies followed by a manual review of cases by geneticists, which is based on the International Classification of Diseases version 10 (ICD-10). Detected anomalies were classified as chromosomal, genetic, and environmental, isolated, and multiple congenital anomalies. Patients with more than one involved system were recorded as multiple anomalies. Congenital malformations with abnormal karyotyping were included in the group of chromosomal abnormalities independently of the type of anatomically defined anomalies.

Statistical analysis

The data obtained from the medical history were grouped and distributed accordingly in a database in Microsoft Excel. They were processed with statistical software SSPS 26. A descriptive analysis of data was carried out, with which certain conclusions were obtained, and part of the results are the basis for calculating further statistical research. Using the normality test, it was checked whether the sample was selected from a population with a normal distribution. Mean value standard deviation, and structure percentages were determined. The difference between them is determined by the difference test (Difference test. A p-value of less than 0.05 is considered statistically significant.

Results

Table 1. Prevalence and incidence in the examined period

Year		US screening Ist trimester	US screening II trimester
2019	No.	469	834
	malformations	77	3030
	incidence	1.5%	3.6%
2020	No.	480	845
	malformations	4	14
	incidence	0.8%	1.6%
2021	No.	425	901
	malformations	14	30
	incidence	3.3%	3.3%
prevalence	2019-2021	1.8%	2.9%

The incidence of severe fetal malformations prenatally diagnosed with ultrasound screening at the Clinic for Gynecology and Obstetrics in Remedika Hospital-Skopje, in 2019 in the first trimester it is 1.5%, and in the second trimester 3.6%

The incidence of severe fetal malformations was prenatally diagnosed with ultrasound screening in

2020 in the first trimester, it is 0.8%, and in the second trimester 1.6%

In 2021 in the first trimester it is 3.3%, and in the second trimester 3.3%

Table 2. Patient characteristics

	N	Average	Minimum	Maximum	SD
Age	99	31.0	20	46	5.439800
Gestation					
Ist trimester	25	12.7	11.5	14.2	0.778888
IInd trimester	74	21.1	14.5	32.0	2.188508
Nationality					
Macedonian			68	68.7	
Albanian			31	31.3	
Parity					
First			54	54.5	
Second			22	22.2	
Third			15	15.2	
Fourth			4	4.1	
Fifth			3	3.0	
Sixth			1	1.0	
Chronic diseases					
Non			89	89.9	
Homozigot factor V,MTHFR			1	1.0	
Wilmsov Tu -operated			1	1.0	
heterozigot MTHFR			1	1.0	
Hashimoto			1	1.0	
Hypotireoidisam			2	2.0	
Homozigot MTHFR,FBG,MTRR			2	2.0	
Astma bronchiale			1	1.0	
Homozigot MTHFR			1	1.0	
Drugs during pregnancy					
Vitamins			99	100.0	
Antibiotics			1	1.0	
Gestagens			11	12.0	
LMH			3	3.0	
Eutirox			3	3.0	
Conception method					
IVF			9	9.1	
Spontnaeus			88	88.9	
AIH			2	2.0	
Karyotypization					
NIPT - Turner Sy			1	1.0	
NIPT- Down Sy			1	1.0	
Edwards Sy			1	1.0	
Down Sy			1	1.0	
NIPT - deletio 10q26			1	1.0	
Patau Sy,Trisomia 13			1	1.0	
Pozitiv			6	6.1	
Negativ			26	26.3	
No data			67	67.6	
Gender					
Female			34	34.4	
Male			41	41.4	
No data			24	24.2	
Chorionicity					
Singleton			41	41.4	
Triplets			24	24.2	
Twins			34	34.4	

The prevalence of severe fetal malformations prenatally diagnosed with ultrasound screening at the Clinic for Gynecology and Obstetrics in Remedika Hospital-Skopje, in the examined period 2019-2021 in the first trimester is 1.8%, and in the second trimester 2.9% (Table 1).

The average age of the patients is 31.0±5.4 years, ranging from 20 to 46 years old. According to the nationality, 68.7% are Macedonian and 31.3% Albanian,

the percentage difference is significant for p<0.05 (Difference test, p=.0000).
More than half of the mothers 54.5% have first parity, the percentage difference between the first parity versus the rest is significant for p<0.05 (Difference test, p =.0000).
Among mothers, chronic diseases are registered in 10 (10.1%), and not in 89.9%, the percentage difference is significant for p<0.05 (Difference test, p =.0000).
During pregnancy, all patients/mothers took vitamins, and 3.0%, in addition to vitamins, took Eutirox and LMH, 12.0% took gestagens.
Comorbidity is not registered in 93.9% of the mothers, but three mothers did not give an answer. 98 of the patients do not smoke and do not take drugs, and one patient does not give an answer.
88.9% of mothers became pregnant spontaneously, 9.1% became pregnant with IVF, and two mothers with AIH, the percentage difference is significant for p<0.05 (Difference test, p =.0000)
Karyotyping is positive in 6 (6.1%). Karyotyping is positive and genetic chromosomal malformation is proven in 6.0% (Down Sy, Edwards Sy, Turner Sy, Patau Sy, Trisomia 13, deletio (10q26) and so on. Karyotyping is negative in 26.3% (26) of the examined. In 67.6% there is no data.
41.4% is male, 34.4% female gender, and in 24.2% gender cannot be determined, the percentage difference is significant for p<0.05 between the male gender and the inability to determine the gender (Difference test, p=.0099). The other percentage differences are insignificant.
When determining chorionicity in the largest percentage of 41.4% are singletons, 34.4% are twins and 24.2% are triplets, the percentage difference is significant for p<0.05 between singletons and triplets (Difference test, p=.0099). The other percentage differences are insignificant. The average gestational week of detection in the first trimester is 12.7±0.8, the average gestational week of detection in the second trimester is 21.1±2.2 (Table 2).

Table 3. Presentation of systemic malformation

EUROCAT Classification	
List of included anomalies	
Nervous system	
Neural Tube Defects-Anencephalus and similar, Encephalocele, Spina Bifida	3
Hydrocephalus	1
Severe microcephaly	
Arhinencephaly/holoprosencephaly	2
Agenesio/hypoplasio vermis	1
Agenesio corpus callosum	2
Eye	
Anophthalmos/micropthalmos	
Congenital cataract	
Congenital glaucoma	
Ear, face and neck Ear, face and neck	
Anotia	
Hygroma colli, NT > 95th centile	12
Congenital heart defects	
Severe CHD	
Common arterial truncus	
Double outlet right ventricle	
Transposition of great vessels	1
Single ventricle	
Ventricular septal defect (VSD)	30
Atrial septal defect (ASD)	
Atrioventricular septal defect (AVSD)	
Tetralogy of Fallot	2
Tricuspid atresia and stenosis	
Ebstein`s anomaly	
Pulmonary valve stenosis	
Pulmonary valve atresia	
Aortic valve atresia/stenosis	1
Mitral valve anomalies	
Hypoplastic left heart	2
Hypoplastic right heart	1
Coarctation of aorta	
Aortic atresia/interrupted aortic arch	
Total anomalous pulm venous return	
PDA as only CHD in term infants (>=37 weeks)	
Respiratory	

Choanal atresia	
Cystic adenomatous malf of lung	
Hydrothorax	3
Oro-facial clefts	
Cleft lip with or without palate	1
Cleft palate	2
Digestive system	
Oesophageal atresia with or without tracheo-oesophageal fistula	
Duodenal atresia or stenosis	
Atresia or stenosis of other parts of small intestine	
Ano-rectal atresia and stenosis	1
Hirschsprung's disease	
Atresia of bile ducts	
Annular pancreas	
Diaphragmatic hernia	3
Abdominal wall defects	
Gastroschisis	1
Omphalocele	
Urinary	
Bilateral renal agenesis including Potter syndrome,unilateral agenesia	2
Multicystic renal dysplasia	2
Congenital hydronephrosis	4
Bladder exstrophy and/or epispadia	
Bladder exstrophy and/or epispadia	
Renis duplex, pyelon duplex	2
Megacystis	2
Limb	
Limb reduction defects	
Club foot - talipes equinovarus	3
Hip dislocation and/or dysplasia	
Polydactyly	2
Syndactyly	
Sandal gap	3
Other anomalies/syndromes	
Skeletal dysplasias	4
Craniosynostosis	
Congenital constriction bands/amniotic band	
Situs inversus	
Conjoined twins	
Congenital skin disorders	
VATER/VACTERL	
Vascular disruption anomalies	
Lateral anomalies	
Teratogenic syndromes with malformations	
Fetal alcohol syndrome	
Valproate syndrome	
Maternal infections resulting in malformations	
Genetic syndromes + microdeletions	1
Chromosomal	
Down Syndrome	2
Patau syndrome/trisomy 13	1
Edward syndrome/trisomy 18	1
Turner syndrome	1
Klinefelter syndrome	

Discussion

The current study showed that in the investigated period of the Clinic for Gynecology and Obstetrics in "Remedika Hospital"-Skopje in the period from January 2019 to December 2021. 3954 antenatal screenings were performed in the first and second trimester of pregnancy, where 6(6.1%) chromosomal anomalies, 64(64.6%) isolated anomalies, and 29(29.3%) were multiple anomalies.

From the view of (Table 3) can be noted that the following sistematic malformation are present.

Anomalies of the nervous system are registered 9 (9.1%), of which one-third (33.3%) belong to Neural Tube Defects Anencephalus and similar, and Spina Bifida.

In the group of ear, neck, and face anomalies there are 12 (12.1 %) are all Hygroma colli, NT>95th centile.
The highest percentage of congenital heart defects is registered, namely 37(37.4%). The percentage difference in relation to the representation of other anomalies is statistically significant for p<0.05 (Difference test, p= .0000). Of them, 30 (81.1%) belong to the systemic anomaly Ventricular septal defect (VSD).
Urinary malformations are registered 12 (12.1%). Of the represented urinary malformations, 33.3% are due to congenital hydronephrosis.
Respiratory anomalies are registered 3 (3.0%) namely hydrothorax.
Orofacial clefts are registered as 3 (3.0%).
3(3.0%) anomalies are registered in the digestive system.
Defects of the abdominal wall Gastroschisis is registered once.
Talipes equinovarus malformations [3], Polydactyly [2], and Sandal gap [3] are registered in 8(8.1%).
6 (6.1%) chromosomal anomalies are represented, of which 2(33.3%) are Down Syndrome.
Comparisons with other studies in relation to European countries show a similar incidence, with a prevalence of either defect of the central nervous system or of the cardiovascular system [1,7,14,21,22,25].
Over the past 30 years, screening methods such as prenatal ultrasound screening, genetic testing, and bio-chemical testing have increasingly been offered to pregnant women. These screening measures increase the detection rate of fetal anomalies [15]. A significant number of fetal anomalies are surgically correctable, hence the need for timely, accurate and reliable prenatal diagnosis with a multidisciplinary approach is maximally adopted and in utero surgical correction is offered where which is feasible [14]. Although many malformations can be identified, it is recognized that some may be missed, even with sonographic equipment in the best hands, or they may develop later in pregnancy. Before starting the examination, the specialist should counsel the patient regarding the potential benefits and limitations of routine fetal ultrasound scanning [23].

Conflict of interest statement. None declared.

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Original article

INFLUENCE OF DONOR TYPE IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION ON EFFICACY AND SURVIVAL IN PATIENTS WITH ACUTE LEUKEMIA - RETROSPECTIVE SINGLE CENTER ANALYSIS

ВЛИЈАНИЕ НА ВИДОТ НА ДАРИТЕЛОТ ПРИ АЛОГЕНАТА ТРАНСПЛАНТАЦИЈА НА ХЕМАТОПОЕТСКИ МАТИЧНИ КЛЕТКИ ВРЗ ЕФИКАСНОСТА НА ПРОЦЕДУРАТА И ПРЕЖИВУВАЊЕТО НА ПАЦИЕНТИТЕ СО АКУТНА ЛЕУКЕМИЈА - РЕТРОСПЕКТИВНА АНАЛИЗА

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Abstract

Introduction. Performing allogeneic stem cell transplantation in leukemia patients, especially in patients with AML is often an imperative in the treatment approach in these patients. Mainly this is due to the biggest curative potential that this therapeutic procedure has, backed up by the immunological effect of donor T cells, defined as graft *versus* leukemia effect. It is a standard to do HLA typing of patients and their families to search for potential donors for allogeneic HSCT as upfront planning of the treatment approach for a patient. But, sometimes there is no compatible sibling donor available, so an alternative must be made with unrelated and haploidentical donors. This safe and justified.
Methods. In our retrospective analysis we included 85 patients with acute leukemia treated on the University Clinic for Hematology in Skopje from 2010 to 2022, where an allogeneic HSCT was done. The majority of patients were diagnosed with AML. The inclusion criteria of patients analyzed in this study were: age >14 and ≤70 years, confirmed diagnosis of AML or ALL, achieved CR confirmed with analysis of bone marrow specimen either by flow cytometry, molecular analysis of cytological analysis with <5% of blasts in bone marrow. Patients that were MRD+and had higher number of blasts (5-10%) were also included and those with Karnnofsky score ≥70%. For statistical analysis of data obtained in this study the software Graph Pad Prism 9 was used. The survival rates were analyzed using the Kaplan-Meier method. For all statistical tests, $p < 0.05$ was considered to be statistically significant.
Results. Of the analyzed group, 49(57.6%) patients were male and 36(42.4%) female. Of all patients, the majority, 69(81.1%), were diagnosed with AML, while 16 (18.9%) with ALL. According to the type of donor, we used sibling donors in the majority of patients 46 (54.1%), in 29(34.1%) matched unrelated donors (MUD) and in 10(11.8%) haploidentical donors. The OS of patients with sibling allogeneic HSCT was 56.7% for 24 months; in MUD allogeneic HSCT was 61.6%, which did not show any statistical significance, but that was not the case in patients with haploidentical HSCT where the OS was 33.3% in 24 months. According to the type of donor, in allogeneic HSCT from sibling donor the TRM was 7.2% and in unrelated/haploidentical HSCT was 15%, which was not statistically significant. There was no statistical difference in the relapse rate; it was 28.5% during a 24-month-period in patients with MUD/haploidentical transplantation, and in MRD transplantations.
Conclusion. Allogeneic HSCT is well established therapeutic option and an imperative in treating leukemia patients. In lack of sibling donors, MUD allogeneic HSCT is an adequate alternative providing effective therapeutic approach, making the haploidentical HSCT the least preferable option.

Key words: AML, ALL, allogeneic, related, unrelated, haploidentical

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Апстракт

Алогената трансплантација на хематопоетски матични клетки (ХМК) кај пациентите со акутна леукемија, особено кај пациентите со АМЛ, често претставува императив во терапискиот пристап кај овие пациенти. Ова најмногу се должи на големиот куративен потенцијал кој го имаа оваа тераписка процедура, а се должи на имунолошкиот ефект на донорските Т клетки, дефиниран как графт версус леукемија ефект. Стандардна клиничка пракса, во почетокот на лекувањето кај овие пациенти да се нап-

рави ХЛА типизација на пациентот и неговата фамилија со цел да се бара потенцијален дарител на хематопоеткси матични клетки со цел реализација на алогена трансплантација. Но, некогаш нема потенцијален сроден дарител на ХМК, па затоа потребно е да се направи алтернатива со барање на несроден или хаплоидентичен дарител на ХМК кој би се искористил за реализирање на алогената трансплантација. Но, се поставува прашање дали овој пристап е оправдан кај овие пациенти.

Методи. Во оваа ретроспективна анлиза беа вклучени 85 пациенти со акутна леукемија лекувани на Универзитетската клиника за хематологија во Скопје, во периодот од 2010 до 2022 година и кај кои е реализирана алогена трансплантација на ХМК. Најголемиот дел од пациентите беа дијагностицирани со АМЛ. Инклузионите критериуми за пациентите кои беа предмет на анализа во оваа студија беше возраст >14 години и ≤70 години, дијагноза за АМЛ или АЛЛ, постигната комплетна ремисија потврдена со анализа на примерок од коскената срцевина со проточна цитометрија, молекуларни анализи или цитолошка анализа со <5% на бласти во коскената срцевина, но исто така и пациентите кои беа позитивни за минимална резидуална болест и имаа повисок број на бласти (5-10%) исто така беа вклучени во оваа анализа, и Karnnofsky score ≥70%. За статистичка анализа на податоците беше користен софтвер Graph Pad Prism 9. Стапките на преживување беа анализирани со примена на Kaplan-Meier-овиот метод. За сите статистички тестови, P < 0.05 се сметаше за статистички сигнификантно.

Резултати. Од анализираната група на пациенти, 57.6% беа мажи, додека 42.4% жени. Најголемиот дел, 81.1% беа дијагностицирани со АМЛ, додека останатите 18.9% со АЛЛ. Според типот на дарител на ХМК, кај 54.1% беа користени сродни дарители на ХМК, кај 34.1% компатибилни несродни дарители, додека кај 11.8% хаплоидентични дарители. Вкупното преживување (OS) кај сродните алогени трансплантации на ХМК изнесуваше 56.7% за 24 месеца, додека кај несродните алогени трансплантации на ХМК 61.6%, при што не беше потврдена статистичка сигнификантност, што не беше случај со хаплоидентичните трансплантации на ХМК каде OS изнесуваше 33.3% за 24 месеци и беше сигнификантно полошо. Морталитетот асоциран со трансплантацијата (TRM) асоциран со типот на дарителот, кај алогените ТХМК од сроден дарител изнесуваше 7.2%, додека кај несродните/хаплоидентичните алогени ТХМК 15% и не беше потврдена статистички сигнификантна разлика. И во стапката на релапс, немаше статистичка сигнификантност, односно изнесуваше 28.5% за период од 24 месеца кај пациентите со несродни/хаплоидентични алогени ТХМК и кај пациентите со сродна алогена ТХМК.

Заклучок. Алогената трансплантација на ХМК претставува етаблирана тераписка опција и претставува императив во третманот на пациентите со акутна леукемија. При недостаток на сроден дарител на ХМК, примената на компатибилен несроден дарител на ХМК претставува адекватна опција која обезбедува ефективен тераписки пристап кај пациентите, додека хаплоидентичната трансплантација на ХМК е последната тераписка опција која би била во избор за третман на пациентите.

Клучни зборови, АМЛ, АЛЛ, алогена, сродна, несродна, хаплоидентична

Introduction

Treatment of patients with acute leukemia represents a true challenge for hematologists worldwide. Performing allogeneic stem cell transplantation, especially in patients with AML is often an imperative in the treatment approach to these patients. Mainly this is due to the biggest curative potential that this therapeutic procedure has, backed up by the immunological effect of donor T cells, defined as graft *versus* leukemia effect. But balancing between true therapeutic benefit and complications that lead to higher non relapse mortality of the allogeneic stem cell transplant can sometimes impose a challenging and difficult decisions. The incidence of acute myeloid leukemia (AML) is around 3 to 4 cases per 100,000, and the median age of diagnosis is 63 years [1]. Achieving complete remission (CR) is crucial in patients with AML, as the main reason worsening the survival rates of these patients is the relapse of the disease rather than primary treatment failure. The standard of care induction first-line therapy in AML patients is the combination of cytosine arabinoside (Ara-C) and anthracycline (daunorubicin or idarubicin) [2]. After achieving CR, consolidation therapy with high dose ARA-C follows [3]. It is estimated that the rates of CR after standard induction therapy are around 70%, and patients with favorable disease have good overall survival (OS) rates of around 60%. But this can not be said for patients with intermediate and with adverse risk of AML. Also, targeted therapies are used in combination with standard, chemotherapy regimens in patients with specific mutations. For example, in patients with mutated FLT3, the kinase inhibitor midostaurin (there are also other types of FlT-3 inhibitors) is added to the standard induction and consolidation therapy of AML patients [4]. Also, other agents, like hypomethylating agents, including azacytidine and decitabine, may be a treatment option in monotherapy or combination therapy with venetoclax, a BCL2 inhibitor [5]. In general, the treatment approach after achieving first CR, according to the EBMT recommendations, is performing allogeneic hematopoietic stem cell trans-

plantation (HSCT) in all AML patients, except in patients with favorable disease that are in CR1 and are minimal residual disease negative (MRD-). In addition, allogeneic HSCT in patients with primary refractory disease, or without CR is questionable due to the poor survival rates (Figure 1) [6]. The performance status of a patient is also very important as a risk adapted approach, where we can identify a patient with comorbidities in whom allogeneic HSCT may not be beneficial. But, the advent of reduced intensity conditioning (RIC) and the bigger availability of donors is allowing to increase the number of patients where allogeneic HSCT can be safely done [7].

Acute lymphoblastic leukemia (ALL) is more often

Disease	Disease status	MSD allo	MUD allo	MMAD allo	Auto	CAR-T
Haematological malignancies						
AML[a]	CR1 (favourable risk and MRD–)[b]	GNR/II	GNR/II	GNR/II	CO/I	
	CR1 (favourable risk and MRD+)[b]	S/II	CO/II	CO/II	GNR/II	
	CR1 (intermediate risk)[b]	S/II	CO/II	CO/II	CO/I	
	CR1 (adverse risk)[b]	S/II	S/II	S/II	GNR/I	
	CR2	S/II	S/II	S/II	CO/II	
	APL Molecular CR2	S/II	CO/II	GNR/III	S/II	
	Relapse or refractory	CO/II	CO/II	CO/II	GNR/III	

Fig. 1. Indications for allogeneic HSCT in AML patients
S-standard of care, CO-clinical option, GNR-generally not recommended, I/II/III-grade of evidence

encountered in pediatric population. In adults, it has an incidence of 1.7 per 100,000 population. It has a worse prognosis compared to AML. Treatment of patients with ALL consists of chemotherapy regimens used for prephase treatment, induction, treatment intensification/ consolidation with intention to achieve CR, and then proceed with maintenance therapy or allogeneic HSCT. Usually, the treatment is consisted of combination of cytostatic drugs like vincristine, cyclophosphamide, corticosteroids, with or without L-aspariganase, methotrexate, ARA-C [8]. Sometimes addition of monoclonal antibodies like rituximab are indicated [9] and TKI, like imatinib, for Ph+ ALL patients. Usually, allogeneic HSCT in CR1 in ALL patients, according to the EBMT is indicated in patients with high-risk disease (Figure 2) [10], although the criteria for risk stratification of ALL patients can vary among different study groups.

Factors considered by all study groups	Factors considered by majority of study groups	Factors considered by some study groups
Inadequate response during/ after consolidation: • MRD $>10^{-4}$/detectable at any level	Inadequate response to induction I: • No hematological CR • MRD $>10^{-3}$ after induction	Initial CNS involvement
Age (various cut points)	High initial WBC: • $>30 \times 10^9$/L in B-ALL • $>100 \times 10^9$/L in T-ALL	Adverse immunophenotype: • Early T-precursor • Mature T • Pro-B
BCR:ABL1	Other genetic factors: • KMT2A rearrangements • Hypodiploidy • Complex karyotype	

Fig. 2. Definition of high-risk ALL patients

Usually, every day clinical practice has to do a HLA typing of patients and the family has to search for potential donors for allogeneic HSCT as upfront planning of the treatment approach for the patient. But, sometimes there is no compatible sibling donor available, so an alternative must be made with unrelated and haploidentical donors. This is safe and justified. According to the CIBMTR, there is a constant trend towards an increased use of alternative donors for allogeneic HSCT as a safe approach in AML and ALL patients. So, we have made an analysis of using related and unrelated donors for allogeneic HSCT in patients with acute leukemia treated at the University Clinic for Hematology in Skopje, Republic of North Macedonia.

Material and methods

In our retrospective analysis we included 85 patients with acute leukemia treated at the University Clinic for Hematology in Skopje from 2010 to 2022, where an allogeneic HSCT was done. The majority of patients were diagnosed with AML. They were initially treated with the standard induction regimen "7+3" [antimetabolite cytosine arabinoside (Ara-C) and 3 days of an anthracycline (i.e., daunorubicin or idarubicin)]. If CR was not achieved after standard induction, confirmed with reevaluation of bone marrow after standard induction therapy, we continued with FLAG-Ida regimen (fludarabine 30 mg/m^2 and AraC 2 g/m^2 for 5 days,

idarubicin 10 mg/m^2 for 3 days, and G-CSF 5 microg/kg from day 0 until neutrophil recovery). If CR was achieved, the patient was considered a candidate for hematopoietic stem cell transplantation (HSCT) as mandatory further treatment of the disease. If CR was confirmed after induction therapy, a minimum of 2 courses of consolidation regimen with a high-dose ARA-C was the next therapeutic approach. In patients with ALL, we administered either Hyper C-VAD or BFM regimen. Also, in some patients classified as primary refractory, or had an early relapse of the disease, FLAG-Ida was given. The intend was to achieve a CR before allogeneic HSCT. However, even patients who were not in CR, particularly those that were MRD +, were also candidates for allogeneic HSCT, even though worse survival rates were expected. In these patients we intended to shorten the length of the immunosuppression therapy, aiming towards GVL effect.

Our tendency was to do HLA typing in all potential candidates for allogeneic HSCT, searching for sibling donors. If no sibling donors were found, our next step was to search for potential unrelated donors. Finally, haploidentical allogeneic HSCT was also optional. *In vivo* T cell depletion with ATG was a standard in the conditioning regimen in patients undergoing unrelated donor HSCT. Even in patients with sibling donor allogeneic HSCT, ATG was used for reduction of GvHD rates. Mainly it was indicated in patients where we had higher expectation for GvHD occurrence (for example when we used female donors, especially females giving previous births, information on permissive mismatch in the high-resolution HLA typing, etc.). The ATG was omitted in patients with sibling donor transplant and disease with a poor risk, or that were MRD + avoiding the increased risk for relapse because of T cell depletion and reduction of GVL effect. We used the standard dose of ATG 5-10 mg/Kg TT, administered on days -3-2-1 before transplant. The interval between ATG and the infusion of the allograft was debated, but we used the recommendation that closer to transplant, the higher the levels of circulating ATG leading to a more effective GVHD protection [11,12].

As GvHD prophylaxis, in patients with myeloablative conditioning (MAC), we mainly administered calcineurin inhibitor with a short course of methotrexate given on days +1, +3, +6, +11 posttransplant, which is the most used protocol worldwide and it is a standard approach in many transplantation centers [13]. In patients where we used reduced intensity conditioning (RIC), the most frequently used GvHD prophylaxis was the combination of CNI (cyclosporine A) and MMF (mycophenolate mofetil) [14]. In the haploidentical setting, the gold standard is giving posttransplant cyclophosphamide (PTCY). In our group of patients, we applied the classical Baltimore's PTCY prophylaxis with cyclophosphamide 50 mg/kg on days +3 and +4 followed by CNI/MMF [15]. Although rare, but also adding ATG to PTCY in the haploidentical setting was optional, because there are data that show that patients with AML who underwent haploidentical transplantation and received ATG+PTCY (associated with MMF +CsA) as GVHD prophylaxis can lead to lower rates of cGVHD of all grades [16].

The inclusion criteria of patients analyzed in this study were: age >14 and ≤70 years, confirmed diagnosis of AML or ALL, achieved CR confirmed with analysis of bone marrow specimen either by flow cytometry, molecular analysis of cytological analysis with <5% of blasts in bone marrow. Patients who were MRD+and had a higher number of blasts (5-10%) were also included and Karnofsky score ≥70%.

The exclusion criteria manly were based on age, patients older than 70 years were excluded, also patients that were not in CR, and patients in CR but with inadequate hematological recovery (Neutrophils <1,000/μL; Platelets < 50,000/μL), and Karnofsky score <70%.

For statistical analysis of data obtained in this study the Graph Pad Prism 9 software was used. The survival rates were analyzed using the Kaplan-Meier method, and in the multifactorial analysis Cox proportional hazard model. For all statistical tests, $p<0.05$ was considered to be statistically significant.

Results

Of the analyzed group, 49 (57.6%) patients were male and 36 (42.4 %) female (Figure 3).

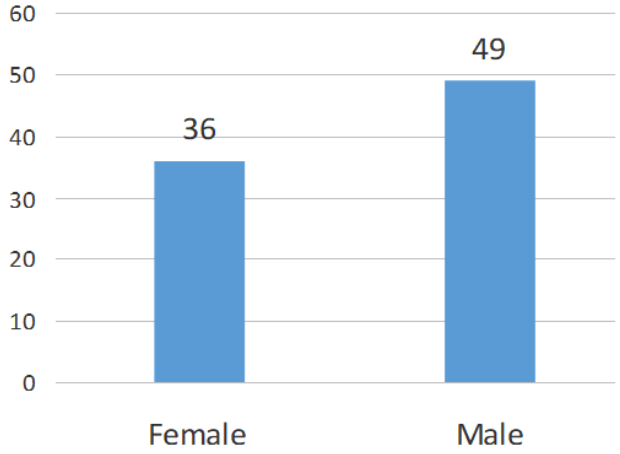

Fig. 3. Sex distribution of patients

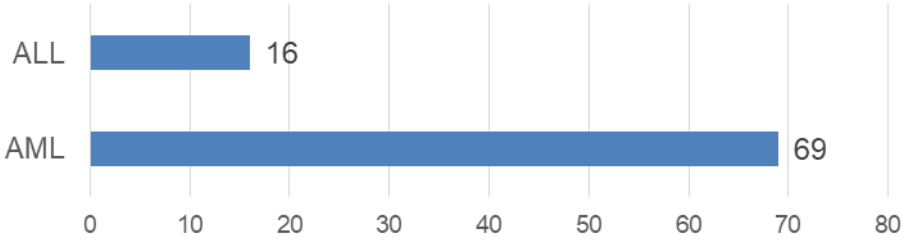

Fig. 4. Diagnosis of patients

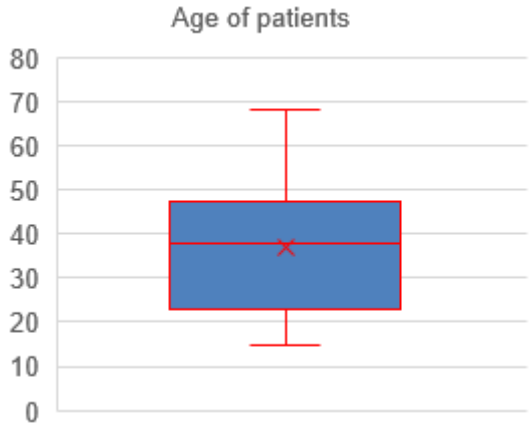

Fig. 5. Age of patients

Of all patients, the majority, 69 (81.1%) were diagnosed with AML, while 16 (18.9%) with ALL (Figure 4). This was probably due to the fact that AML is more frequent in adults, and additionally not all ALL patients are transplanted in CR1.

The median age of the transplanted patients was 39.5 years (Figure 5).

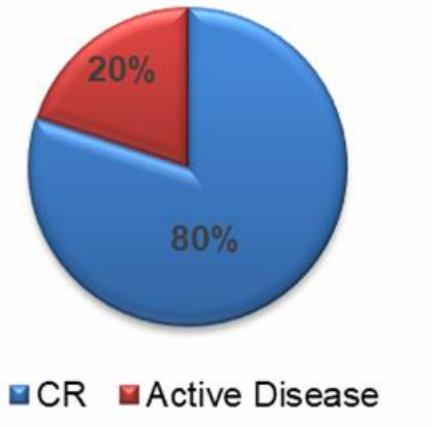

Fig. 6. Disease status before transplant

80% of all patients were transplanted in CR, while 20% were not in CR, or were MRD positive before the allogeneic HSCT (Figure 6). In these patients, we tailored the immunosuppression therapy posttransplant towards shortening with regular chimerism monitoring and monitoring of molecular markers if they were initially present. Impending relapse was an indication for DLI. The median time from diagnosis till performing the transplantation was 7.66 months.

Analyzing the risk profile of the disease, we noted that majority of patients were with intermediate risk profile (45%), 24% had a good risk profile and 31% had an adverse disease (Figure 7).

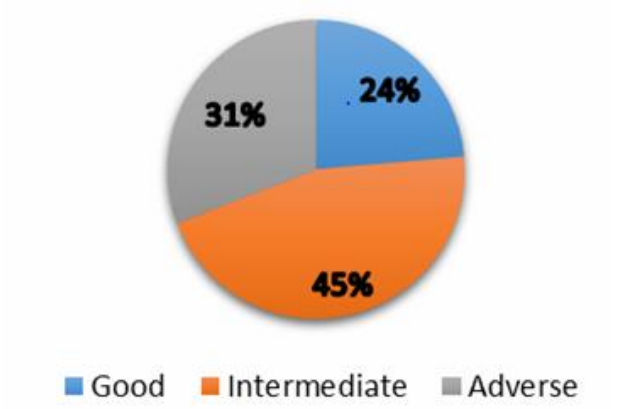

Fig. 7. Risk profile of the disease

Almost all patients were transplanted in CR-1 (95.6%), and the rest in CR-2 (4.4%) (Figure 8).

The majority of patients (94.1%) were conditioned with myeloablative conditioning regimens (MAC), mainly BY-Cy +/- ATG. The rest (5.9%) received reduced intensity conditioning (Figure 9).

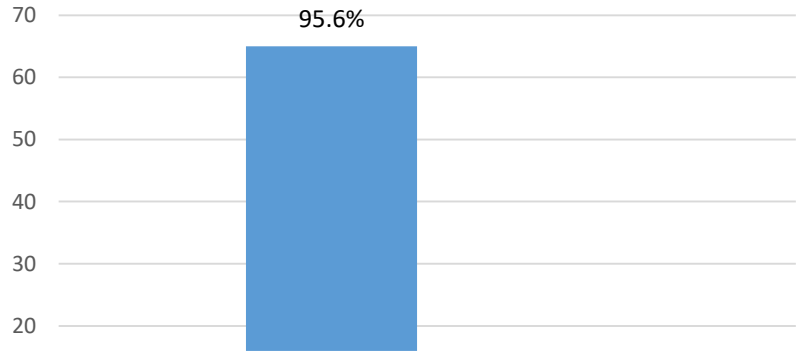

Fig. 8. Disease status before transplant

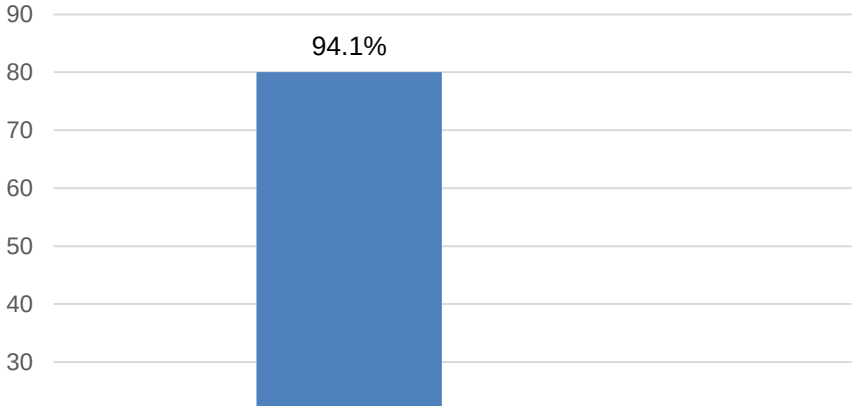

Fig. 9. Conditioning intensity

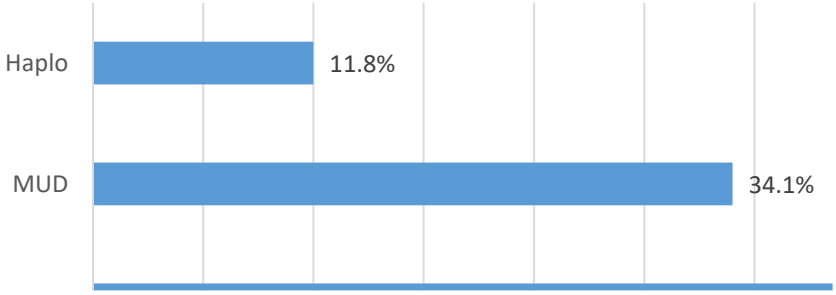

Fig. 10. Donor type used for allogeneic HSCT

According to the type of donor, in the majority of patients 46(54.1%) we used sibling donors, in 29(34.1%) matched unrelated donors (MUD) and in 10(11.8%) haploidentical donors (Figure 10).

In patients with sibling transplantation, the rate of some degree of GvHD was 30.4%, while in patients with MUD was 27.6%, which was not statistically different (Figure 11).

ATG was used in conditioning of 54.1% of patients (all with MUD and some MRD transplants) (Figure 12). ATG was administered on days -3-2-1 before the graft application, and we used a standard dose of ATG at 5 to 10 mg/kg. There were no serious adverse events during administration of ATG leading to discontinuation

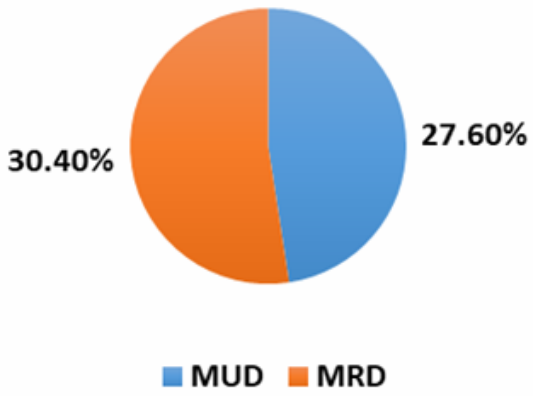

Fig. 11. Rate of GvHD

of the drug. We used a standard premedication with chloropyramine, methylprednisolone and acetaminophen. It led to a significant reduction of GvHD rates, with no important influence on infective complications leading to worsened TRM (Figure 13).

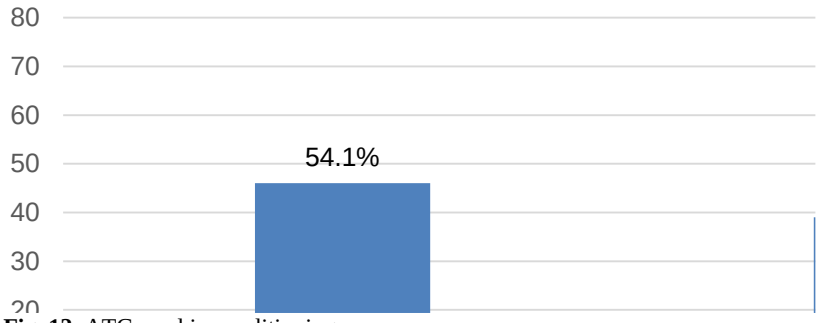

Fig. 12. ATG used in conditioning

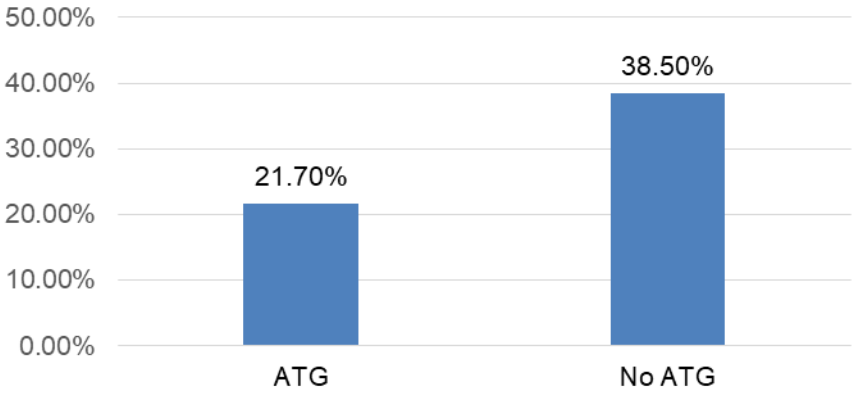

Fig. 13. Rate of GvHD in ATG and non ATG patients

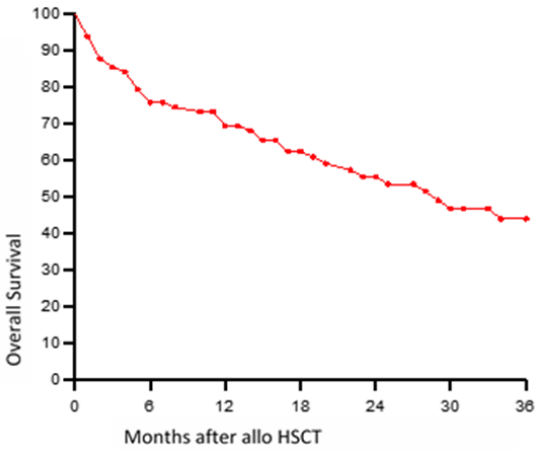

Fig. 14. Overall survival (OS) of patients

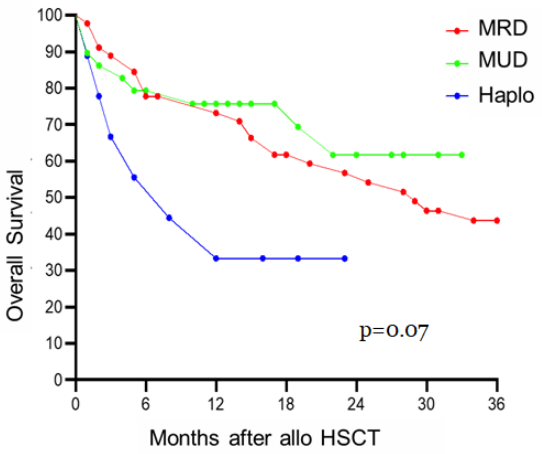

Fig. 15. OS in MRD, MUD and haploidentical transplants

The OS of all patients for 24 months was 55.6% (Figure 14).

The OS of patients with sibling allogeneic HSCT was 56.7% for 24 months, in MUD allogeneic HSCT 61.6%, which did not show any statistical significance, but that was not the case in patients with haploidentical HSCT where the OS was 33.3% in 24 months (Figure 15).

Then we analyzed the disease-free survival between these two groups of patients. The disease-free survival (DFS) in patients with sibling, MUD and haploidentical HSCT in 36 months was 42%, 63.2% and 30% respectively, and showed a statistical significance in favor of the unrelated donors. This is shown in Figure 16.

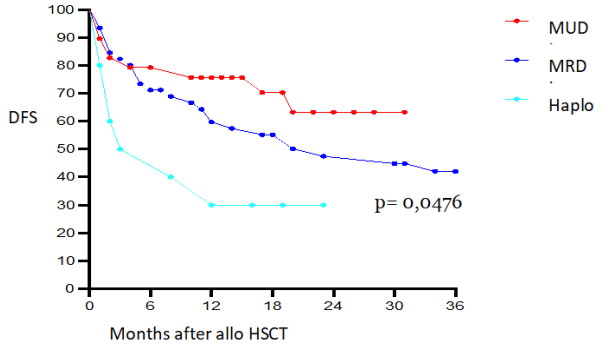

Fig. 16. DFS in patients with MRD, MUD and haploidentical allogeneic HSCT

The overall transplant-related mortality (TRM) was 13.3% (Figure 17). When we analyzed the TRM according to the type of donor, in allogeneic HSCT for sibling donor the TRM was 7.2% and in unrelated/haploidentical HSCT it was 15%, which was not statistically significant (Log-rank (Mantel-Cox) test, P value = 0,1422, Chi square = 2,154 (Figure 18).

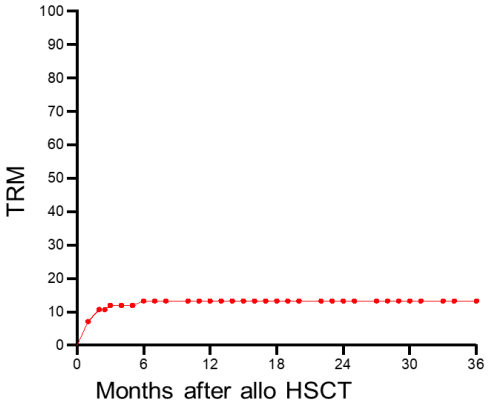

Fig. 17. TRM in all patients

Adding ATG to the conditioning regimen is standard of care for unrelated donor transplants. It is also used in sibling donor transplants where the estimation shows that the benefit of this drug outweighs the expected complications. Figure 19 illustrates OS between patients receiving ATG and patients conditioned without ATG,

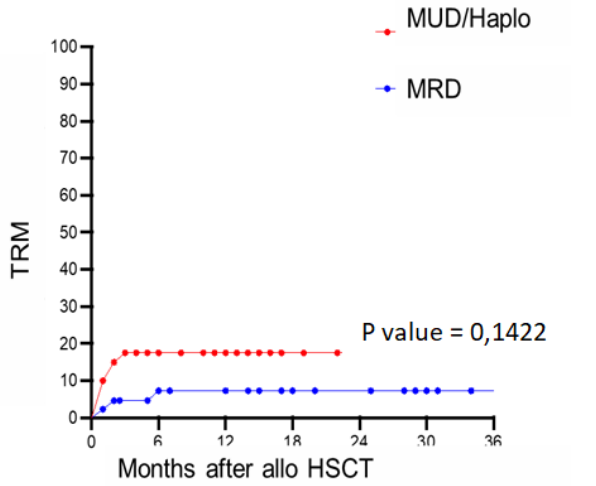

Fig. 18. TRM in MRD and MUD/Haploidentical transplants

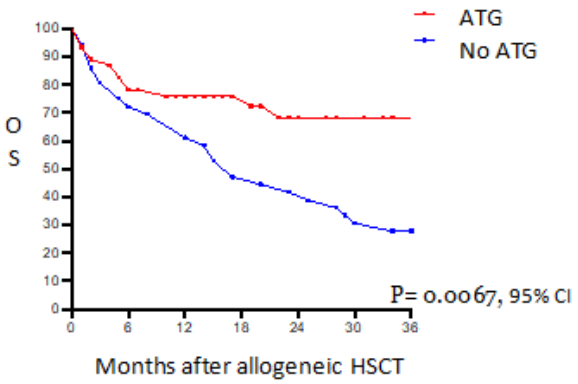

Figure 19. OS in patients receiving ATG and without ATG

and it clearly presents a significant benefit towards better OS in ATG group.

Even in the multifactorial analysis, our data showed that ATG, among other factors had significant influence on OS of the patients (Table 1).

Probably the main goal of allogeneic transplantation is control of the disease, avoiding relapse and finally cured patient. We analyzed the relapse rate between these two groups of patients and did not confirm a significant difference in relapse rates (Figure 20).

Table 1. Multifactorial analysis

Variable	R	Estimate	S.E	95% CI	|t|	P value
EBMT risk score	0.9756	-12.46	5.966	-24.36 to -0.5651	2.089	0.0403
MRD	0.7019	-8.850	26.49	-61.68 to 43.98	0.3341	0.7393
ATG	0.6752	-40.02	23.26	-86.41 to 6.368	1.721	0.0297
Chimerism	0.8848	-56.31	37.30	-130.7 to 18.08	1.510	0.1356

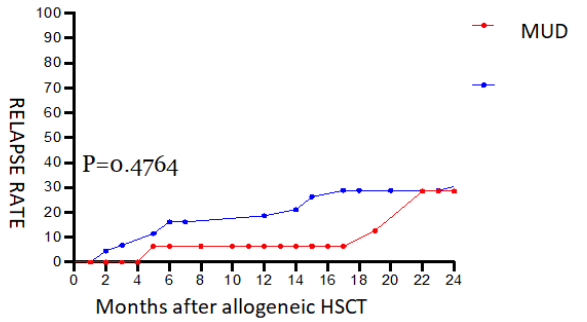

Fig. 20. Relapse rate in patients with MUD and MRD transplants

OS in patients with MRD transplants during 12 months was 76%, and 73.2% in patients with MUD transplants, and we did not confirm any significant difference, with p= 0.5385 (Figure 21).

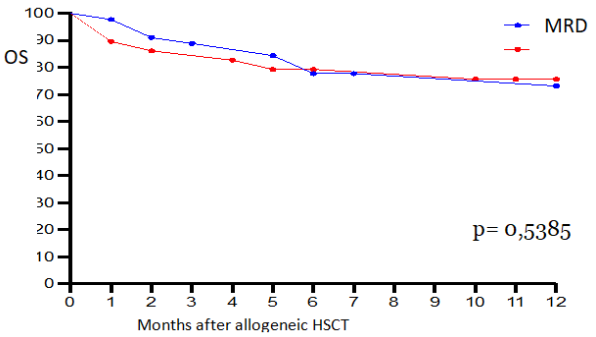

Fig. 21. OS in patients with MRD and MUD transplants

Discussion

Probably every hematologist facing a patient with acute leukemia from the begging of the treatment somehow intends and hopes that the management at the end will lead to performing allogeneic HSCT. This is mainly explained by the fact that this therapeutic procedure has the biggest potential to cure the malignant hematological disease, and to a large extent this is due to the immunologically based GVL effect. So, it is recommended that HLA typing should be done early when facing a leukemia patient, searching for a potential sibling donor of HSC in the family. But often a sibling donor is not available. In these cases, there is a possibility to search for unrelated donor in the world registry of potential donors of HSC. And, even if there is no potential unrelated donor, then haploidentical transplantation is a possibility. However, sometimes we all ask ourselves whether the choice of other donors, despite a sibling donor, is justified.

In our analysis, there was no significant difference among patients regarding sex, 57.6% were males, and 42.4 % females. The median age of the patients was 39.5 years. The majority were diagnosed with AML, and the higher incidence of this disease detected in the adult population when compared to ALL. When an allogeneic HSCT in a leukemia patient is planned to be done, it is recommended that the disease is in CR. In our group of patients, 80% were in CR, but we also included 20% of patients with active disease, where we tailored the immunosuppression to a shorter period of time inducing higher GVL effect. In 3 patients where we confirmed an impending relapse according to the molecular and chimerism analyses we gave donor lymphocyte infusions (DLI). All of this was done and justified to maintain the disease under control as well as to give patients a chance of better survival, knowing that active disease mainly meant refractory disease to the applied chemotherapy regimens. Also, 76% of patients were with intermediate or poor prognosis.

The majority of patients were transplanted in CR-1 and that explains the fact that our center tends to do an allogeneic transplant in patients with leukemia as a must do in the management of patients. A larger number of patients, 94.1%, were conditioned using MAC, mainly Bu-Cy +/- ATG.

When we do a MUD allogeneic transplant, there is a fear of greater percentage of GvHD, and greater extent of GvHD. But, our analysis did not show a significant difference of GvHD rates. In MRD, the rate of GvHD was 30.4% and in MUD 27.6%. This might be explained by the fact that in MUD, in the conditioning regimen ATG is mandatory, while in MRD allogeneic transplant, we used ATG in selected patients. In fact, we used ATG in 54.1% of patients. The analysis between ATG and non-ATG patients showed a significantly lower rate at any grade of GvHD in ATG group where GvHD rate was 21.7%, and in non-ATG group 38.5%. This approach did not worsen the infectious complications, and hance, did not have a dismal effect on TRM. In fact, when we analyzed the OS, it was statistically better in ATG group compared to non-ATG group (p value 0.0067). Even data of the multifactorial analysis showed that ATG, among other factors, had significant influence on OS of patients.

The OS of all patients at 24 months was 55.6%, having in mind that we included both patients with ALL and patients who were not in CR. When we analyzed the OS separately, according to the donor type, the results showed that in a period of 24 months, there was no statistical significance in the OS between the unrelated and sibling donor transplants. However, this was not the case with haploidentical transplants, where the OS was significantly worse. DFS, for the same time interval, was significantly better in the MUD allogeneic transplantations.

The overall transplant-related mortality (TRM) was 13.3%. But when we divided the TRM according to the donor type, we did not confirm a statistical significance in the difference between MRD and MUD transplants (P value 0.1422).

One of the main reasons for failure of allogeneic transplant in leukemia patients is disease relapse. Usually, the relapse is characterized by a very poor prognosis if reinduction of CR is attempted. So, it is best if relapse is somehow prevented, either by tailoring the immunosuppression therapy, using targeted therapy or using DLI. We analyzed the relapse rate in our patients, expecting that it would be higher in MUD transplants because of the standard use of ATG in the conditioning regimen. But, the results showed no statistical difference in the relapse rate, or to be more precise, it was 28.5% during a period of 24 months in patients with MUD/haploidentical transplantation, and in MRD transplantations (p value 0.4764).

The OS in patients with MRD transplants during 12 months was 76%, and in patients with MUD transplants 73.2%; we did not confirm any significant difference (p=0.5385).

Conclusion

Allogeneic HSCT is well established therapeutic option and an imperative in treating leukemia patients. In lack of sibling donors, MUD allogeneic HSCT is an adequate alternative providing effective therapeutic approach, making the haploidentical HSCT the least preferable option.

Conflict of interest statement. None declared.

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MMP

Original article

THE EFFECT OF ATOSIBAN BEFORE FRESH EMBRYO TRANSFER IN PATIENTS WITH REPEATED EMBRYO IMPLANTATION FAILURES

ЕФЕКТОТ НА АТОСИБАН ПРЕД СВЕЖ ЕМБРИО ТРАНСФЕР КАЈ ПАЦИЕНТКИ СО ПОВТОРУВАЧКИ НЕУСПЕШНИ ИМПЛАНАЦИИ НА ЕБРИОНИТЕ

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Abstract

Patients with recurrent implantation failure (RIF) may have more uterine contractions. Several studies suggested that atosiban administration before embryo transfer resulted in higher pregnancy rates in RIF patients. This study aimed to examine the effects of atosiban as oxytocin antagonists given before fresh embryo transfer (ET) in patients with RIF on pregnancy outcomes.
A retrospective clinical study was performed. A total of 160 infertile women with RIF undergoing *in vitro* fertilization (IVF) with fresh embryo transfer were allocated into the atosiban (n=80) and the control (n=80) groups. All patients had infertility due to tubal factor, hormonal anovulatory disorders, male factor or unexplained reasons. The treatment group received intravenous administration of atosaban about 30 min before embryo transfer with a bolus dose of 6.75 mg over 1-2 minutes. In the control group, the same number of cycles atosiban was not administrated before ET. The clinical pregnancy rate per cycle and implantation rate per transfer were 37.5% and 22.78% in the atosiban-treated group, which were significantly higher than in the control group (21.25% and 13.58%, respectively, P=0.01). The miscarriage rates of groups 1 and 2 were 16.66% and 27.27%, respectively (P=0.01). These results have indicated that atosiban increase the implantation rate and pregnancy rate after IVF in fresh ET cycle for women undergoing repeated embryo implantation failures. These results suggest that atosiban treatment before embryo transfer is effective in priming of the uterus for implantation and could improve pregnancy outcomes in RIF patients.

Keywords: atosiban, recurrent implantation failure, fresh embryo transfer, pregnancy rate

Апстракт

Пациентките со повторувачки неуспешни имплантации (ПНИ) може да имаат поизразени контракции на утерус. Повеќе студии сугерираат дека давање на атосибан пред ембрио трансфер (ЕТ) кај ПНИ пациентки доведува до згоелмена стапка на бременост. Оваа студија имаше за цел да го испита ефектрот на атосибан како окситоцински антагонист даден пред свеж ембрио трансфер кај пациентки со повторувачки неуспешни имплантации во однос на постигната бременост.
За таа цел беше реализирана ретроспективна студија. Вкупно 160 жени со инфертилитет со повторувачки неуспешни имплантации кој беа во ин витро фертилизација (ИВФ) постапка со свеж ембрио трансфер беа поделени на група со атосибан (Н=80) и контролна група (Н=80). Суте пациентки беа со дијаноза за инфертилитет поради тубарен фактор, хормонски-ановулаторни нарушувања, машки фактор или идиопатски инфертилитет. Кај групата на пациентки која беше третирана беше администриран атосибан 30 минути пред ембрио трасферот во болус доза од 6.75 мг во период од една до две минути. Во контролната група која ја сочинуваа жени со ист број на циклуси не примаа атосибан пред ЕТ. Клиничката стапка на бременост и стапката на имплантација по циклус беа 37.5% и 22.78% во групата со атосибан што беше сигнификатно повиско отколку кај контролната група (21.25% и 13.58%, за Р= 0.01). Стапката на абортуси кај групата 1 и 2 беа 16.66% и 27.27% (Р=0.01). Овие резултати индицираат дека атосибанот ја зголемува стапката на имплантација и стапката на бременост после ИВФ со свеж ЕТ кај жени со повторувачки неуспешни импланатации на ембриони. Овие резултати сугерираат дека третманот со атосибан пред ембрио трансфер има ефект во припрема на утерусот за имплантација и може да го подобри постигнувањето на бременост кај пациетни со ПНИ.

Клучни зборови: атосабан, повторувачки неуспешни имплантации, свеж ембрио трансфер, стапка на бременост

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Introduction

In vitro fertilization-embryo transfer (IVF-ET) is an assisted reproduction technology in which oocytes are extracted from an infertile woman and fertilized *in vitro*. The resulting embryo is then transferred into the uterine cavity [1]. Although tremendous progress has been made in the IVF-ET technique, the delivery and pregnancy rates associated with it remain low, at approximately 20% and 29%, respectively; in Europe and USA 32% and 39%, respectively, in the USA [2,3]. Determining how the pregnancy rate can be increased and how improved pregnancy outcomes can be achieved are issues that need to be solved by every reproductive center. It is widely believed that the quality of the embryos and the conditions in the intrauterine environment play vital roles in the success of IVF-ET [4]. However, women who experience repeated implantation failure (RIF) do no achieve successfully pregnancies despite the use of high-quality embryos. Those failures may result in tremendous mental and economic pressure on these infertile couples. The definition of RIF is not standardized. It can be defined as a failure to achieve implantation in at least three consecutive IVF attempts when 1-2 high quality embryos are transferred in each cycle [5]. Several factors have previously been proposed as potential contributors to implantation failure [6]. The uterus in a non-pregnant state has dynamic characteristics as a result of the periodic contractions of the smooth muscles in the myometrium. The myometrium exhibits continuous contractility known as uterine peristalsis, contributing to the transportation of sperm and facilitating the successful implantation of the embryo [7]. It is suggested that decreased uterine peristalsis during the phase of implantation aids in the successful implantation of the embryo. However, in certain individuals undergoing *in vitro* fertilization (IVF) treatment, it has been proposed that abnormal uterine peristalsis during embryo transfer may contribute to the failure of implantation [8,9]. The occurrence of uterine peristalsis during embryo transfer (ET) has been found to be linked with reduced rates of clinical pregnancy in both fresh and frozen-thawed cycles of ET [8]. Oxytocin is an endogenous hormone synthesized by the hypothalamus and subsequently released by the posterior pituitary gland. Its primary function is to induce uterine contractions through the activation of cell membrane receptors [10]. Previous studies have identified the presence of vasopressin type V1a and oxytocin receptors within the myometrium of a non-pregnant uterus [11]. Moreover, the presence of oxytocin receptors in the non-pregnant uterus exhibits variability during the menstrual cycle, with lower levels observed during the follicular phase and higher levels during the luteal phase. Additionally, the expression of these receptors is enhanced by supraphysiological amounts of estradiol [12]. The reduction of uterine contractility in non-pregnant patients can be efficiently achieved through the inhibition of oxytocin receptors [6]. Atosiban is a receptor antagonist for vasopressin V1a and oxytocin. It selectively acts on the uterus to suppress uterine contractions and was initially used solely to prevent premature delivery [13]. In 2007, Pierzynski *et al.* were the first to use atosiban to facilitate IVF-ET in an animal model (rabbit). They also evaluated the effect of atosiban on human sperm motility in their study [14]. Their results indicated that clinical applications involving the application of atosiban for the proposed indications may be safe for embryos because it did not harm preimplantation rabbit embryo development or human sperm motility. The results of this embryo toxicity study encouraged Pierzynski *et al.* to proceed with a clinical experiment involving the use of atosiban in a woman with RIF in 2007 [15]. The woman was 42 years old and had a history of seven failed IVF-ET attempts. The atosiban decreased the patient's uterine contractile activity and resulted in successful embryo implantation and a normal twin diamniotic pregnancy. Pierzynski *et al*. were therefore the first to apply atosiban in IVF-ET in a clinical setting. Since then, atosiban has gradually been increasingly applied in IVF-ET, and a similar result was achieved in a case report conducted by Liang *et al*. in 2008 [16]. The first prospective study which showed that atosiban may benefit subjects with RIF undergoing IVF/embryo transfer with cryopreserved embryos was conducted by Lan *et al*. in 2012 [17].

The impact of atosiban on pregnancy outcomes in women undergoing IVF has been investigated in recent years, and more efficient sets of parameters have been evaluated, including the implantation, clinical pregnancy, live birth, miscarriage, multiple pregnancy and ectopic pregnancy rates. However, the findings failed to reach a consensus [18-22].

This study aimed to examine the effects of atosiban as oxytocin antagonists given before fresh embryo transfer (ET) in patients with RIF on pregnancy outcomes.

Materials and methods

A retrospective study was conducted. Participants with RIF came to the Department of human reproduction at Acibadem Sistina Hospital from January 2018 to April 2024 to perform the fresh IVF-ET cycle. The essential information and follow-up data of the pregnancy outcomes were collected from our electronic medical record system. A total of 160 patients were found to be eligible for this retrospective study. All included patients signed informed consent for IVF-ET and medication. All couples previously had been evaluated by day 3 hormone levels, preovulatory 2D/3D US evaluation, hysterosalpingography, semen analysis, and hysteroscopy and laparoscopy, if indicated. The age range of the women was 25-39 years. The predominant diagno-

ses were male factor, tubal factor or unexplained infertility. The inclusion criteria were that the participant had a medical history of RIF; normal basal FSH level per our laboratory (≤10 mIU/mL); the morphology of uterus was normal evaluated by the 2D/3D transvaginal ultrasound, hysterosalpingography (HSG) or hysteroscopy (HSC); and at least one or two top-quality embryos after IVF/ICSI. Exclusion criteria were: age more than 39 years; severe male factor (requiring testicular sperm extraction); endometriosis; endocrine disorders (hyperthyroidism or hypothyroidism, hyperprolactinemia, premature ovarian failure); diminished ovarian reserve (FSH >12 IU/ml); any evidence of clinically relevant systemic disease (e.g. diabetes mellitus type 1); congenital uterine anomaly or distortions of the uterine cavity or apparent endometrial pathologies (submucous myoma, synechia, polyps, etc.); hydrosalpinges and patients with difficult transfer. Women undergoing ovulation induction were routinely down-regulated with triptorelin acetate (Decapeptyl, 0.1 mg; ER-KIM, Ilac San.; 0.1 mg/d) or busereline acetate SC (Suprefact 7 ml; Aventis Pharma Deutchland GmbH; 0.5 mg/d) starting from the 21st day of the preceding cycle in long down-regulation protocol. The analogue was continued until the day of HCG. After the down-regulation, ovulation induction was performed by daily injections of 150-300 IU of highly purified human FSH (Fostimon, IBSA) or 150-300 IU of follitropin alfa (Gonal F, Serono) The ovulation was triggered by 10,000 IU of hCG (Choriomon 5000 IU Amp, IBSA; or Pregnyl, 5,000 IU amp, Organon Ilac San.) or by a subcutaneous injection of 250 μg recombinant human chorionic gonadotropin (Ovitrelle; Merk Serono, Germany), when there were at least three leading follicles with a diameter of >18 mm. After 34-36 hours, egg collection was performed by transvaginal ultrasound. *In vitro* fertilization or intracytoplasmic sperm injection and embryo transfer was performed in all patients. Luteal phase support was performed by progesterone (Utrogestan, 200 mg caps, one vaginal capsule three times per day; Besins I˙scovesco Lab., Paris, France or 8% Crinone gel one dose per day). To identify patients with potentially difficult ET in order to measure the uterine cavity depth and direction of the cervix and the uterus, on the day of first ultrasound axme for the control of ovarian stimulation, the distance between the fundus of the uterine cavity (end of the endometrial image) and the internal ostium of the cervical canal was measured by transvaginal ultrasound. Two to three days after oocyte recovery, usually two, but occasionally three embryos per patient were replaced depending upon the age of the patient, the indication for IVF, the number of previous IVF attempts, and the number and quality of embryos available for replacement. The highest quality embryos were selected for transfer, with quality being assessed based on cell number and number of cytoplasmic fragments. Embryos were classified as follows: grade 1: perfectly symmetrical with no fragmentation; grade 2: perfectly symmetrical with slight fragmentation (20% fragments) (23,24). Embryos of Veeck grades 1 or 2 were considered high quality.

The use of atosiban followed the patient's wishes, after informing patients of the possible effects, reactions and costs of atosiban. In addition to the medications used routinely in our IVF program, patients in the atosiban group received intravenous atosiban 30 min before ET with a bolus dose of 6.75 mg, within 1–2 min infusion time. Patients in the control group received treatment plan as similar as that in the atosiban group, except for the atosiban. All transfers were performed in the same IVF operating room suite with the patient in the lithotomic position, with a comfortably full bladder and the cervix was exposed using a bivalve speculum. The exocervix was cleaned and endocervical mucus was removed with a sterile catheter connected to a syringe containing culture medium. The catheter (Soft-trans embryo transfer catheter set, Sydney COOK, Australia) was first filled with transfer medium (Sydney COOK, Australia). Next, the transfer medium containing the embryos was loaded into the catheter between air bubbles, and finally more transfer medium was added (maximum total volume: 30 μl). Prior to embryo transfer, the endometrial thickness, the distance from the external cervical os to the fundal endometrial surface, and the point that the tip of the catheter should reach for embryo replacement were measured by means of transabdominal and transvesical (with full bladder) ultrasonography with Toshiba, diagnostic ultrasound system (Nemio SSA-550 A; Shimoishiogami; Japan), with real time transabdominal convex transducer 3.75 MHz. In order to facilitate this measurement, the speculum was withdrawn, if necessary, so that the external cervical os could be seen. Under direct transabdominal ultrasound guidance, the transfer catheter was then advanced through the endocervical canal into the lower uterine segment. Immediate identification of the catheter tip was essential to minimize motion of the catheter and avoid any impact on the endometrium. In all transfers, the medium containing the embryos was gently expelled into the uterine cavity under ultrasound monitoring, with the volume being sufficient to permit the ultrasonographic visualization of the transfer inside the uterine cavity, which was also facilitated by the presence of air bubbles ('transfer bubbles') between the embryos. The transfer and guide catheters were then slowly withdrawn as a unit and inspected for any retained embryos and to detect bleeding. The same transfer technique was maintained in all patients. Clinical pregnancy was defined as the presence of gestational sac by ultrasound with appropriately rising B-hCG levels. The implantation rate was the proportion of embryo trans-

ferred resulting in an intrauterine gestational sac, and miscarriage was defined as pregnancy loss before 12 weeks of gestation.

Statistical analysis

The collected data were input into a computer database, predefined according to a specially prepared form and software for the needs of the study. The data processing as well as their analysis was done with the statistical software Statistica for Windows. We used the $\chi 2$-test to compare qualitative variables, and Student's t-test and Mann-Whitney U-test to compare quantitative variables. Data are reported as means (± SD). The significance level was set at $P < 0.05$.

Results

The mean age ± SD of the patients was 33.32±4.46 years in the control group and 34.82±5.28 years in the treatment group. The causes of their infertility were: tubal factor, hormonal anovulation, unexplained infertility and subfertile male factor infertility. Both groups proved to be comparable regarding the main demographic characteristics, as well as the main cycles parameters (without statistically significant differences) (Table 1).

Table 1. Comparison between main demographic and baseline characteristics of patients in the atosiban and control treatment groups

Parameters	Atosiban group (n=80)	Control group (n=80)	t	Z	p
Age	34.82±5.28	33.32±4.46	-0.79		0.43
Duration of infertility (year)	6.11±3.60	5.83±7.35			0.124
Baseline FSH level (mIU/ml)	6.98±2.05	7.54±2.56	-2.36		0.58
Baseline LH level (mIU/ml)	4.36±2.37	5.12±1.13		4.58	0.78
Baseline estradiol level (pg/ml)	33.75±10.99	36.84±20.33		-0.09	0.92
Male factor of infertility	32(40%)	38(47.5%)	NS		
Tubal factor of infertility	20(25%)	17(21.25%)	NS		
Unexplained infertility	28(35%)	25(31.25%)	NS		

There were no significant differences between the two groups in terms of embryo transfer characteristics. Our results showed no significant differences between the two groups in terms of gonadotropin ampoules administered (33.9±11.4 in atosiban group *vs*. 32.2±12.6 in control group); estradiol levels on the day of HCG day (2157±756 in atosiban group *vs*. 1990±1085 in control group); mean of oocytes retrieval and embryo transferred (ET) (10.6±5.2 *vs.* 9.9±6.1; 2.2±0.6 *vs*. 1.9±1.6, respectively in atosiban group and control group, and grade of transferred embryos (Table 2).

Table 2. Comparison between patients' ovarian stimulation characteristics, oocyte retrieval; embryo transfer characteristics in the atosiban and control treatment groups

Parameter	Atosiban group (n=80)	Control group (n=80)	T test
Estrogen level on HCG day (pg/ml)	2157±756	1990±1085	NS
No of Gonadotropin ampules	33.9±11.4	32.2±12.6	NS
No of retrieved oocytes	10.6±5.2	9.9±6.1	NS
Endometrial thickness on ET day	11.7±3.6	10.4±2.9	NS
No of transferred embryos	2.2±0.6	1.9±1.6	NS

Table 3. Outcomes of IVF–embryo transfers in the atosiban and control treatment groups

Outcomes od IVF/ICSI-ET	Atosiban group (n=80)	Control group (n=80)	p
Implantation rate	37/158 (22.78%)	22/162 (13.58%)	0.01
Clinical pregnancy rate	30/80 (37.5%)	17/80 (21.25%)	0.01
Miscarriage rate	5/30 (16.66%)	6/22 (27.27%)	0.01

In the atosiban treatment group, the clinical pregnancy rate per cycle and implantation rate were 37.5% and 22.78%, respectively, which were significantly higher than those in the control group (21.25% and 13.58%, respectively; *P*=0.01). The miscarriage rate was significantly lower in the study group than in the control group (16.66% and 27.27%, respectively, *P*=0.01) (Table 3). No side effects of atosiban were encountered in patients.

Discussion

RIF is a challenging condition in the field of IVF. The present retrospective study analyzed the effects of atosiban on the pregnancy outcomes of patients with

RIF in IVF-ET cycles. IVF-ET is an advanced technology in dealing with the infertility [2,26]. Its appearance brought hope to the infertile couples. However, the success rate was not satisfactory. Successful IVF-ET was not only depended on the embryo quality, but also attributed to the intrauterine environment. It is estimated that >30% of patients would have uterine contractions to result in poor pregnancy [27-29]. In 1997, a study pointed out that abnormal endometrial peristalsis could reduce the implantation rate and clinical pregnancy rate [30]. One study had also found that frequent and strong uterine contractions 5 minutes after implantation could reduce the live birth rate [31]. Methods to reduce endometrial peristalsis and improve clinical pregnancy outcomes had become a hot topic in assisted reproductive technology (ART) in recent years. The other study had also found that, in the natural conception state, about 30% of embryos can be successfully implanted, while in IVF-ET, the success rate of embryo implantation was only 10-15% [32], which might be related to the hormonal effects and ET procedure.

Embryo transfer is the most delicate step in assisted reproduction treatment. All recommended measures in clinical practice of IVF-embryo transfer, such as manipulating embryos as gently as possible during IVF, avoiding maneuvers that might trigger uterine contractions, using soft catheters and progesterone administration starting on the day of oocyte retrieval, aim at minimizing the uterine contraction frequency on the day of embryo transfer [33,34].

Increased uterine contractions in IVF patients may be due to several factors. Matorras *et al*., 2004, showed the increased duration of embryo transfer catheter into the endometrial cavity gradually decreased the implantation and pregnancy rates by several mechanisms including the increased uterine contractions [35]. Fanchin *et al.*, 1998, reported that uterine contractions at the day of embryo transfer could also be triggered by intensive cervical manipulation [28]. Reports that difficult transfers and the provider performing the embryo transfer may negatively affect the success rate [36-38] raise concern that 'easy' embryo transfer may be a major determinant of a successful transfer. Furthermore, it was suggested that during embryo transfer, excessive movement of the catheter tip may lead to endometrial trauma [39,40] or transcervical embryo expulsion [41]. The present study excluded the patients with difficult transfer for the purpose of ensuring homogenization.

High concentration of circulating estradiol concentrations in IVF patients may also be a causative factor for increased uterine contractions. Richter *et al*., 2004, showed that high concentration of estradiol promoted the oxytocin effect on non-pregnant uterus through increased oxytocin receptor gene expression in the myometrium [42]. Uterine contractions at the time of embryo transfer have been associated with markedly lower implantation and pregnancy rates per embryo transfer [43]. Ultrasound images showed that the increased frequency of intimal peristalsis and various forms of intimal movement would lead to difficult implantation of embryos, miscarriage of implanted embryos during development and ectopic pregnancy [14,15]. So, it speculated that inhibiting contractions and reducing abnormal endometrial peristaltic waves could improve pregnancy rate. The reason of uterine contractions was attributed to the increased levels of oxytocin and PGF2a [12,44]. Oxytocin is a hormone produced by the hypothalamus and released by the posterior pituitary gland, which mainly induced the uterine contractions. PGF2a could also reduce the perfusion of endometrial blood flow. As a safe and effective PGF2a and oxytocin-antagonist, atosiban could compete against the oxytocin receptors on uterine myocytes and inhibit the production of prostaglandin F2a (PGF2a) to reduce the uterine contractions and increase the endometrial blood supply [45]. However, some studies also identified that the positive effects of atosiban was not widespread [21]. It might only improve the IVF pregnancy in patients with RIF or difficult transfers [46-48]. This phenomenon was also confirmed in our outcomes of fresh IVF-ET of patients with RIF.

Another important effect of atosiban is inhibiting the endometrial production of prostaglandin $F_{2\alpha}$, which was shown to decrease endometrial blood supply and embryonic survival in cattle [49]. Oxytocin receptor blockade, apart from the reduction of uterine contractions, is reported to inhibit the stimulation of uterine production of prostaglandins [50].

Additionally, atosiban has been demonstrated to preferentially relax uterine arteries of near-term pregnant rats, decreasing the systolic blood pressure, which in turn may increase uterine perfusion [51]. Such multidirectional modes of action of atosiban may be beneficial, providing a specific treatment for the improvement of uterine receptivity following embryo transfer.

In this study, the rates of miscarriage in the atosiban group were statistically significantly lower than those in the control group. This may have been due to the effects of atosiban on uterine contractility as well as its positive effects on the endometrial environment.

This is our first clinical study that used the administration of oxytocin antagonist before embryo transfer in IVF patients. It may have a beneficial effect on uterine receptivity and implantation providing a decrease in uterine contractile activity, an increase in endometrial perfusion and improvement in endometrial status. However, randomized, prospective placebo-controlled trials with larger series are needed to confirm the findings.

In conclusion, atosiban increases the implantation rate and pregnancy rate and decreases the miscarriage rate. Because it is uterine specific and embryo safe, atosi-

ban may constitute a new treatment opportunity in embryo-transfer procedures.

Conflict of interest statement. None declared.

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Case report

RESECTION OF THE LATE RECCURENCE OF PANCREATIC HEAD CARCINOMA AFTER WHIPPLE PROCEDURE IS SAFE, FEASIBLE AND OFFERS LONG-TERM PROGNOSIS IMPROVEMENT

РЕСЕКЦИЈАТА НА КАСНИОТ РЕЦИДИВ НА ПАНКРЕАТИЧЕН ЦЕФАЛИЧЕН КАРЦИНОМ ПОСЛЕ ВИПЛОВА ПРОЦЕДУРА Е БЕЗБЕДНА, ВОЗМОЖНА И НУДИ ПОДОБРУВАЊЕ НА ДОЛГОРОЧНАТА ПРОГНОЗА

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Abstract

Pancreatic cancer is a severe disease with poor prognosis, the seventh most common cancer cause for death in the world, and the third in the USA. Despite advanced procedures for diagnosis and management, 80% of patients develop recurrence, lethal in the first 2-5 years. One small subgroup with isolated recurrence was the objective of this study, in order to improve the prognosis.

In our practice, of a total of 223 patients who underwent duodenopancreatectomy, we staged 3 patients with resectable recurrent pancreatic carcinoma. Inclusion criteria for selection were: US, CECT scan, PET scan, CEA, CA 19-9, VEGF level. One patient had recurrence on the pancreatic stump, and 2 in the surrounding lymph nodes. They underwent surgery and re-resection of the specimen. The redo was a subtotal pancreatectomy and Roux-en-Y procedure.

There was no perioperative mortality, and morbidity in all 3 patients appeared to be a pancreatic fistula, grade A. Intrahospital stay was 12, 15, 17 days, median 15. Median overall survival was 32 months (14, 22, 61). In comparison with other studies, we had a better postoperative survival, but significantly lower rate of re-operated patients, 1.34% *vs*. 6.4%, due to late diagnosis. The median overall survival was significantly longer compared to patients treated with radio-and chemotherapy only.

Having in mind the available data and our results obtained from the small number of patients treated surgically so far, we can conclude that surgery in these patients with isolated recurrence of the pancreatic carcinoma is feasible, promising a significant improvement of the prognosis. To confirm this active attitude, a multicenter study is required for finding an evidence-based protocol for these patients.

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Keywords: pancreatic cancer, recurrence, re-resection, morbidity, survival

Апстракт

Панкреатичниот карцином е тешка болест со лоша прогноза, седма најчеста причина за смрт во светот, а трета во САД. И покрај напреднатите процедури и технологија за дијагноза и третман, 80 % од пациентите развиваат рецидив, летален во првите 2-5 години. Една мала група на пациенти со изолиран локален рецидив е предмет на оваа студија, со желба да ја подобриме прогнозата.

Во нашата праксам од вкупно 223 пациенти кои беа подложени на дуоденопанкреатектомија, ние одвоивме 3 пациенти со ресектабилен рецидивен панкреатичен карцином. Инклузивните критериуми за селекција беа: УС, КЕЦТ, ПЕТ скен, ЦЕА, ЦА 19-9, ВЕГФ маркери. Еден пациент имаше рецидив на панкреатичниот остаток, а 2 на околните лимфни јазли. Тие беа подложени на рересеција на рецидивот. Реоперацијата се состоеше од субтотална панкреатектомија и Ру ен У процедура.

Немаше пероперативна смртност, а морбидитетот кај сите 3 пациенти се експонираше како панкреатична фистула, тип А. Интрахоспиталниот престој беше 12, 15, 17 дена средна вредност 15. Средно вкупно преживување беше 32 месеци (14, 22, 61). Во споредба со други студии, ние имавме подобро постоперативно преживување, но значајно понизок процент на реоперирани пациенти, 1,34 % спрема 6,4%, пред и се поради доцната дијагноза. Средното вкупно преживување беше значајно подолго во споредба со пациентите третирани само со радио-хемотерапија.

Имајќи ги предвид достапните податоци и нашите резултати добиени од мала група на пациенти кои досега ги третиравме хируршки, можеме да заклучиме дека хируршкиот третман на овие пациенти со изолиран ресецибилен рецидив на панкреатич-

ниот карцином е возможен и ветувачки за значајно подобрување на прогнозата. За да се потврди вакви от активен став потребна е мултицентрична клиничка студија, за да се пронајде протокол базиран на доказ за овие пациенти.

Клучни зборови: панкреатичен карцином, рецидив, ререскција, морталитет, морбидитет, преживување

Introduction

Pancreatic carcinoma is the seventh most common cancer-related cause for death annually in the world, and the third for cancer-related deaths in the USA. Prognosis of patients suffering from pancreatic ductal adenocarcinoma (PDA) is poor; after diagnosis 24% of patients survive 1 year, and 9% survive 5 years. The incidence is variable, i.e., in Europe it is 7.7/100,000, in North America 7.6/100,000 inhabitants. It is more common in males than in females: 5.5 *vs.* 4.0/100,000. Because of the obscure, torpid appearance and course of the disease as well as demanding sophisticated means for disclosure, the diagnosis is often made in an advanced stage, decreasing the possibility for cure and worsening the long-term prognosis. The desirable early diagnosis makes feasible the radical curative treatment, improving the overall survival (OS). Namely, 40 years ago, the 5-year OS was 5%; 20 years ago, it was 20%; and nowadays, up to 40%. There are series of treated patients referring a 5-year survival up to 67%. The treatment is variable due to the stage at diagnosis. It consists of neoadjuvant therapy, surgery, adjuvant therapy. The main role in this treatment is indubitably a radical surgical treatment-pancreatectomy. It may be: proximal duodenopancreatectomy -Whipple procedure (DP), distal pancreatectomy, total pancreatectomy, depending on the topographic presentation of the tumor.
A certain period of time after surgery and completed adjuvant treatment, majority of patients develop and suffer from a recurrent pancreatic carcinoma (RPC), reported in up to 80%. It is usually disseminated, incurable and a cause for death. In a small group of patients, this recurrence is local and isolated, limited to the operative site. It usually emerges in the local lymph nodes, surrounding tissue, or pancreatic remnant, although R0 resection has been reported on pTNM. Such a localized RPC may offer a slight hope for further surgical treatment and expanding the expected disease-free life.
The aim of this study was to present the treatment of this group of patients with resectable and due to this curable RPC, in order to provide further disease-free period of life, and consequently to improve the prognosis of the disease. Nevertheless, a small number of patients with RPC are referred and involved in clinical trials to provide evidence-based data and to enable decision for further management and treatment [2].

Material and methods

In a period of 8 years, we had 223 patients suffering from PDAC, with cephalic presentation. They were submitted to DP, Whipple procedure (WP). From the group who underwent an extensive duodenopancreatectomy (EDP), we analyzed 3 male patients with resectable RPC. The inclusion criterion was diagnosed solitary recurrence, confirmed with CE angio-CT, MRI, and elevated level of Lewis tumor marker CA 19-9, VEGF. Concerning the age and topography of the RPC, these 3 patients were:
65 yrs. - patient 1 (P1) with RPC on the pre aortal lymph nodes, and with infiltration-abutment of the efferent jejunal loop, also the superior mesenteric artery (SMA),
69 yrs.- patient 2 (P2) with RPC on the pancreatic remnant, and
73 yrs. - patient 3 (P3), with RPC in peri pancreatic-retro anastomotic lymph nodes and with infiltration-interface of the portal vein (PV) - superior mesenteric vein (SMV).
The implied procedure was re-resection of the RPC, with subtotal pancreatectomy-blind stump, with perioperative frozen section probe, and the reconstruction with Royx-en-Y limb of the digestive tube (Figure 1). In the first patient (P1), the re-resection was also extended with the efferent hepaticojejunal loop, because of the infiltration by the recurrence (Figure 2). In the same patient, a tangential resection and reconstruction of the SMA was imposed at the emerging site of the first jejunal artery.

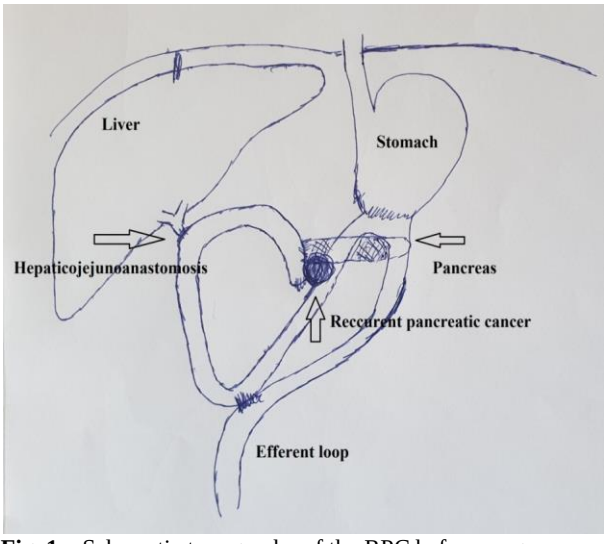

Fig. 1a. Schematic topography of the RPC before surgery

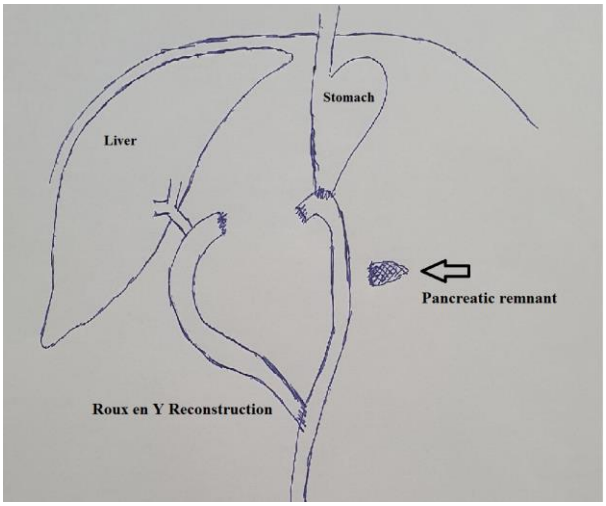

Fig. 1b. Reconstruction of the site after surgery and extirpation of the RPC

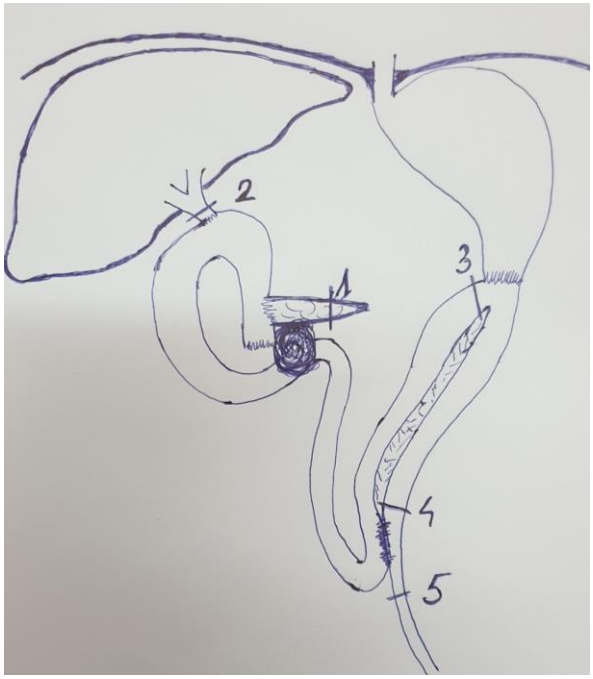

Fig. 2. Specific re-resection procedure in patient P1, with extirpation of the entire previous reconstruction, with 5 re-resection lines: 1. Re-resection line on the pancreatic remnant, 2. Re-resection line on the common hepatic duct, 3. Re-resection line on the afferent hepato-gastric loop, 4. Re-resection line on the efferent gastrojejunal loop, which later goes to Y anastomosis, 5. Distal re-resection line on the efferent jejunal loop, which later goes to hepatojejunoanastomosis

Results

In our series of 223 patients with PDA with cephalic presentation, submitted to DP, WP, 120 underwent a standard duodenopancreatectomy (SDP) with D2 lymph node dissection, while 103 underwent extensive duodenopancreatectomy (EDP) with D3 dissection. In the SDP group of patients, there were 61% males (73 of 120), and 39% females (47 of 120). In the EDP group, 65% (67 of 103) were males and 35% (36) were females. The recruitment was done at the Clinic for Gastroenterohepatology, after US, CECT scan, PET scan, laboratory findings of CEA, Lewis CA 19-9, and VEGF. Inclusion criterion for surgery was operable-resectable PDA, and/or borderline resectable by the subclassification according to SMV/PV invasion, by the protocol and consensus of the ISGPS, JPS, NCCN. Of the total sample (223), 15 patients had borderline resectable carcinoma (BRC), and all of them received EDP. Eight of these 15 patients were subjected to portal vein resection, either longitudinal resection with direct suture without graft, or transversal resection with direct anastomosis, also without venous graft.

Patients with SDP, after certain months of disease-free life, grossly developed disease relapse, mainly metastases in the liver, peritoneal dissemination, or lung metastases, either or not associated with local recurrence; thus, the malignancy recurrence in these cases after follow-up was inoperable and incurable.

In the EDP group, 3 patients developed localized RPC, 2 cases were in local lymph nodes, while 1 was on the pancreatic remnant. These 3 patients, all males, were 65, 69 and 73 yrs. old, and like all others, were treated with chemotherapy by protocol after primary surgery (Table 1).

The onset of the RPC in these three patients, ending disease-free life, was 16 months, 20 months, 23 months after primary surgery (Table 1). Two of them were subclinical, and one patient suffered from jaundice, mild discomfort and tender in the upper abdomen. The jaundice was amid the infiltration of the efferent hepaticojejunal loop with obstruction, by the RPC.

Patients underwent US, CECT scan, and PET scan, verifying the local recurrence without further distal dissemination of the disease, and feasible for surgery.

In all 3 patients, the Lewis CA 19-9 was elevated, in 2 of them VEGF. After the verification of the operability, patients did not undergo another neoadjuvant chemotherapy for the RPC. They were submitted to surgery, and operated. The surgery consisted of massive adhesiolysis, dissection of the RPC-specimen, extirpation, and further reconstruction. The extirpation of the specimen in all three was re-resection of the entire pancreatojejunoanastomosis, proximal resection of the remnant of the pancreas to subtotal pancreatectomy, without pancreatojejunoanastomosis-blind stump. In 2 cases resection of the efferent pancreaticojejunal loop was proximally from and with preservation of the hepaticojejunoanastomosis. In patient 1, suffering from obstructive jaundice, a complete efferent hepaticojejunal loop was also resected, altogether with the hepaticojejunoanastomosis. In the same patient, a partial-marginal resection of the superior mesenteric artery (SMA) was imposed due to infiltration of the RPC, exactly at the origin site of the first jejunal artery, with primary reconstruction (Figure 3). The digestive reconstruction was carried out with Roux-en-Y procedure, in all three cases (Figure 1b.). As mentioned before, one

Table 1. Patients' characteristics

Patient	Age	Staging pTNM at the first op	Type of procedure, number of lymph nodes retrieved	Other treatments	Disease free period (months)	Topography of the recurrence	Post-recurrence procedure	Other treatment after re-resection
1.	65	IIb	Extended Whipple procedure, 28 lymph nodes	Chemotherapy postoperatively	16	Preaortocaval lymph nodes	Re-resection of the pancreatojejunal anastomosis+hepaticojejunal anastomosis+resection of the SMA	Chemotherapy postoperatively
2.	69	IIIa	Extended Whipple procedure, 26 lymph nodes	Chemotherapy postoperatively	20	Pancreatic stump	Re-resection of the pancreatojejunal anastomosis	Chemotherapy postoperatively
3.	73	Ib	Extended Whipple procedure, 21 lymph nodes	Chemotherapy postoperatively	23	Peripancreatic lymphatic tissue, station 18	Re-resection of the pancreatojejunal anastomosis, portal vein resection	Chemotherapy postoperatively

PRC was on the pancreatic stump (patient 2), one was on the peripancreatic lymphatic tissue (patient 3), 18 station bellow the inferior edge of the pancreas, with dorsal abutment-shift of the portal vein, leading to a complete dissection and partial portal vein resection with direct reconstruction, and patient 1, with RPC at the preaortocaval lymph nodes, 16 station, with infiltration of the efferent jejunal loop (jaundice), also first jejunal artery leading to a complete dissection and partial SMA resection with direct reconstruction. We avoided total pancreatectomy; the estimation was to facilitate the postoperative course and management, despite some authors who propose a total pancreatectomy, and refer good results thereafter.

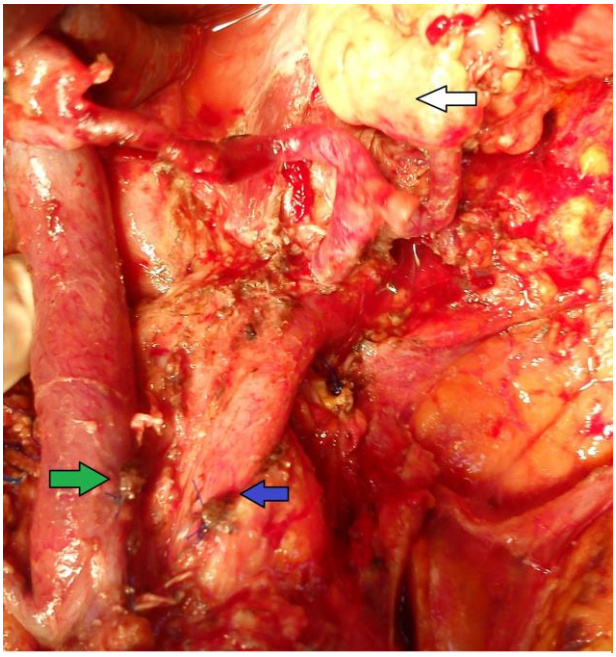

Fig. 3. Partial-tangential resection of the SMA, with primary reconstruction-suture Pancreatic remnant-blind stump after subtotal pancreatectomy, white arrow, Resected and reconstructed SMA, blue arrow, Resected and ligated splenic vein, green arrow

All three patients survived; there was not peroperative mortality. In all three, the morbidity was expressed with present postoperative pancreatic fistula (POPF) A. The POPF was pancreatic occlusion failure (POF) type, explaining the expected desirable postoperative course. In 2 patients, there was a wound infection, and one suffered from delayed gastric emptying. There were no other complications or advert events affecting the morbidity.

Two of the patients with low outlet POPF were dismissed on the 15th and 17th postoperative day, after removal of the drains. The third patient had high outlet of the POPF, over 200 ml/24 h, and was released on the 12th postoperative day with remained soft silicon drain in the neighborhood of the pancreatic stump, presenting controlled POPF (POF) type A. After 3 weeks and decreased outlet, the drain was removed. The postoperative pathohistology findings were R0 in 2 patients, and R1 in patient 2, although perioperative frozen section probes were R0 in all three.

Concerning the survival, the patient with R1 at the reoperation, 11 months after this re-surgery presented with advanced stage of the disease, liver metastases, and died 14 months after surgery. The second one, had 15 months of disease-free period, overall survival of 22 months, and perished with peritoneal dissemination, while the third is still alive, disease-free, 110 months after surgery. The average, medial postoperative overall survival is approximately 48 months.

Discussion

Pancreatic cancer is a severe disease, leading to unsatisfactory outcome after all efforts and improvement of

the management so far. Pertinent data refer 24% survival for 1 year, bellow 10% for 5 years overall [1]. Disappointing data suggest 80% of the radically operated patients who develop RPC, which is even worst for cure, management, and prognosis [1-3]. The treatment of these patients with RPC is still interfered with bias, ambiguity, and insufficient confidence affecting the decision for further treatment. Also, one major group of patients after DP, with high potent malignancy of the PDA, express RPC in the first 6 months, during the adjuvant chemotherapy, defined as local-pancreatic stamp, lymph node, or distant metastases, which is very common for R1 resection [3]. When the relapse of the disease is disseminated with distal metastases, the option is clear, chemotherapy and radiotherapy [4], **but the big dilemma is what is to be done with patients with localized-isolated RPC?** A highly demanding surgical procedure and technique on one hand, and uncertain perioperative and postoperative outcome and benefit on the other hand, lead most of the clinicians to negative attitude concerning surgery of RPC. Therefore, so far, a multimodal radiochemoterapy has been proposed [4]. The lack of data and clinical trials involving these particular patients devoid us from a clear protocol for further management, i.e., surgical treatment. The main ambiguity concerning surgery of RPC is: Does surgery contribute to be of benefit for patients' quality of life, or does it improve OS? One retrospective analysis from 2009, (PRISMA-Preferred Reporting Items for Systematic reviews and Meta-Analysis), revealed the extension of the OS after surgical treatment of the isolated local RPC to 26 months, disease-free period 14 months on average, comparing to multimodal radio and chemothrerapy treatment only, where median survival was 14 months [5]. In our study, patients had an average 48 month-postoperative medial survival after surgical removal of the RPC (14, 22, 110 months). On the other hand, we had only 3 resectable RPC from 223 cases, which is 1.34%, while in other studies there were 6.4%, 82 from 1281 cases [5]. This outcome for the postoperative OS in our small series is likely to be better comparing to other centers and studies (48 *vs.* 26), and particularly may be amid early diagnosis of the RPC in these three patients, which was at the time of asymptomatic onset of the recurrence (with an exception of one patient, suffering from jaundice because of the efferent loop obstruction), based on increased level of tumor markers (CA 19-9, VEGF), PET scan and CECT, without gross infiltration of the surrounding tissues. Also is inevitable good quality control of the radicality of the re-do procedure, maintained in one single attending surgeon. On the other hand, a significantly smaller rate of resectability reveals late diagnosis generally, when surgery is no more applicable and beneficial. Another study was conducted on 17 patients re-operated for suspected or proven RPC. Of 17, re-resection was possible in only 5 patients, while others underwent a palliative procedure. Of these 5, 3 were proven chronic pancreatitis postoperatively, while only 2 RPC. Only 2 of 14 patients with malignancy were operable and feasible for re-resection and re-anastomosis [6]. Because of the high perioperative mortality (6 %) in this series, this study would suggest a higher level of selection of patients undergoing surgery for RPC. Improved selection and more strict inclusion criteria may be achieved by EUS guided biopsy, PET Scan, tumor markers, etc., thus avoiding re-do surgery for benign reasons.

One German study revealed encouraging data. Patients with re-resection of the isolated RPC, had a significantly longer median survival after resection, 26.0 months, in comparison with non-resected patients, 10.8 months, ($p=0.0104$). This outcome favors the decision of aggressive approach if isolated RPC is diagnosed, as a feasible and curative procedure with low perioperative morbidity (25%) and mortality (1.8%) [7].

Moreover, probably the most beneficial and promising subgroup of these patients are those with RPC of the pancreatic stamp. In this series, six patients with isolated RPC of the stump were submitted to total pancreatectomy. There was no morbidity or mortality in the series. The median survival after reoperation was 27.5 months, also significantly higher than in non-operated patients [8].

Another similar retrospective study was conducted on 11 patients, suffering from RPC of the pancreatic stump. After the completion of the pancreatectomy, the result of the median survival was even better and more encouraging, up to 44 months [9-11]. It is also important to mention that there was no significant difference in the mortality between the results in the studies, (Fisher's Exact Test = 1.622), $p>0.05$ ($p=0.540$).

These promising results, although from a small series and various centers, motivated the clinicians to observe and much more freely and easily to adopt the aggressive and beneficial attitude to RPC [10,11]. In order to improve the management and prognosis in these patients, surgeons have made a further step, an aggressive approach in patients with solitary lung and liver metastases, which would be an issue for discussion in the future [12]. The only absolute contraindication remains peritoneal carcinosis.

Comparing the results from our series to those referred by other studies, we support and adopt the aggressive attitude to radical surgical treatment for isolated RPC. The main goal should be the improvement of OS, and particularly disease-free period. Moreover, the firm suggestion for surgery is low morbidity and mortality, despite the demanding and challenging procedure (in our series none).

Conclusion

The results regarding the active surgical attitude to

isolated RPC overwhelm the conservative approach with radio-and chemotherapy only. The meticulous dissection, extirpation of the RPC with R0, malignancy-free margin, is the aim which imposes to be reached. It is a safe and feasible procedure and treatment in specialized institutions with experienced surgical teams. The mainstream favoring this approach are the obtained data-results for median and overall survival after radical re-operation.

However, the number of patients treated in this manner is still small, and further high volume clinical multicenter trials would have to be conducted in order to achieve consensus concerning this management.

Finally, the additional issue we observed, supplementing this ensemble of topics of RPC is the extension of the primary done DP. In our series, all three patients with resectable RPC were recruited from the extensive duodenopancreatectomy group with extended lymph node dissection, D3. This fact, despite the small series, may suggest the extensive duodenopacreatectomy leads to a higher frequency of resectable RPC, an observation also deserving further high clinical trials.

Conflict of interest statement. None declared.

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Case report

RHABDOMYOLYSIS IN PATIENT WITH INFLUENZA A - CASE REPORT:

РАБДОМИОЛИЗА КАЈ ПАЦИЕНТ СО ИНФЛУЕНЦА А

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Abstract

Introduction. Rhabdomyolysis is a serious condition characterized by muscle breakdown. Common causes include exertion, crush, and drugs. Infection, many more viral infections such as influenza A and B, coxsackie viruses, Epstein-Barr virus, herpes simplex, adenovirus, echovirus, HIV, and cytomegalovirus are also recognized as the reason for rhabdomyolysis.
Case report. We present a case of a 47-year-old male patient with fever, muscle pain, and dark urine whose nasopharyngeal swab detected Influenza A infection. Initial laboratory analysis revealed extremely elevated levels of creatine kinase (CK) necessitating hospital treatment. Following a ten-day course of treatment, the patient was discharged without complications such as acute renal failure or myositis.
Conclusion. Influenza A is a common infection that causes outbreaks and epidemics in cold months that do not always cause respiratory complications. Rhabdomyolysis is a rare but serious complication that should be recognized and treated because of the high risk of morbidity and mortality.

Keywords: Influenza, rhabdomyolysis, creatin kinase, myoglobin

Апстракт

Вовед. Рабдомиолиза е сериозна состојба при која доаѓа до брз распад на мускулите. Најчести причини се тежок напор, краш синдром, употреба на лекови. Од инфективни причинители, вируси како инфлуенца А и Б, коксаки, Епштајн-бар вирус, херпес симплекс, аденовирус еховирус, ХИВ, цитомегаловирус исто така се вбројуваат во причини за рабдомиолиза.

Приказ на случај. Ви претставуваме случај на 47-годишен маж со покачена температура, болки во мускулите и темна урина на чиј назофарингеален брис беше детектирана инфлуенца А. Во иницијалните лабораториски анализи беше нотирана екстремно висока вредност на креатин киназа (СК) по што следеше прием во болница за хоспитален третман и следење. По десет дена хоспитален престој, пациентот беше испишан без посериозни компликации како што се акутна бубрежна слабост или миозит.
Заклучок. Инфлуенца А е честа причина за појава на епидемија во студените месеци која не секогаш предизвикува респираторни компликации. Рабдомиолизата е ретка, но сериозна компликација од инфлуенца вирусот која треба да се препознае и лекува поради високиот ризик од морбидитет и морталитет.

Клучни зборови: инфлуенца, рабдомиолиза, креатин киназа, миоглобин

Introduction

Rhabdomyolysis is a complex medical condition involving the rapid dissolution of damaged or injured skeletal muscle. As a result, intracellular muscle components are released into the bloodstream, including myoglobin, creatine kinase (CK), lactate dehydrogenase, and electrolytes [1]. The clinical presentation may vary, ranging from an asymptomatic increase in serum levels of enzymes released from damaged muscles to conditions such as volume depletion, metabolic and electrolyte abnormalities, and acute kidney injury (AKI). The diagnosis is confirmed when the serum creatine kinase (CK) level is >1000 U/L or at least 5x the upper limit of normal. Other important tests to request include serum myoglobin, urinalysis, and a full metabolic panel including serum creatinine and electrolytes. The trigger for this condition could be from different etiology like toxins, drugs, and exertion. Acute viral infections with influenza A and B, coxsackie viruses, Epstein-Barr virus, herpes simplex, parainfluenza, adenovirus, echo-

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virus, HIV, and cytomegalovirus are associated with rhabdomyolysis [2]. The mechanisms of developing rhabdomyolysis by influenza virus are still elusive. One of the mechanisms proposed is that muscle damage was caused by viral invasion directly or indirectly through the induction of an immune-mediated action [3]. Prompt recognition of rhabdomyolysis is important to allow for timely and appropriate treatment [4]. We present a case of a male patient with rhabdomyolysis and influenza A infection.

Case presentation

47-year-old male patient came into our department with myalgia, cough, and fever lasting for three days. First day he was examined by a doctor but third day from the onset of symptoms he noticed dark urine along with generalized skeletal muscle pain severe enough to impair walking which was the motive for his medical examination in our emergency ambulance. The patient has a history of bronchial asthma on therapy with bronchodilators and hyperlipidemia controlled with statins. On admission, he appeared in moderate distress. His vital signs were as follows BP 115/70 mmHg, HR 95/min, temperature was 37.8, oxygen saturation was 95% on room air. The physical examination demonstrated dehydration, general weakness, and mild muscle tenderness. Auscultation revealed characteristic wheezing. Chest radiography showed no signs of respiratory complications (Figure 1).

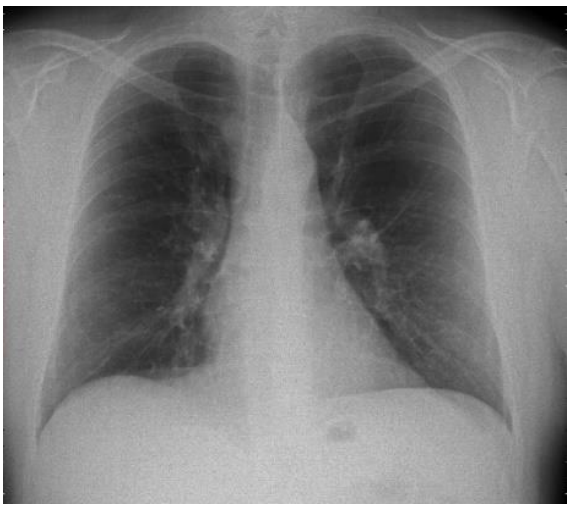

Fig. 1. Chest radiography of patient

His therapy with statins was discontinued. Initial laboratory evaluation shows an extremely high level of creatine kinase CK-100 420 U/L lactate dehydrogenase LDH 3101 IU/ml myoglobin 4753, uroanalysis shows the presence of red blood cells in sediment. His nasopharyngeal swab detected Influenza A. Laboratory findings of elevated creatine kinase, myoglobin levels, and uroanalysis in conjunction with clinical symptoms, supported the diagnosis of rhabdomyolysis. We started therapy with oral neuraminidase inhibitor Oseltamivir and intensive parenteral hydration with crystalloid solutions to prevent acute kidney injury (AKI). Ultrasonography of the abdomen and urinary tract was performed, and no signs of kidney damage were observed. By the third hospital day, urine become with normal color and the value of CK and myoglobin was lowered. Hydration was crucial for promoting diuresis and preventing renal injury, while antiviral therapy aimed to suppress influenza viral replication. On day eight patient were examined by neurologist and electromyography (EMG) and somatosensory evoked potentials SSEP were performed. The result goes in addition to a chronic parcel radical lesion of L3 and L5 levels mutually asymmetrically of a medium degree. Collateral reinnervation has been established. Additionally, m.deltoideus l.sin registered a myopathic route suggesting a primary muscle lesion. This result suggests that there was no danger of myositis or polyradiculopathy. The trend of blood investigation at admission, during the hospital stay and at discharge is shown in Table 1. Despite extremely high levels of pointed enzyme urea and creatinine were in the referent values all the time. On day ten patient was discharged home with creatin kinase 606 U/L and myoglobin 105 U/L/, clinically stable with no signs of

Table 1. Sequential laboratory results during hospitalization

	Reference values	1 day	2 day	3 day	5 day	9 day	10 day
Hb*	120-180 g/l	163		134	133	123	124
Le*	4-10 x 10^9/l	5.10		2.60	4.50	4.10	4.10
ALT*	10-51 U/L	85	87	97	95	62	55
AST*	10-47 U/L	535	607	658	336	72	47
CK*	30-170 U/L	100420	99717	84927	18652	1484	606
CK MB*	<16 U/L		953	795	187	30	23
LDH*	120-246 IU/ml	3101	3073	2356	371	181	196
Myoglobin	<75 ng/ml		4573	3403	787		105
urea	1,7-8,3 mmol/l	5.8	3.9	2.6	3.2	2.9	2.6
Creatinine	62-133 μmol/I	82	68	64	62	63	61
CRP*	0-10 mg/l	18		4	1	1	

Hb*-Hemoglobin, Le*-Leukocytes, ALT*-Alanin aminotransferase, AST*-Aspartate aminotransferase,

CK*-Creatin kinase, CK MB*-Creatine kinase MB, LDH*-Lactat dehydrogenase, CRP*-C reactive protein

further complications. It appears that the patient experienced rhabdomyolysis, likely as a complication of influenza A virus, leading to dark urine and myalgia. However, with appropriate management, including hydration and antiviral therapy, the patient recovered without long-term complications. At the follow-up two weeks after he was feeling well and the laboratory findings showed creatine kinase level in referent values - CK 125 U/L (Table 1).

Discussion

The most common cause of virus-associated rhabdomyolysis is influenza [5,6]. It's crucial to understand the incidence and risk factors associated with this condition. Although the exact mechanisms underlying influenza-associated rhabdomyolysis are not fully elucidated, current understanding suggests a multifactorial process involving direct viral invasion of muscle cells, release of inflammatory cytokine, and viral toxin causing myonecrosis leading to rhabdomyolysis [7]. A study of 18 cases in the 2009 influenza epidemic found that 62% of the patients had elevated creatin kinase levels above 200 U/L. An increase in creatin kinase levels was also correlated with the severity of patients' illnesses [8]. Current influenza guidelines do not address creatine kinase measurement, which is an important marker in rhabdomyolysis. Our case suggests CK measurement should be part of the workup for hospitalized patients with acute influenza infection.

This case underscores the importance of vigilance in monitoring influenza patients for potential complications, especially those with pre-existing medical conditions. Despite initially presenting with symptoms suggestive of influenza, including myalgia, cough, and fever, the development of dark urine and severe myalgia necessitated further evaluation and intervention. Timely intervention and collaborative care can lead to favorable outcomes and prevent long-term sequelae. Diagnosing rhabdomyolysis in the setting of influenza can be challenging, particularly in cases where symptoms overlap with those of the underlying viral infection. Healthcare providers must be vigilant in assessing patients with influenza for signs of rhabdomyolysis and consider appropriate diagnostic testing, such as serum creatine kinase levels and urinalysis, to confirm the diagnosis. Treatment typically involves aggressive fluid resuscitation to maintain renal perfusion, promote the clearance of myoglobin and prevent kidney injury. Public health efforts aimed at promoting vaccination uptake, particularly among high-risk populations, are essential for mitigating the burden of influenza-related morbidity and mortality. Overall, further research into the epidemiology, pathophysiology, and optimal management of influenza-associated rhabdomyolysis is warranted to improve clinical outcomes and enhance our understanding of this potentially serious complication.

Conclusion

Influenza A is a common infection that causes outbreaks and epidemics in cold months that do not always cause respiratory complications. Recognizing rhabdomyolysis in patients with influenza is paramount for early diagnosis and intervention. Although our patient's condition with a history of asthma favored a severe form of influenza and respiratory complications, he suffered from rhabdomyolysis. This case shows that timely recognition prevents further serious complications. In addition to early detection and treatment, implementing preventive strategies such as annual influenza vaccination can help reduce the risk of severe influenza infection and its associated complications, including rhabdomyolysis.

Conflict of interest statement. None declared.

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УПАТСТВО ЗА ПРИЈАВА НА ТРУД ОД СОРАБОТНИЦИТЕ НА ММП

"Македонски Медицински Преглед" (ММП) е стручно списание на Македонското лекарско друштво, првенствено наменето на лекарите од општа практика, специјалистите од одделните медицински дисциплини и истражувачите во областа на базичните медицински и други сродни науки.

Списанието ги има следниве рубрики и категории на трудови:
1. Изворни трудови
2. Соопштувања за клинички и лабораториски искуства
3. Прикази на случаи
4. Од практика за практика
5. Едукативни статии
6. Вариа е (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.

ТЕКСТ НА РАКОПИСОТ

Сите ракописи се испраќаат во електронска форма на електронската адреса (mld@unet.com.mk ; info@mld.mk) 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.

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. email); 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 SI 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.

1. **ПРИЛОЗИ** Како прилог-документација на трудовите предложени за печатење, може да се доставаат 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.

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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):
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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.

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