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LUNG TRANSPLANTATION AT THE ZAGREB UNIVERSITY HOSPITAL CENTER, ZAGREB, CROATIA

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Objective: Lung transplantation has become a standard of care for patients with a variety of non-malignant end-stage lung diseases. The aim of the study was to report on the safety and feasibility of lung transplantation at the Zagreb University Hospital Center. ***Methods:*** In this single center retrospective observational study, all consecutive patients undergoing lung transplantation at the Zagreb University Hospital Center from April 2021 until December 2022 were included. The only inclusion criterion was surgery for lung transplantation. Patient demographic and operative characteristics were reported, as well as early outcomes, including 30-day mortality, hospital stay, intensive care unit stay, duration of mechanical ventilation, and incidence of primary graft dysfunction. The degree of primary graft dysfunction was graded based on the International Society for Heart and Lung Transplantation criteria at 72 hours after transplantation with grades 0 to 3. ***Results:*** During the 21-month study period, 19 patients were successfully transplanted. There was no 30-day mortality. There was one late death at 18 months after transplantation. Median in-hospital stay was 32 days, ranging from 21 to 62 days. Mean mechanical ventilation duration was 105±58 h and median of intensive care unit stay was 6 days, ranging from 4 to 15 days. Only two (11%) patients had the highest grade 3 primary graft dysfunction. Of the remaining patients, 16 (84%) had none (grade 0) and one (5%) patient had mild primary graft dysfunction (grade 1). ***Conclusion:*** Our results suggest that lung transplantation is safely performed at the Zagreb University Hospital Center. Initial results with no operative mortality are encouraging. Further follow-up and experience are needed to make inferences on long-term outcomes of our lung transplantation patients.

Key words: lung transplantation, end-stage lung disease, extracorporeal membrane oxygenation, primary lung graft dysfunction, survival, operative mortality

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INTRODUCTION

Over the past 35 years, lung transplantation has become a viable option for patients with a variety of non-malignant end-stage lung diseases. Although the first human lung transplantation was performed back in 1963 (1), only a few procedures were performed until the mid-80s, when the operation became a clinical reality. Since the year 2000, a steady growth of lung transplant procedures has been reported with approximately 4500 procedures annually in recent years (2).

Despite favorable results in recent years, lung transplantation remains burdened with the risk of mortality and morbidity, which are related to primary graft dysfunction (PGD) and chronic lung allograft dysfunction (CLAD) (3). Long-term results of lung transplantation are not yet as good as other solid-organ transplants. Early outcomes have been considerably improved by technical advances in graft procurement, preservation, implantation, perioperative care, immunosuppression, and postoperative medical management. However, long-term survival has improved minimally over the last two decades. Various complications with delayed onset, such as CLAD or opportunistic infection, continue to significantly impact recipient quality of life, survival, and long-term outcomes (4).

Primary graft dysfunction is defined as lung injury that occurs within the first 72 hours following lung transplantation as reflected by the appearance of diffuse allograft edema/infiltration on chest radiograph. Severe PGD is the most common cause of early mortality and has also been associated with later dysfunction of the graft (5). Therefore, those surviving this initial insult remain at a risk of long-term morbidity and mortality. It has been reported that the routine application of veno-arterial extracorporeal membrane oxygenation (VA ECMO) during lung transplantation has significantly decreased the rates of severe PGD after lung allograft transplantation (6).

Single lung transplantation used to be more frequent than bilateral, while today the number of bilateral transplants has surpassed the number of single lung transplants (2). First lung transplantation in Croatia was performed in 2003 at the Jordanovac Department of Thoracic Surgery in Zagreb. In the years to follow, lung transplantation was not performed in Croatia until 2021. During this period, patients requiring lung transplantation were transplanted in Vienna (7). Close collaboration with the Department of Thoracic Surgery at Vienna University Hospital was developed, which led to the first bilateral lung transplantation in Croatia on April 17, 2021.

AIM

The aim of this study was to report on the safety and feasibility of lung transplantation at the Zagreb University Hospital Center, and on our initial experience with the procedure.

METHODS

This single center retrospective observational study included all consecutive patients undergoing lung transplantation at the Zagreb University Hospital Center, Zagreb, Croatia, from April 2021 until December 2022. The only inclusion criterion was surgery for lung transplantation. There were no exclusion criteria. Both adult and minor patients were included in the study. The institutional Review Board of the Zagreb University Hospital Center approved the study. It was conducted according to the Declaration of Helsinki. Written informed consent was waived due to the retrospective nature of the study. Individual medical records were reviewed for demographic, clinical, and laboratory data. Patient demographic and operative characteristics were reported, as well as outcomes during the follow-up period, which was completed in January 2023.

Surgery for transplantation

Bilateral thoracosternotomy, also known as clamshell incision, in the fourth intercostal space was performed in all cases. Internal thoracic arteries were ligated and severed. This approach provides the best exposure to both hila and the heart. This was particularly important since central cannulation for VA ECMO support was used (Figure 1).

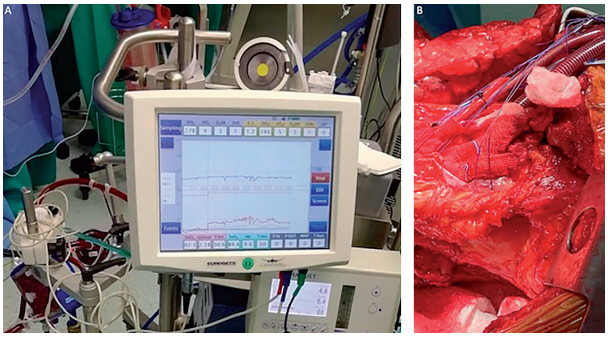

Figure 1. *Intraoperative extracorporeal membrane oxygenation support: (A) intraoperative veno-arterial extracorporeal oxygenation monitoring during lung transplantation; (B) central cannulation of the aorta and the right atrium via clamshell incision for veno-arterial extracorporeal membrane oxygenation.*

Pneumonectomy was performed in a standard manner with stapling of the pulmonary artery and pulmonary veins as peripheral as possible. The bronchus was prepared centrally and divided two rings from the carina. Thereafter, the lung was taken out of the chest cavity. During the entire procedure of pneumonectomy, special attention was always taken to avoid phrenic nerve injury. On the left side, the recurrent laryngeal nerve should also be preserved. In patients with significant adhesions, the lung was mobilized with caution to avoid injuries to the phrenic nerve or vital structures.

The first step in implanting the lung was the formation of bronchial anastomosis. Topical cooling was provided with ice slush. A bacteriologic swab was taken from the donor lung, and the bronchial system was flushed with saline to remove residual mucus. The bronchial anastomosis was performed in a single running suture technique using double-armed 4-0 polydioxanone, starting at one end of the cartilaginous part and going over the membranous portion and then using the same single running suture for the anterior cartilaginous part. After completion of the bronchial anastomosis, the left atrial anastomosis was performed. A Satinsky clamp is placed centrally in the left atrium to ensure a sufficient cuff for the left atrial anastomosis. The anastomosis was performed with a 4-0 running polypropylene suture, at a level where myocardial

muscle tissue is present. Thereafter, the recipient pulmonary artery was centrally clamped and opened. The pulmonary artery anastomosis is again performed in a running technique using a 5-0 polypropylene suture. This anastomosis sequence was used in all our cases (Figure 2).

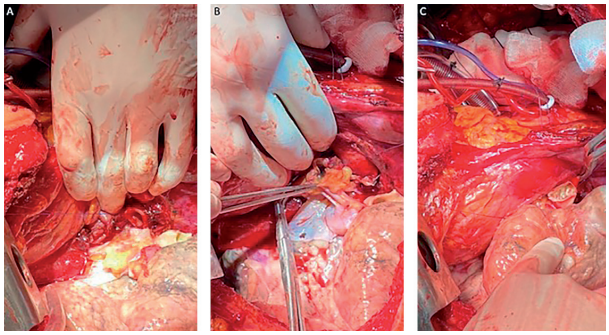

Figure 2. *Anastomosis sequence for lung transplantation: (A)bronchial anastomosis is performed in a single running suture technique. A double-armed 4-0 polydioxanone is used, starting at one end of the cartilaginous part of the bronchus and going over the membranous portion and then using the same single running suture for the anterior cartilaginous part; (B)once a sufficient left atrial cuff of the recipient has been prepared, the left atrial anastomosis is performed with a 4-0 running polypropylene suture, at the level where myocardial muscle tissue is present; (C) pulmonary artery anastomosis is again performed in a running technique using a 5-0 polypropylene suture.*

Before exposing the newly implanted lung to innate circulation, initial immunosuppression with 1 g methylprednisolone was administered. All other immunosuppressants were started upon arrival to the intensive care unit (ICU). Second important step prior to complete releasing the clamps is retrograde and antegrade flushing of the newly implanted lung. Flushing was done to remove the remainder of the preservation solution (Perfadex Plus, Göteborg, Sweden) and to deair the vascular bed in the donor lung. Thereafter, the sutures were knotted and all clamps removed. At this moment of lung transplantation, the ischemic period, started during organ procurement, was completed and lung protective ventilation was started. The same procedure was done for the contralateral lung. We instituted bilateral lung transplantation in all cases.

After implantation of both lungs, meticulous hemostasis was performed with special attention to the donor pulmonary ligament, pericardium, and location of dense adhesions. Fibrin glue and hemostatic gauzes were applied on the vascular suture lines. Chest drainages were placed in the costodiaphragmatic sinus and anterior to the hilus towards the apex on each side. Additionally, a small Jackson-Pratt drain was placed in the posterior aspect of each pleural cavity.

Extracorporeal membrane oxygenation

All patients were transplanted with a preemptive intraoperative central VA ECMO support (Figure 1). Central cannulation sites were the right atrium for inflow cannula and the ascending aorta for outflow cannula. Elongated One-Piece Arterial Cannula (EOPA, Medtronic Inc, Minneapolis, MN, USA) was used for the aorta and a curved-tip cannula also from Medtronic was used for the right atrium. In brief, the circuit consists of the inflow and outflow cannulas, blood pumping device, oxygenator, and integrated heat exchanger. Either Cardiohelp (Getinge AB, Göteborg, Sweden) or Biomedicus (Medtronic) centrifugal pump were used. Hollow fiber membrane oxygenators incorporated in the HLS or PLS Set also from Getinge were applied. Biocompatible surface coating lines were used. All patients received a bolus dose of 50-60 IU/kg unfractionated heparin before cannulation, and the dose was modified based on the coagulation status of the patient and surgeons' preference. Activated clotting time was not monitored routinely.

During pneumonectomy, theVA ECMO flow was set to 50% of predicted cardiac output and adapted according to hemodynamic and gas exchange demands. After implantation of the second lung, flow was gradually reduced, and the patient weaned from ECMO. Function of the lungs was evaluated 10 minutes after decannulation and immediately after chest closure. If lung function did not meet the predefined quality criteria, or if there was clear worsening between measurements, a peripheral VA ECMO system was inserted in a femoro-femoral configuration (Figure 3) and patients were transferred to the ICU support.

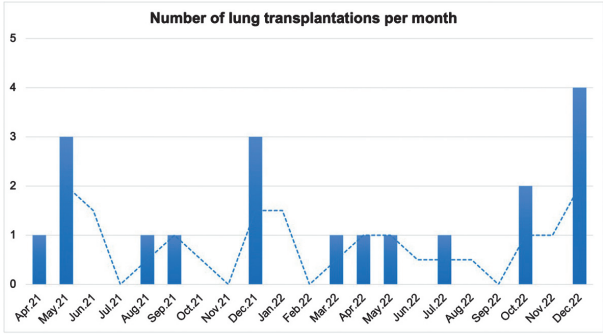

Figure 3. *Number of lung transplantations with trendline performed at the Zagreb University Hospital Center each month from April 2021 until December 2022.*

Immunosuppression, infection prophylaxis and rejection management

After intraoperative administration of methylprednisolone, all patients received induction therapy with alemtuzumab (Campath, Sanofi, MA, USA) upon

arrival to the ICU. Alemtuzumab is a recombinant monoclonal antibody directed against cell surface glycoprotein CD52, which is expressed on several immune cells and its activation induces immune cell depletion (8). Maintenance immunosuppression protocol is provided in Table 1 and is based on the low-dose immunosuppression protocol from the Vienna Lung Transplantation Group (9). Perioperative infectious prophylaxis was based on broad-spectrum antibiotics or adapted to resistance testing. All patients received continuous pneumocystis prophylaxis with trimethoprim-sulfamethoxazole. Prophylactic inhalation therapy with amphotericin B and gentamicin was provided during the initial one-month period after transplantation. Cytomegalovirus (CMV) prophylaxis included CMV-specific human hyperimmune globulins together with valganciclovir for a minimum of 3 months. Surveillance bronchoscopy with transbronchial biopsy and bronchoalveolar lavage was performed during initial hospitalization and at 2, 3, 6 and 12 months after transplantation or whenever clinically indicated. Likewise, all patients underwent chest computed tomography during initial hospitalization prior to bronchoscopy and on follow-up visits. Additional diagnostic scans were performed in case of lung function deterioration, e.g., in case of suspected acute rejection episode or infections. Biopsies were classified according to the International Society of Heart and Lung Transplantation (ISHLT) criteria (10). In case of marked drop in lung function or suspicion of acute rejection, after exclusion of probable/definitive antibody mediated rejection (11), patients were treated with high-dose corticosteroids, and in case of non-response, reinduction with alemtuzumab or an interleukin-2 receptor antagonist (daclizumab) was administered followed by extracorporeal photopheresis.

Table 1 *Maintenance immunosuppression protocol.*

Time after LuTx	Tacrolimus (ng/mL)	Prednisolone (mg)	MMF (mg)*
0-3 months	8-10	25	–
3-6 months	6-8	20	–
6-9 months	6-8	15	–
9-12 months	6-8	10	–
12-24 months	5-7	5	500 twice a day
>24 months	4-6	5	500 twice a day

*LuTx, lung transplantation; MMF, mycophenolate mofetil; *in case of obstructive chronic lung allograft dysfunction, 750-1000 mg twice a day after 12 months.*

Outcome measures

Primary outcome of interest was operative mortality. Operative mortality was defined as death within 30 days after surgery or in-hospital death. Patients were followed-up monthly in the outpatient clinic. Follow-up was terminated in January 2023 for the purpose of this study. There were no patients lost to follow-up. Secondary outcomes of interest were in-hospital stay, ICU stay, duration of mechanical ventilation, and rates of PGD at 72 hours following transplantation. A grading system for PGD incorporating arterial oxygen partial pressure to ractional inspired oxygen (PaO_2/FiO_2) ratio and chest radiograph findings was used. Based on this system, each patient was graded with PGD grade 0-3 as described in the 2017 consensus group statement of the ISHLT (12).

Statistical analysis

Continuous variables were expressed as mean±standard deviation (SD) or median with range, whereas categorical variables were described with frequencies and proportions. To assess distribution, the Shapiro-Wilk test for normality was applied. It was used to determine which variables were normally distributed (mean, SD) and which were non-normally distributed (median, range). Independent (unpaired) Student's t-test was used for normally distributed continuous variables. Fisher exact test was chosen for analysis of categorical variables. All analyses were performed in Microsoft Excel for Mac and R version 4.2.2 for Mac (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

In April 2021, after being discussed by the multidisciplinary team, first patients suffering from end-stage chronic obstructive pulmonary disease/emphysema were placed on the transplant waiting list and reported to the Eurotransplant. During the study period, 27 patients were listed overall. Of the patients that were listed, 19 (70%) were transplanted. One patient died due to sepsis while being on the waiting list. This was a patient with end-stage lung disease on VV ECMO after being infected with severe acute respiratory syndrome coronavirus 2 (SARSCoV2) and having contracted coronavirus disease 2019 (COVID-19). A history of COVID-19 was present in 5 (26%) transplanted patients, not related to the indication for lung transplantation. The number of lung transplantations that were performed monthly during the study period was, as expected, highly unpredictable and it ranged from 0 to 4 procedures *per* month (Figure 4). There were 15 (79%) male and 4 (21%) female patients that were transplanted. Median age of these patients was 56, ranging from 12 to 68 years. Underlying diagnoses for transplantation were diverse and are reported in Table 2 alongside with patient comorbidities.

There was one pediatric case. The pediatric patient was transplanted under the indication of graft *versus* host disease (GvHD). After a matched sibling donor hematopoietic stem cell transplantation for acute lymphocytic leukemia, the patient developed GvHD of the lungs. Once the risk of disease recurrence was significantly reduced, he was put on the waiting list and successfully transplanted.

Table 2 *Baseline lung transplant patient characteristics, matching factors, and surgical data.*

		N=19
Demographics and comorbidities		
Age (years)		56 (12-68)
Female		4 (21%)
Height (m)		1.69±0.11
Weight (kg)		67±13
BMI (kg/m^2)		23±3
Arterial hypertension		2 (11%)
Hyperlipidemia		2 (11%)
Diabetes		2 (11%)
GERD		3 (16%)
History of COVID-19		5 (26%)
Secondary PH		8 (42%)
Matching		
ABO matching	Identical	15 (79%)
	Compatible	4 (21%)
High risk CMV mismatch		3 (16%)
Gender mismatch		9 (47%)
Underlying diagnosis		
COPD		6 (32%)
ILD		6 (32%)
AATD		3 (16%)
PH		1 (5%)
CF		1 (5%)
Other		2 (11 %)
Type of transplantation		
Double lung		19 (100%)
Size reduction		5 (26%)
Ischemia right lung (min)		351±59
Ischemia left lung (min)		464±66
Prolonged postoperative ECMO		
VA ECMO		1 (5%)
VV ECMO		1 (5%)

AATD, alpha-1 antitrypsin deficiency; BMI, body mass index; CF, cystic fibrosis; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; GERD, gastroesophageal reflux disease; HFNC, high flow nasal cannula; ILP, interstitial lung disease; LuTx, lung transplantation; PH, pulmonary hypertension; VA, veno-arterial; VV, veno-venous.

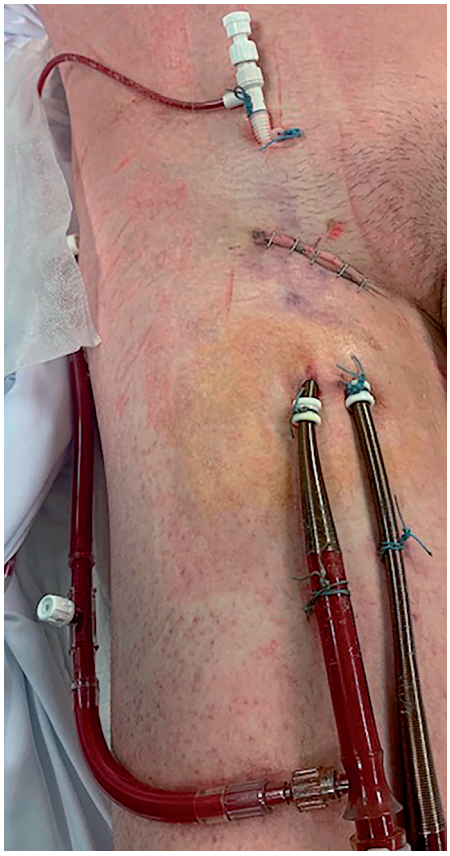

Figure 4. *Prolonged postoperative veno-arterial extracorporeal membrane oxygenation support in the groin: 15 Fr arterial and 17 Fr venous cannulas were placed in the right common femoral artery and vein with an 8 Fr distal reperfusion line. Prolonged postoperative veno-arterial extracorporeal membrane oxygenation support is started immediately after transplantation prior to leaving the operating room in recipients with pulmonary hypertension and in patients with questionable graft function at the end of implantation.*

Donor characteristics are reported in Table 3. Fourteen donor lungs came from within Croatia and the remaining 5 came from abroad. As expected, donors were younger than recipients (p=0.002). The mean age of donors was 38±14, ranging from 14 to 56 years. There were more females among donors than among recipients (p=0.045). Gender mismatch was observed in 9 (47%) transplanted patients. There were 15 (79%) identical ABO blood group matches. All the four compatible ABO matches were from O donors for either A or B recipients. High-risk CMV mismatches (D+/R) were observed in 3 (16%) cases. Donor reports revealed PaO_2 at 100% FiO_2 in the range from 233 to 637 mmHg. During procurement, blood gas analyses were reassessed. In case of PaO_2<300 mmHg with 100% FiO2 and 5 mmHg of positive end-expiratory pressure, the lungs were rejected. Following a satisfactory blood gas analysis, final decision to continue with lung transplantation was made in each case after visual inspection of the explanted lungs (Figure 5).

Table 3. Lung transplantation donor characteristics.

	N=19
Age (years)	38±14
Female	11 (58%)
Height (m)	1.70±0.09
Weight (kg)	72±15
BMI (kg/m^2)	24±3
Arterial hypertension	7 (37%)
Diabetes	1 (5%)
Smoking	5 (26%)
pO_2 (mmHg)	466±101
pCO_2(mmHg)	40 (28-75)

BMI, body mass index; pO_2 partial pressure of oxygen; pCO_2 partial pressure of carbon dioxide

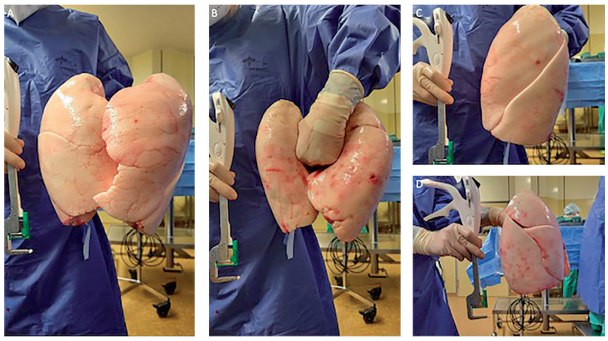

Figure 5. *Final decision to continue with lung transplantation was made during lung procurement based on blood gas analysis, palpation, compliance assessment, and visual inspection of the explanted lungs: (A) anterior view of the explanted lung with the linear stapler aside to aid in size assessment; (B) posterior view; (C) left lateral view; (D) right lateral view.*

All patients received bilateral lung transplantation. Lung volume reduction was undertaken in 5 (26%) patients. Lung volume reduction was based on surgeons' preference. In these cases, the middle lobe and lingula were resected. Lung ischemia times ranged from 237 to 475 min on the right side and 329 to 575 min on the left side. Prolonged ECMO support was necessary in two cases. One was related to high-grade PGD immediately after transplantation. The other one did not show any signs of diffuse lung edema on chest radiograph. Prolonged ECMO support was started immediately after transplantation prior to leaving the operating room. Both patients were successfully weaned from support. One was weaned after four days, and the other one, only one day after transplantation.

The need to reexplore the chest cavity for bleeding was necessary in one (5%) patient. The patient was taken back to the operating room due to an excessive chest tube output on the same day of transplantation. Bleeding was surgically managed, and the patient recovered fully. There was one ECMO related complication requiring an intervention. It was related to leg ischemia due to right external iliac artery thrombosis after peripheral VA ECMO removal. Surgical thrombectomy was performed without any further sequels for the patient.

Hospital stay, mechanical ventilation duration and ICU days are reported in Table 4. High-grade PGD at 72 hours after transplantation is characterized with diffuse lung edema on chest radiograph. Most patients were grade 0 and the incidence of high-grade PGD was low. Detailed information on each grade is reported in Table 4. There were no cases of PGD grade 2 at 72 hours following transplantation.

Table 4. *Outcome measures.*

		N=19
Mechanical ventilation (h)		105±58
ICU time (days)		6 (4-15)
In-hospital stay (days)		32 (21-62)
PGD grade at 72 hours	0	16 (84%)
	1	1 (5%)
	3	2 (11%)

PGD, primary graft dysfunction; ICU, intensive care unit

There were no operative deaths. All patients were successfully discharged from the hospital after the index procedure. Two patients transplanted in December 2022 were recovering well but still in the hospital while this manuscript was in preparation. There were no late deaths except for one. The patient died 18 months after transplantation due to sepsis and multiorgan failure. Compliance to medical therapy was a major issue in this case.

DISCUSSION

In this article, we report our initial experience with lung transplantation at the Zagreb University Hospital Center, Zagreb, Croatia. Lung transplantation was the only solid organ transplantation that was not routinely performed in Croatia until recently. Since April 2021, lung transplantations are routinely performed at our center and Croatian patients no longer need to travel abroad for lung transplantation. Based on the results of this study, we can say that lung transplantation was safely performed at our center and the initial outcomes are encouraging. Further follow-up is needed to give an insight into long-term results.

Survival is probably the most robust and straight forward assessment of outcome after lung transplantation. Large registries can easily provide information on survival estimates based on multicenter international data. The ISHLT registry has accumulated data on more than 64,000 lung transplant recipients. It annually reports survival estimates and has become a quality benchmark in the field. According to the recent registry report, the median survival for adult recipients since 2010 is 6.7 years, but bilateral lung recipients appear to have a better median survival than single lung recipients (7.8 *versus* 4.8 years) (2). However, it is not entirely clear if the survival advantage is directly related to the choice of procedure or rather to the recipient characteristics. Better allograft longevity and overall favorable outcomes of lung transplantation today are a result of refinement in the processes of recipient and donor selection, surgical techniques, immunosuppression, and other post-transplant treatment regimens. Despite these improvements, survival outcomes of lung transplantation recipients remain inferior to those of other solid-organ transplant procedures. For instance, the median survival after heart transplantation is around 12 years (13).

The underlying diagnosis has a major impact on survival after transplantation. Certain diagnoses carry higher risks of operative complications and PGD. It is important to emphasize that some diagnoses are linked to particular age groups. Recipients with chronic obstructive pulmonary disease are older and they have the best one-year survival but lower ten-year survival compared to those with cystic fibrosis or primary pulmonary hypertension (14). Recipients with primary pulmonary hypertension have the lowest one-year survival but their ten-year survival approaches those with cystic fibrosis (2). In the more recent epoch of lung transplantation, improvement in one-year survival is notable, although the difference is not so obvious at 5 years. This might be due to management strategies that have been more effective at reducing early complications rather than the later ones.

Primary graft dysfunction is still one of the main risks at short-term outcome after lung transplantation. It is a form of acute respiratory distress syndrome characterized with diffuse alveolar damage, which occurs between early hours and a few days after transplantation. In the ISHLT registry, PGD is defined as lung injury that occurs within the first 72 hours following lung transplantation as reflected by the appearance of diffuse lung edema on chest x-ray. A grading system for PGD incorporates the PaO_2/FiO_2 ratio and chest radiograph findings (12). Severe PGD is the most common cause of early mortality after lung transplantation and has also been associated with later allograft dysfunction (5). It accounts for more than 20% of deaths in the first 30 days (2,15). Several groups have shown that severe PGD is associated with increased perioperative mortality and impaired 1-year survival rates (16). Proper intraoperative allograft management is crucial to avoid PGD. Based on the ISHLT PGD working group data, severe graft dysfunction is observed in up to 20% of patients within 72 hours after lung transplantation (12). In our patient cohort, we observed severe graft dysfunction in only 2 (11%) patients. One of these patients was managed with VA ECMO support immediately after transplantation before leaving the operating room. Weaning from ECMO was successful within four days of transplantation.

The optimal strategy and use of extracorporeal circulation during lung transplantation has been a matter of ongoing discussion. Some centers apply intraoperative support only in unstable patients, whereas others use it routinely. In our center, we opted for routine intraoperative VA ECMO support in all our transplant cases. Cardiopulmonary bypass has been a more traditional approach mainly used in the early days of lung transplantation, whereas VA ECMO came into use at a later date. When used properly, the side effects associated with routine application of intraoperative central VA ECMO are nonsignificant (17).

Lung transplantation does not necessarily require the use of extracorporeal circulation. However, the use of intraoperative VA ECMO guarantees hemodynamic and respiratory stability during single-lung ventilation period. This is particularly important in patients with severe pulmonary hypertension and those with preexisting hemodynamic instability. It also facilitates lung protective ventilation strategies and allows for prolonged and controlled reperfusion of the newly implanted allograft. Finally, a routine use of intraoperative VA ECMO support has been associated with exceptionally low PGD rates and even improved survival, particularly in patients with primary pulmonary hypertension (6,17).

Chronic lung allograft dysfunction and infections are the leading cause of death and the main limiting factor in long-term survival after lung transplantation. Up to 30% of fatal outcomes are due to CLAD (2). There are two main phenotypes of CLAD: bronchiolitis obliterans syndrome (BOS), so called oCLAD, and restrictive allograft syndrome (RAS), so called rCLAD plus mixed forms. Survival after the onset of BOS is about 50% after only three years (2). Our understanding of CLAD mechanism and risk factors is still insufficient. Currently available therapies for CLAD are not effective. Infections led by bacterial bronchitis and pneumonia are the most common complications at all time points after lung transplantation. Fungi, CMV, community acquired respiratory viruses and mycobacteria all contribute to the overall infectious burden (18-21).

Matching age, height, and size are established requirements in a lung allocation program to ensure successful transplantation and optimal outcomes. Conventionally, ABO blood group identical matching has been used in lung allocation. Sometimes an ABO compatible donor is used instead of an ABO identical one due to the scarcity in donors and the individual assessment of each recipient. Matching an ABO compatible donor rather than identical to the recipient did not reveal any negative consequences in our limited experience. It has been reported that recipients who receive ABO compatible matched allografts show a similar survival rate to recipients who receive ABO identical ones (22). ABO blood group compatible matching might potentially shorten lung transplant waiting list times. Other donor and recipient factors considered during matching include human leukocyte antigen status, gender, CMV, Epstein Barr virus and toxoplasma serology, smoking status, and screening through bronchoscopy and chest x-ray.

An overall shortage of lung donors remains a major limiting factor for the number of transplants performed annually (23). *Ex vivo* lung perfusion technology allows for lung perfusion and reconditioning in an environment which may reduce lung injury in some cases. This might allow for transplantation from donors previously deemed unsuitable (24,25). Recently, studies are emerging on new target temperature for static lung preservation. Conventionally, lungs are preserved on ice for about 6 to 9 hours before transplantation at 4°C. This limits availability of organs across locations where the distance would prolong ischemia time beyond the 9-hour threshold. It has been reported that increasing the static storage temperature to 10°C preserves mitochondrial function and reduces mitochondrial injury in animal models (26,27). A successful clinical application has been reported in five human lung transplant recipients after up to 16 hours of static storage at 10°C (26).

This was a non-randomized retrospective observational study with all the limitations associated with the study design. There were only 19 subjects in the study, making it a small sample size study; therefore, statistical comparisons were not feasible. The study might be underpowered to make strong conclusions on the matter. A larger sample with longer follow-up is warranted.

CONCLUSION

The results of our study have shown once again that lung transplantation is a feasible option for patients with end-stage lung disease refractory to medical therapy. Not only it is feasible but safe and effective. Even under the circumstances of the novel coronavirus pandemic, we managed to establish a lung transplantation program at our center. An imperative to perform life-saving lung transplantations was a major driver in this process. Regional hospitals and pulmonologists are urged to refer their patients with advanced stage non-malignant lung disease for lung transplantation evaluation to our center. Only with the support from referring physicians will the program continue its grow that long term.

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SAŽETAK

TRANSPLANTACIJA PLUĆA U KLINIČKOM BOLNIČKOM CENTRU ZAGREB U HRVATSKOJ

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Cilj: Transplantacija pluća postala je standard skrbi za pacijente s nizom nemalignih plućnih bolesti u završnom stadiju. Cilj ovog istraživanja bio je izvijestiti o sigurnosti i izvedivosti transplantacije pluća u Kliničkom bolničkom centru Zagreb u Hrvatskoj. ***Metode:*** U ovu retrospektivnu opservacijsku studiju uključeni su svi uzastopni pacijenti koji su bili podvrgnuti transplantaciji pluća u Kliničkom bolničkom centru Zagreb od travnja 2021. do prosinca 2022. godine. Jedini kriterij za uključivanje bio je kirurški zahvat transplantacije pluća. Zabilježene su demografske i operativne karakteristike pacijenata, kao i rani ishodi, uključujući 30-dnevnu smrtnost, boravak u bolnici, boravak na jedinici intenzivne njege, trajanje mehaničke ventilacije i incidenciju primarne disfunkcije presatka. Stupanj primarne disfunkcije presatka ocijenjen je na temelju kriterija Međunarodnog društva za transplantaciju srca i pluća 72 sata nakon transplantacije ocjenama od 0 do 3. ***Rezultati:*** Tijekom dvadesetjednomjesečnog razdoblja istraživanja transplantacija je uspješno primijenjena u 19 pacijenata. Nije bilo 30-dnevne smrtnosti. Dogodila se jedna kasna smrt 18 mjeseci nakon transplantacije. Medijan boravka u bolnici bio je 32 dana, u rasponu od 21 do 62 dana. Prosječno trajanje mehaničke ventilacije bilo je 105±58 h, a medijanboravka u jedinici intenzivne njege bio je 6 dana, u rasponu od 4 do 15 dana. Samo dva (11 %) bolesnika imala su primarnu disfunkciju presatka najvišeg stupnja 3. Od preostalih bolesnika 16 (84 %) ih nije imalo nikakav (stupanj 0), a jedan (5%) bolesnik imao je blagi, stupanj 1. ***Rasprava:*** U ovom članku prikazujemo naše početno iskustvo s transplantacijom pluća. Transplantacija pluća bila je jedina od transplantacija solidnih organa koja se donedavno u Hrvatskoj nije rutinski izvodila, s napomenom da je prva transplantacija pluća u Hrvatskoj učinjena još 2003. godine u Klinici za torakalnu kirurgiju Jordanovac, ali se program transplantacije nije tada nastavio. Od travnja 2021. godine transplantacije pluća rutinski se izvode u našem centru i hrvatski pacijenti više ne moraju putovati u inozemstvo radi transplantacije pluća.Sveukupni nedostatak donora pluća i dalje je glavni ograničavajući čimbenik za broj transplantacija koje se izvode na godinu. Svega 20%-30% doniranih pluća iskoristi se za transplantaciju. Potrebno je kontinuirano unaprjeđenje i razvoj strategija koje će povećati broj donora i uporabljivih plućnih presadaka. ***Zaključak:*** Naši rezultati pokazuju da se transplantacija pluća sigurno izvodi u Kliničkom bolničkom centru Zagreb. Početni rezultati bez operativnog mortaliteta su ohrabrujući. Daljnje praćenje i iskustvo potrebni su za donošenje zaključaka o dugoročnim ishodima naših pacijenata s transplantacijom pluća.

Ključne riječi: transplantacija pluća, završni stadij bolesti pluća, ekstrakorporalna membranska oksigenacija, primarna disfunkcija plućnog presatka, preživljenje, operativna smrtnost

PRECIPITIRAJUĆI ČIMBENICI I KLINIČKA OBILJEŽJA DIJABETIČKE KETOACIDOZE

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Uvod: Dijabetička ketoacidoza (DKA) jedna je od najozbiljnijih akutnih komplikacija šećerne bolesti (ŠB). Pojedina istraživanja su pokazala da su infekcije precipitirajući čimbenik u polovice ispitanika. Nekoliko novijih istraživanja naglašava da je loše pridržavanje liječenja također česti uzrok DKA. ***Cilj:*** Identificirati najčešće precipitirajuće čimbenike za DKA u Republici Hrvatskoj. ***Ispitanici i postupci:*** Ovo retrospektivno multicentrično istraživanje uključivalo je bolesnike sa ŠB-om tipa 1 ili tipa 2 s dijagnozom DKA između 1. siječnja 2014. i 31. prosinca 2018. i liječenih u 5 različitih središta za liječenje ŠB-a: Dubrovnik, Našice, Split, Zagreb i Osijek. U analizu je uključena samo prve epizoda DKA. Pacijenti koji boluju od steroidnog ŠB-a i ŠB-a zbog endokrinih poremećaja kao što su akromegalija i Cushingov sindrom bili su isključeni. ***Rezultati:*** Istraživanjem je obuhvaćeno 160 bolesnika (55 % muškaraca), od kojih je 68% imalo ŠB tip 1. Srednja dob ispitanika bila je 42 godine (od 18 do 89). Najčešći uzrok DKA bila je infekcija (57 %), zatim slabo kontrolirani ŠB (37 %) i prva prezentacija ŠB-a (9 %), dok je u 7% bolesnika DKA bila uzrokovana ostalim uzrocima kao što su kvar inzulinske pumpe, moždani ili srčani udar. U skupini bolesnika s infekcijama najčešće su bile infekcije mokraćnog sustava (30 %), probavne infekcije (30 %) i infekcije respiratornog trakta (19 %), dok je 21 % bolesnika imalo druge izvore infekcije. U 36 ovih bolesnika uz infekciju je bio prisutan i prethodno loše kontroliran ŠB, a u 12 % DKA uzrokovana infekcijom bila je prvo očitovanje bolesti. U bolesnika sa ŠB-om tipa 2 infekcije su češće bile uzrok DKA nego u bolesnika sa ŠB-om tipa 1 ($P < 0,05$). U bolesnika sa ŠB-om tipa 1, slabo regulirana glikemija je češće uzrok DKA (31%) nego u bolesnika sa ŠB-om tipa 2 (18 %). ***Zaključak:*** Najčešći precipitirajući čimbenici za razvoj DKA su infekcije i loša regulacija ŠB-a. Potrebna je bolja edukacija bolesnika o važnosti redovite primjene inzulina i korekcije terapije tijekom akutne bolesti.

Ključne riječi: šećerna bolest, ketoacidoza, precipitirajući čimbenici, hiperglikemija, inzulin, infekcija

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UVOD

Dijabetička ketoacidoza (DKA) jedna je od najozbiljnijih akutnih komplikacija šećerne bolesti (ŠB) (1). Obilježena je trijadom koju čine hiperglikemija, ketoza i metabolička acidoza. Rezultat je relativnog ili apsolutnog manjka inzulina uz stvaranje kontraregulacijskih hormona poput glukagona, kortizola, katekolamina i hormona rasta (2). Godišnja incidencija DKA procijenjena je na 10 - 30/1000 bolesnika sa ŠB-om,

dok smrtnost od DKA iznosi 0,5 % - 1 %, iako u pojedinim zemljama koje imaju slabiju zdravstvenu zaštitu, smrtnost se kreće u rasponu od 6 % pa do čak 44 % (3,4). Iako se DKA ponajprije javlja u šećernoj bolesti tip 1 (DM tip 1), istraživanja i iskustva iz kliničke prakse pokazuju da je sve veća zastupljenost DKA kod ŠB-a tipa 2 (5,6). U oba tipa ŠB-a za nastanak DKA najčešće je potreban neki precipitirajući čimbenik (7,8). U pojedinim istraživanjima pokazano je da su infekcije bile precipitirajući čimbenik u polovice ispitanika (9,10). S druge strane, nekoliko novih istraživanja naglašava da je slaba adherencija prema terapiji također čest uzrok koji susrećemo u DKA (1,11). Pored visokog pobola i značajne smrtnosti, DKA je i financijsko opterećenje za zdravstveni sustav. U Sjedinjenim Američkim Državama oko pola milijuna bolničkih dana godišnje otpada na DKA te se procjenjuje da se godišnje izdvaja oko 2,4 milijarde američkih dolara za liječenje DKA (12).

Cilj našeg istraživanja bio je utvrditi najčešće precipitirajuće čimbenike, kao i laboratorijske i kliničke značajke DKA u Republici Hrvatskoj. Poznavanje navedenih čimbenika može nam pomoći u razvoju mjera prevencije nastanka DKA i edukaciji bolesnika i medicinskih djelatnika o pravovremenom prepoznavanju rizičnih situacija za nastanak DKA, što bi u konačnici trebalo rezultirati i manjom pojavnošću DKA.

ISPITANICI I POSTUPCI

U ovo retrospektivno, multicentrično istraživanje uključeni su bolesnici sa ŠB-om tipa 1 ili tipa 2, stariji od 18 godina, s dijagnosticiranim DKA u razdoblju od 1. siječnja 2014. godine do 31. prosinca 2018. godine, liječeni u 5 različitih bolničkih središta (Opća bolnica Dubrovnik, Opća bolnica Našice, Klinički bolnički centar Split, Klinički bolnički centar Zagreb i Klinički bolnički centar Osijek). Ako je bolesnik imao više epizoda DKA, samo prva je bila uključena u analizu. Bolesnici sa steroidnim ŠB-om i ŠB-om u sklopu drugih endokrinih poremećaja, poput akromegalije i Cushingovog sindroma, isključeni su iz istraživanja. Demografski podatci (dob i spol), epidemiološka obilježja, klinički podatci i nalazi krvnih pretraga preuzeti su iz medicinske dokumentacije.

ŠB je definiran prema kriterijima ADA (prema engl. *American Diabetes Association*, Američko dijabetološko udruženje) koji uključuju: glukoza natašte ≥ 7 mmol/l ili glukoza 2 sata nakon opterećenja glukozom ≥ 11,1 mmol/L ili slučajno izmjerena glukoza > 11,1 mmol/L uz klasične simptome hiperglikemije ili hiperglikemične krize ili glikolizirani hemoglobin A1c (HbA1c) > 6,5 % (13). Kriteriji za dijagnozu ŠB-a tipa 1 bili su nizak c-peptid uz prisutnost jednog ili više autoimunih biljega (GAD, prema engl. *glutamic acid decarboxylase*, dekarboksilaza glutamične kiseline, ICA, prema engl. *islet cell antibodies*, protutijela na otočiće gušterače, IA2, prema engl. *insulin antibodies,* protutijela na inzulin) (13). Ispitanici koji nisu ispunjavali navedene kriterije kategorizirani su kao ŠB tipa 2. Dijagnoza DKA postavljena je na temelju kriterija ADA: glikemija > 13,9 mmol/l, pH < 7,3 ili bikarbonati (HCO_3^-) < 18 mmol/l te ketonemija ili ketonurija (14). Prema stupnju DKA bolesnici su podijeljeni u skupine s blagim (pH 7,25 - 7,3, HCO_3^- 15 - 18 mmol/l), umjerenim (pH 7,0 - 7,24, HCO_3^- 10 - 15 mmol/l) i teškim DKA (pH <7, HCO_3^-< 10 mmol/l) (14). Neadekvatna regulacija ŠB-a definirana je kao vrijednost HbA1c veća od 8 % ili neadherencija prema terapiji ŠB-a, uz neadekvatnu prehranu i stil života u posljednjih 6 mjeseci. Klinički značajna hipokalijemija definirana je kao vrijednost kalija < 3,3 mmo/L (15). Za provedbu istraživanja dobivena je suglasnost Etičkog povjerenstva zdravstvenih ustanova u kojima je istraživanje provedeno.

STATISTIČKA ANALIZA

Statistička analiza podataka napravljena je u statističkom programu SPSS 23.0 (IBM Corp., Armonk, USA). Normalnost raspodjele podataka ispitivana je Kolmogorov-Smirnovljevim i Shapiro-Wilkovim testovima. Parametrijski podatci prikazani su kao srednja vrijednost ± standardna devijacija, a neparametrijski podatci pomoću vrijednosti medijana (raspon, min. – maks.). Usporedba srednjih vrijednosti parametara dvaju nezavisnih uzoraka učinjena je pomoću Studentovog t-testa, odnosno pomoću jednofaktorske analize varijance (ANOVA) s post-hoc Tuckey analizom, ako se promatralo više od dva nezavisna uzorka. Za ispitivanje razlike između neparametrijskih obilježja dvaju nezavisnih uzoraka korišten je Mann-Whitneyev test, a za više od dva nezavisna uzorka Kruskal-Wallisov test, dok je za usporedbu učestalosti upotrebljen χ^2 test. Za procjenu povezanosti neparametrijskih obilježja upotrijebljena je Spearmanova korelacija. Vrijednosti *P* manje od 0,05 smatrane su statistički značajnima.

REZULTATI

U istraživanje je uključeno 160 bolesnika (55 % muškaraca) od kojih je ŠB tip 1 imalo 68 %, a ŠB tip 2 32 %. Medijan dobi ispitanika bio je 42 godine (od 18 do 89). Klinička obilježja bolesnika uključenih u istraživanje prikazana su u tablici 1.

Tablica 1. *Obilježja bolesnika s tipom 1 i tipom 2 šećerne bolesti (ŠB), N = 160.*

Obilježje	Svi	ŠB tip 1	ŠB tip 2	*P**
Broj bolesnika (%)	160 (100)	109 (68)	51 (32)	
Dob (godine)	42 (18 - 89)	32 (18 - 89)	56 (20 - 87)	< 0,01†
Spol, n (%)				
Muškarci	88 (55)	56 (51)	32 (63)	0,233‡
Žene	72 (45)	53 (49)	19 (37)	
Trajanje hospitalizacije (dani)	9 (4 - 40)	7,5 (4 - 28)	12,5 (4 - 40)	< 0,05†
Smrtni ishod, n (%)	3 (2)	1 (33)	2 (67)	0,192‡
pH	7,165 ± 0,014	7,162 ± 0,018	7,169 ± 0,022	0,203§
Koncentracija glukoze (mmol/L)	30,7 ± 1,3	27,9 ± 1,6	35,4 ± 2,2	0,054§
Koncentracija kalija (mmol/L)	4,9 ± 0,1	4,9 ± 0,1	4,9 ± 0,2	0,67§
Stupanj DKA, n (%)				
Lagan	33 (21)	21 (19)	12 (23)	0,535‡
Umjeren	92 (57)	63 (58)	29 (57)	0,911‡
Teški	35 (22)	25 (23)	10 (20)	0,635‡
Precipitirajući čimbenici DKA				
Infekcija	91 (57)	56 (51%)	35 (68%)	< 0,05‡
Infekcija (samostalno)	47 (52)	33 (59%)	14 (40%)	0,715‡
Infekcija i prva prezentacija ŠB	11 (12)	4 (7%)	7 (20%)	< 0,05‡
Infekcija i nereguliran ŠB	33 (36%)	19 (34%)	14 (40%)	0,144‡
Prva prezentacija ŠB	15 (9%)	10 (9.3%)	5 (10%)	0,899‡
Neregulirani ŠB	43 (27%)	34 (31.3%)	9 (18%)	0,072‡
Ostalo	11 (7%)	9 (8.4%)	2 (4%)	0,505‡

Podatci su prikazani kao srednja vrijednost ± standardna devijacija, odnosno medijan (raspon, min. – max.). DKA – dijabetička ketoacidoza. Koncentracije prikazane u mmol/L. P - razlike između bolesnika sa ŠB-om tipa 1 i ŠB-om tipa 2; †Mann-Whitneyev test; ‡χ2 test; §Studentov t-test*

Najčešći uzrok DKA bila je infekcija (57 %), a potom slijede loše reguliran ŠB (37 %) i prva prezentacija ŠB-a (9 %), dok je u 7 % bolesnika DKA bila posljedica drugih uzroka, kao što su kvar inzulinske pumpe, moždani ili srčani udar.

U skupini bolesnika s infekcijom najčešće se radilo o infekciji mokraćnog (30 %), probavnog (30 %) i respiratornog sustava (19 %), dok je 21 % bolesnika imalo infekcije drugih sijela (meningitis, celulitis, encefalitis). U 36 % ovih bolesnika uz infekciju je bila prisutna i prethodno loše reguliran ŠB, a u 12 % DKA uzrokovan infekcijom bio je prva manifestacija bolesti (tablica 1). U bolesnika sa ŠB tipa 2 infekcije su bile češći uzrok DKA nego u bolesnika sa ŠB tipa 1 ($P < 0,05$). S druge strane, u bolesnika sa ŠB tipa 1 loše regulirana glikemija bila je češći uzrok DKA nego u bolesnika sa ŠB-om tipa 2.

S obzirom na stupanj DKA, većina bolesnika (78 %) je imala laki ili umjereno teški stupanj, dok je udio bolesnika s teškim stupnjem DKA iznosio 22 %. Nije bilo razlike u uzrocima nastanka između pojedinih stupnjeva DKA (tablica 2).

Bolesnici s umjerenim i teškim stupnjem DKA imali su više koncentracije kalija i glukoze u usporedbi s bolesnicima s lakim stupnjem DKA (< 0,05). Klinički značajnu hipokalijemiju imala su 2 bolesnika (1,25 %), jedan s teškim, a drugi s umjereno teškim DKA.

Trajanje hospitalizacije zbog DKA iznosilo je 9 (4 - 40) dana, pri čemu su bolesnici sa ŠB-om tipa 2 bili hospitalizirani značajno dulje od onih sa ŠB-om tipa 1 [12,5 (4 - 40) dana prema 7,5 (4 - 28) dana; $P < 0,05$]. Nasuprot tome, nije utvrđena razlika u trajanju hospitalizacije između pojedinih stupnjeva težine DKA. Troje bolesnika (2 %) u dobi od 40, 70 i 70 godina umrlo je od DKA; dvoje ih imalo teški stupanj, a jedan umjereno teški stupanj DKA.

Tablica 2. *Obilježja bolesnika s lakim, umjerenim i teškim stupnjem dijabetičke ketoacidoze (DKA), N = 160.*

Obilježje	Svi	Lak stupanj DKA (n = 33)	Umjeren stupanj DKA (n = 92)	Težak stupanj DKA (n = 35)	*P**
Broj bolesnika, (%)	160	33 (21)	92 (58)	35 (22)	
Dob (godine)	42 (18 - 89)	40 (19 - 89)	46 (18 - 79)	41,5 (18 - 87)	0,3†
Spol, n (%)					
Muškarci	88 (55)	21 (64)	53 (58)	14 (40)	0,109‡
Žene	72 (45)	12 (36)	39 (42)	21 (60)	
Trajanje hospitalizacije (dani,)	9 (4 - 40)	6 (4 - 28)	10 (4 - 40)	10 (4 - 28)	0,209†
Smrtni ishod, n (%)	3 (2)	0 (0)	1 (33)	2 (67)	0,154‡
pH	7,165 ± 0,014	7,241 ± 0,025	7,155 ± 0,014	7,079 ± 0,043	< 0,01§,
Koncentracija glukoze (mmol/l)	30,7 ± 1,3	26,1 ± 1,7	32,3 ± 1,9	32,9 ± 3,2	< 0,05§
Koncentracija kalija (mmol/l)	4.9 ± 0,1	4,5 ± 0,2	4,9 ± 0,14	5,4 ± 0,4	< 0,05§
Tip šećerne bolesti (ŠB), n (%)					
tip 1	109 (68)	21 (64)	63 (68,5)	25 (71)	0,784‡
tip 2	51 (32)	12 (36)	29 (31,5)	10 (29)	
Precipitirajući čimbenici DKA, n (%)					
Infekcija	91 (57)	20 (61)	53 (58)	18 (51)	0,73‡
- Infekcija (samostalno)	47 (52)	12 (60)	27 (51)	8 (44)	0,474‡
- Infekcija i prva prezentacija ŠB-a	11 (12)	3 (15)	7 (13)	1 (6)	0,545‡
- Infekcija i nereguliran ŠB	33 (36)	5 (25)	19 (36)	9 (50)	0,561‡
Prva prezentacija ŠB-a	15 (9)	3 (9)	10 (11)	2 (6)	0,671‡
Nereguliran ŠB	43 (27)	6 (18)	24 (26)	13 (37)	0,204‡
Ostalo	11 (7)	4 (12)	5 (5)	2 (6)	0,409‡

Podatci prikazani kao srednja vrijednost ± standardna devijacija, odnosno medijan (raspon, min. – max.). DKA – dijabetička ketoacidoza. P - razlike između bolesnika s lakim, umjerenim i teškim stupnjem DKA, †Kruskal-Wallisov test; ‡χ2 test; § ANOVA*

RASPRAVA

DKA je jedna od najčešćih i najozbiljnijih akutnih komplikacija ŠB-a koja se najčešće javlja u bolesnika sa ŠB-om tipa 1 u kojih postoji potpuni nedostatak sekrecije inzulina, no nerijetko se javlja i u bolesnika sa ŠB-om tipa 2. Niz istraživanja analizirao je uzroke i klinička obilježja bolesnika s DKA u različitim populacijama, a rezultati su se razlikovali ovisno o dizajnu istraživanja i obilježjima ispitanika (1,11,16,17). U cilju da dodatno istražimo precipitirajuće čimbenike, klinička obilježja i ishode liječenja bolesnika s DKA, proveli smo multicentrično, retrospektivno istraživanje koje je uključivalo 160 bolesnika iz pet bolničkih središta koji se bave liječenjem bolesnika sa ŠB-om.

U više od polovice bolesnika u ovom istraživanju uzrok DKA bila je infekcija, samostalno ili u kombinaciji s loše reguliranom šećernom bolesti, a pri tom se najčešće radilo o infekcijama mokraćnog i probavnog sustava. U oko trećine bolesnika predisponirajući čimbenik za nastanak DKA bila je loša regulacija ŠB-a, dok se u nešto manje od 10% bolesnika radilo o prvom očitovanju ŠB-a. I u drugim istraživanjima infekcije i loša regulacija ŠB-a bile su najčešći predisponirajući čimbenici za nastanak DKA (18–22). Najvjerojatniji uzrok su nedovoljna educiranost bolesnika o primjeni inzulinske terapije tijekom akutne bolesti te nesuradljivost bolesnika u liječenju inzulinom zbog psihosocijalnih i socioekonomskih razloga (19,23).

Ishodi liječenja bolesnika s DKA značajno se razlikuju među pojedinim istraživanjima ovisno o stupnju organizacije zdravstvenog sustava u pojedinoj populaciji i obilježjima bolesnika. Dosadašnja istraživanja pokazala su da se stopa smrtnosti od DKA kreće u rasponu < 1 % - 30 % i značajno je niža u razvijenim zemljama u usporedbi sa zemljama u razvoju (3,4,16). Starija životna dob, koja je povezana s teškim komorbiditetima, utvrđena je kao najvažniji prediktor smrtnosti u bolesnika s DKA (3,23,24). Nedavno istraživanje je pokazalo da smrtnost nije povećana samo tijekom hospitalizacije zbog DKA, nego još jedno dulje vrijeme nakon oporavka (17). U našem istraživanju stopa smrtnosti tijekom hospitalizacije zbog DKA je bila relativno niska (2 %), što je sukladno podatcima u drugim razvijenim zemljama (16, 25). Od troje bolesnika koji su umrli, dvoje su imali teški stupanj, a jedan umjereno teški stupanj DKA.

Razlike u smrtnosti između pojedinih istraživanja mogu se dijelom pripisati i razlikama u udjelu bolesnika sa ŠB-om tipa 2 u istraživanoj populaciji, budući da su ti bolesnici stariji i imaju više popratnih bolesti, što sve doprinosi većem riziku smrtnog ishoda (17). Slično tome, i dulje trajanje hospitalizacije koje je utvrđeno u bolesnika sa ŠB-om tipa 2, neovisno o stupnju težine DKA, može se pripisati dobi i popratnim bolestima (23,26,27). Udio bolesnika sa ŠB-om tipa 2 u dosadašnjim se istraživanjima kretao u rasponu od 12 % do 56 %, dok je u našem istraživanju iznosio 32 %.

Retrospektivni dizajn istraživanja, relativno mali broj ispitanika i nedostatak podataka o dugoročnoj smrtnosti bolesnika najveća su ograničenja ovog istraživanja koja treba uzeti u obzir pri interpretaciji rezultata.

ZAKLJUČAK

Ovo istraživanje je pokazalo da su infekcije i loša regulacija ŠB-a najčešći precipitirajući čimbenici u nastanku DKA. Stoga je u procesu liječenja ŠB-a posebnu pažnju potrebno obratiti edukaciji bolesnika o važnosti redovite primjene inzulina, kao i korekciji terapije tijekom akutne infekcije.

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SUMMARY

PRECIPITATING FACTORS AND CLINICAL FEATURES OF DIABETIC KETOACIDOSIS

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Introduction: Diabetic ketoacidosis (DKA) is one of the most serious acute complications of diabetes mellitus (DM). In some studies, infections have been found to be a precipitating factor in more than half of the subjects. On the other hand, several recent studies emphasize that poor treatment adherence is also a common cause of DKA. ***Objective:*** To identify the most common precipitating factors for DKA in Croatia. ***Patients and Methods:*** This retrospective, multicenter study included DM type 1 or DM type 2 patients diagnosed with DKA between January 1, 2014 and December 31, 2018, and treated in 5 different DM treatment centers, i.e., Dubrovnik, Našice, Split, Zagreb and Osijek. Only the first episode of DKA was included in the analysis. Patients receiving steroids and DM due to endocrine disorders such as acromegaly and Cushing's syndrome were excluded. ***Results:*** The study included 160 patients (55% of men), of whom 68% had DM type 1. The mean age of the respondents was 42 (18-89) years. The most common cause of DKA was infection (57%), followed by poorly controlled DM (37%) and first presentation of DM (9%), while in 7% of patients DKA was due to other causes such as insulin pump failure, stroke or myocardial infarction. In the group of patients with infections, urinary tract infections (30%), gastrointestinal infections (30%) and respiratory tract infections (19%) were most common, whereas 21% of patients had other sources of infection. In 36% of these patients, the infection was associated with previously poorly controlled diabetes, and in 12% of them, DKA caused by the infection was the first manifestation of the disease. In patients with type 2DM, infections were more often the cause of DKA than in patients with type 1DM ($p<0.05$).Poorly controlled glycemia appeared to be a more frequent cause of DKA in patients with type 1 DM (31%) than in patients with type 2 DM (18%). ***Conclusion:*** The most common precipitating factors for the development of DKA were infections and poor diabetes management. Better education of patients about the importance of regular insulin administration and correction of therapy in acute illness could reduce the risk of DKA.

Key words: diabetes, mellitus, ketoacidosis, precipitating factors, hyperglycemia, insulin, infection

POST-COVID-19 CONDITION IN SOLID ORGAN TRANSPLANT RECIPIENTS: A SINGLE-CENTER EXPERIENCE

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Solid organ transplant (SOT) recipients who survived acute COVID-19 can develop post-COVID-19 condition. Post-COVID-19 condition can affect multiple organ systems, and its prevalence is up to 70% in general population. Data regarding post-COVID-19 condition in SOT recipients are scarce. The aim of our study was to investigate the prevalence and characteristics of post-COVID-19 condition in SOT recipients.The study included SOT recipients who had kidney transplantation in Merkur University Hospital between 2007 and 2020, and who survived COVID-19. Between July 2020 and June 2021, 78 transplanted patients (kidney only or combined with pancreas or liver) had acute COVID-19, of which 13 patients died. The study was conducted in the form of survey and included 60 patients who all gave informed consent for participation in the study. Post-COVID-19 condition experienced 40 (67%) patients, and most common symptoms were fatigue (43%) and shortness of breath (30%), followed by hair loss (27%), insomnia (22%), sweating (22%), and decline in the quality of life (20%). There was no difference between patients with post-COVID-19 condition and those without post-COVID-19 condition regarding gender, age, transplanted organ(s), time from transplantation to COVID-19, or need of hospitalization due to COVID-19. In conclusion, post-COVID-19 condition was frequent among SOT (kidney) patients, with fatigue and shortness of breath as the most common symptoms, as in general population. Thus, unfortunately, COVID-19 contributed to their comorbidity burden at longterm as well.

Key words: post-COVID-19, SARS-CoV-2, kidney transplantation

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INTRODUCTION

Solid organ transplant (SOT) recipients represent a frail population susceptible to various infective complications, including infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for coronavirus disease 2019 (COVID-19) (1). COVID-19 is a multi-organ disease with a broad spectrum of manifestations. Given the diversity of organ systems that can be affected by COVID-19, survivors might have persistent postinfection sequels, called post-COVID-19 condition (2). Post-COVID-19 is defined by Delphi consensus as a condition that occurs in patients with COVID-19 usually three months from the onset, with symptoms that last for at least two months, and cannot be explained by an alternative diagnosis (2). Given that post-COVID-19 is a relatively new condition, its exact prevalence in general population is unknown, but according to one study, up to 70% of patients experienced long-term consequences of COVID-19 (3). It seems that the prevalence of post-COVID-19 condition does not depend of the severity of acute COVID-19, indicating that hospitalized patients and outpatients, including asymptomatic ones, are both at risk of experiencing long-term consequences of COVID-19 (4). Post-COVID-19 condition is often associated with multiple organ systems, although fatigue, shortness of breath, and cognitive impairment are reported as most common. Furthermore, negative impact on the quality of life and socioeconomic status is also relevant (3). It has been shown that kidney disease is associated with a higher risk of in-hospital mortality among COVID-19 patients (5). Kidney transplant recipients (KTR) have often allograft dysfunction and are more susceptible to acute kidney injury (6). Studies have reported higher mortality rate in KTR with COVID-19 compared to non-transplant patients (7-9). Immunocompromised patients, such as SOT recipients, are more susceptible to COVID-19, have a

greater risk of disease progression and prolonged recovery compared to non-transplant patients (10). It is expected that this population is at an increased risk of experiencing post-COVID symptoms. Nevertheless, data regarding post-COVID-19 condition in SOT recipients are scarce (3). The aim of our study was to investigate the prevalence and characteristics of post-COVID-19 condition in SOT recipients.

PATIENTS AND METHODS

Kidney transplant recipients who had transplantation in Merkur University Hospital between 2007 and 2020 and who survived COVID-19 from July 2020 to June 2021 were asked to participate in the study. Data regarding COVID-19 were regularly documented in all KTRs who were followed-up at our outpatient clinic. The diagnosis of COVID-19 was confirmed by the reverse transcription polymerase chain reaction test from nasopharyngeal/oropharyngeal swab.

Between July 2020 and June 2021, 78 patients had acute COVID-19, of which 13 patients did not survive. All patients who survived acute COVID-19 were contacted to participate in the study, of which five refused it. So, 60 patients were included in the study. The study was conducted in the form of questionnaire on regular follow up, or in the form of telephone interview during the year 2021. All participants gave informed consent for participation in the study. The following post-COVID-19 symptoms were analyzed: fatigue, shortness of breath, cough, anosmia, arthralgia, myalgia, headache, dysgeusia, inappetence, hair loss, dizziness, insomnia, sweating, diarrhea, inability to concentrate, anxiety, depression, memory loss, and decline in the quality of life.

Statistics

Continuous variables were expressed as median (interquartile range, IQR), and categorical variables as absolute number (percentage). Differences between the groups were analyzed with Mann-Whitney test for continuous variables, and using χ^2-test or Fisher exact test when appropriate for categorical variables. Statistical analysis was performed using the STATISTICA (version 12.0 Stat Soft Inc, Tulsa, OK, USA) software.

RESULTS

The study included 60 SOT recipients (65% of male), median age at transplantation 45 (IQR 37-55) years, who had COVID-19 during 2020 and 2021. There were 45 kidney only transplant recipients, 13 pancreas and kidney, and 2 liver and kidney transplant recipients. Median time from transplantation to acute COVID-19 was 5 (IQR 2-10) years. Twenty of them were hospitalized due to COVID-19. All participants survived COVID-19. Among the COVID-19 survivors, 40 (67%) had post-COVID-19 symptoms. The most common symptoms were fatigue (43%) and shortness of breath (30%), followed by hair loss (27%), insomnia (22%), sweating (22%), and decline in thequality of life (20%) (Figure 1). Regarding duration of post-COVID-19 symptoms, patients reported fatigue, hair loss, insomnia, and decline in the quality of life as most persistent (longer than three months), while none of the patients had anosmia, dysgeusia, or inappetence for more than three months. Among patients with post-COVID-19, hair loss was more common among females compared to males (70.6% *vs.* 17.4%; p=0.001), whereas no gender difference was found in other post-COVID-19 symptoms. There was no difference between patients with post-COVID-19 condition and those without post-COVID-19 condition according to gender (p=0.15), age (p=0.882), transplanted organ(s) (p=0.562), time from transplantation to COVID-19 (p=0.312) or need of hospitalization due to COVID-19 (p=0.395) (Table 1).

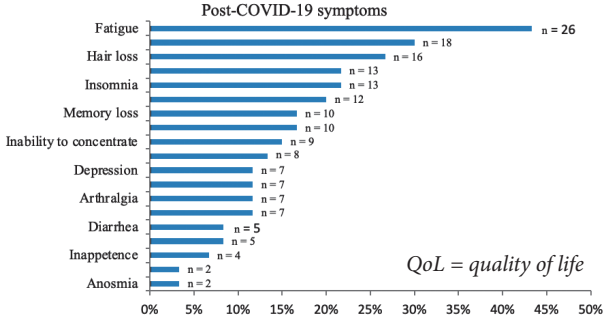

Figure 1. *Prevalence of post-COVID-19 symptoms among solid organ transplant recipients (N=60).*

Table 1. *Solid organ transplant recipients with post-COVID-19 and without post-COVID-19 (N=60)*

Variable	Post-COVID-19 Yes (n=40)	Post-COVID-19 No (n=20)	p
Gender (male), n(%)	23 (57.5)	16 (80)	0.15*
Age#(years)	48 (36.0-53.5)	41 (36.5-56.5)	0.882†
Type of transplantation, n (%) Kidney SPKT SLKT	 30 (75) 8 (20) 2 (5)	 15 (75) 5 (25) 0	0.562*
Time from transplantation to COVID-19 (years)#	6.3 (2.6-19.5)	3.9 (1.9-9.3)	0.312†
Need ofhospitalization, n (%)	15 (37.5)	5 (25)	0.395*

**χ2-test or Fisher exact test; #median (interquartile range, IQR); †Mann-Whitney test; SPKT = simultaneous kidney-pancreas transplantation; SLKT = simultaneous liver-kidney transplantation*

DISCUSSION

Ameta-analysis which included 47 950 patients showed a prevalence of 80% of post-COVID-19 in general population (3). We analyzed post-COVID-19 symptoms in SOT recipients, of which three-quarters were only kidney recipients, while the rest had combined SOT. Our research showed a high prevalence of post-COVID-19 among SOT recipients (67%), without taking into account the severity of acute COVID-19. To date, small observational studies on post-COVID-19 condition in SOT recipients have been published (11,12). According to the Polish study which included 67 KTRs, the prevalence of post-COVID-19 syndrome was 70%, similar as in our research (12). Another study reports clinical complications in 45% of KTR following acute COVID-19, whereas laboratory abnormalities were found in 71% of patients (11). In our study, self-reported fatigue and shortness of breath were most common, followed by hair loss, insomnia, sweating, and reduced quality of life. On the other hand, anosmia and dysgeusia were uncommon. In line with our results, Malinowska *et al*. report that fatigue (43%), dyspnea (34%) and hair loss (31%) were the most common symptoms, whereas smell disorder was the least common one (3%) (12). In addition, Basic Jukic *et al*. also report shortness of breath and tiredness as the most common clinical complications among KTRs (11). In a meta-analysis which included 15 studies with 47 910 patients, almost 60% of patients had fatigue, followed by headache (44%), attention disorder (27%), hair loss (25%), and dyspnea (24%) (3). According to published studies, it seems that post-COVID-19 symptoms including fatigue and dyspnea are frequent in general population and in SOT recipients. One-quarter to almost one-third of patients will experience hair loss after acute COVID-19 (3,12). We found a significantly higher prevalence of hair loss among female patients. Several studies also report a higher prevalence of hair loss after acute COVID-19 among female patients from general population (13-15). Hyperinflammatory response, called cytokine storm occurring during acute COVID-19, may initiate hair loss, together with other factors such as depression and anxiety, which can occur afterwards (18). Numerous clinical complications in KTRs, such as worsening of hypertension, *de novo* diabetes mellitus, skin changes, etc., can occur following acute COVID-19, but they seem to be infrequent (11). Not all sequels of post-COVID-19 last equally. In a study conducted among KTRs in India, fatigue was the most common symptom but its prevalence decreased significantly with time (17). Also, improvement in the quality of life was observed in the majority of patients as follow up time was prolonged (17). On the other hand, in our research, fatigue, hair loss, insomnia, and decline in the quality of life were the most persistent symptoms (lasting beyond three months). In both studies, duration of anosmia was short. This difference in the duration of symptom persistence could be because of the characteristic of the study population, younger patients in India compared to our population, and severity of acute COVID-19 (17). All patients in India required hospitalization *versus* 37.5% of our patients who had post-COVID-19 symptoms (17). We did not find any difference according to gender, age, type of transplanted organ(s), time from transplantation to COVID-19, or need of hospitalization due to acute COVID-19 between patients who developed post-COVID-19 condition and those who did not experience any of post-COVID-19 symptoms. Our results on the prevalence of post-COVID-19 in SOT recipients, with fatigue and shortness of breath being most common, are comparable to the previously published results in general population, as well as in KTRs (3,12).

Our study had several limitations. First was a small study sample. Second, we did not have data on treatment during acute COVID-19. Third, timing of follow up after recovery from acute COVID-19 at our outpatient clinic was not equal in all patients. Fourth, we did not use a widely and validated questionnaire regarding post-COVID-19 symptoms. Fifth, the nature of telephone interview could influence data quality compared to data obtained by face to face interview. However, the main strength of the study are particular epidemiological data on Croatian SOT patients in COVID-19 pandemic during the first pandemic years, inadequately present in the literature so far. Additional strength is that we analyzed miscellaneous post-COVID-19 symptoms (a total of 19 symptoms). Further studies should focus on investigating this relatively new syndrome, risk factors and its consequences in immunocompromised patients such as SOT recipients.

In conclusion, kidney transplanted patients frequently experienced post-COVID-19, irrespective of their gender, age, kidney only or combined SOT or time from transplantation, or even hospitalization, with fatigue and shortness of breath as the most common symptoms, as in general population. Unfortunately, COVID-19 also contributed to their comorbidity burden at longterm.

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SAŽETAK

STANJE POST-COVID-19 U PRIMATELJA BUBREŽNOG PRESATKA: ISKUSTVO JEDNOG CENTRA

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Bolesnici s presađenim organom koji su preboljeli COVID-19 mogu razviti sindrom post-COVID-19. Sindrom post-COVID-19 obilježen je mogućnošću zahvaćanja više organskih sustava, a javlja se u do 70 % bolesnika u općoj populaciji. Podatci o sindromu post-COVID-19 među bolesnicima s presađenim organom su oskudni te je stoga cilj ovoga istraživanja bio utvrditi učestalost i obilježja sindroma post-COVID-19 u navedenoj populaciji. Istraživanje je uključilo bolesnike koji su imali presadbu bubrega u Kliničkoj bolnici Merkur u razdoblju od 2007. do 2020. godine i koji su preživjeli COVID-19. U razdoblju od srpnja 2020. do lipnja 2021. godine 78 bolesnika (s presađenim samo bubregom ili bubregom i gušteračom ili bubregom i jetrom) preboljelo je COVID-19, od kojih je 13 umrlo. Istraživanje je provedeno u obliku ankete, a uključilo je 60 bolesnika koji su dali dobrovoljni pristanak za sudjelovanje. Sindrom post-COVID-19 razvilo je 40 (67 %) ispitanika, a najčešći simptomi bili su umor (43 %), kratkoća daha (30 %), gubitak kose (27 %), nesanica (22 %), znojenje (22 %) i smanjenje životne kakvoće (20 %). Nije nađeno razlike između bolesnika sa sindromom post-COVID-19 i onih koji nisu razvili sindrom post-COVID-19 s obzirom na spol, dob, vrstu presađenog organa, vrijeme od presadbe organa do COVID-19 ili potrebe za hospitalizacijom zbog COVID-19. Zaključno, prema rezultatima našeg istraživanja možemo reći da je sindrom post-COVID čest među bolesnicima s presađenim solidnim organom (bubregom) te da su umor i kratkoća daha najčešći simptomi, kao i u općoj populaciji. COVID-19 je tako, nažalost, i dugoročno doprinio njihovom ukupnom pobolu.

Ključne riječi: post-COVID-19, SARS-CoV-2, transplantacija bubrega

THE EFFECT OF PHYSICAL ACTIVITY AND FITNESS LEVEL ON RETINAL MICROCIRCULATION

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Aim: To examine differences in retinal microcirculation between people with different degrees of physical fitness. *Methods:* The subjects were divided into athletes and non-athletes. Both groups took part in two examinations. The first examination was performed before short-term exercise and the second one immediately after it. First group consisted of 25 athletes (50 eyes), and the second group of 25 non-athletes (50 eyes) who were not previously exposed to acute physical stress. Athletes are defined as people who have been engaged in some form of regular physical activity for at least 5 years, and have met certain criteria according to the International Physical Activity Questionnaire (IPAQ). Non-athletes were those who were physically inactive or at least not regularly engaged in physical activity during the same period and did not meet the IPAQ criteria. The subjects were men and women between 18 and 26 years of age who did not have any cardiovascular disease, used drugs affecting the cardiovascular system, nor had an eye disease or a refractive error greater than spherical equivalent of +/-3 diopters. The examination consisted of optical coherence tomography angiography (OCT-A) imaging pre- and post-workout. The parameters taken into account were vascular density (VD) at three different macular areas according to the standard Early Treatment Diabetic Retinopathy Study (ETDRS) grid, i.e., central zone, inner zone and full area; perfusion density (PD), also at the three mentioned zones; and the area of foveal avascular zone (FAZ) in both eyes. The research also included a standardized survey on physical activity of the subjects (IPAQ), which was completed before the examination. The acute physical exercise consisted of the standardized incremental cycling ergometer test (ICET), which was performed on a stationary exercise bike for 5 minutes, at a given load of 12 degrees. On statistical processing of the data obtained, SPSS for Windows (version 13.0, SPSS Inc., Chicago, Illinois, USA) software was used. *Results:* Baseline measures of VD and PD were similar between the groups. FAZ surface was significantly increased in the athlete group compared with non-athletes both at baseline and after short-term exercise. VD was significantly higher in athletes post-exercise compared with the non-athlete group. Central PD was also significantly increased after exercise in the athlete group, and not in the non-athlete group. *Conclusion:* The results obtained in this study demonstrated that athletes exhibited a more intensive vascular reaction to exercise. The parameters in basal conditions did not show significant difference between the two groups, except for FAZ which was larger in athletes. Significant differences present post-workout in other measured values indicated a more dynamic vascular system in physically active individuals.

Key words: physical activity, retina, blood vessels, OCT-A, ICET

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INTRODUCTION

It is known that physical activity influences the cardiovascular system. Both acute and chronic physical activity have an impact on blood vessels, leading to structural and functional changes. Changes and adaptation depend on the level of physical load and can be modulated with oxidative stress and inflammation induced by exercise (1). With aging, our cardiovascular system reduces its compliance (2). This loss in compliance is caused by hypertrophy of the vascular smooth muscles, replacing smooth muscles with connective tissue, and gaining a higher level of cross-linking of the connective tissue. Physical activity has a protective effect on blood vessels through several mechanisms. First, there is an increase in blood pressure and in pulse, which leads to stretching of blood vessels that counteracts the formation of connective tissue cross-links. Second, during exercise, skeletal muscles have an impact on the vessels in a way that they cause vasodilata-

tion that has a similar effect as mentioned previously. Third, physical activity leads to increase in the release and synthesis of nitric oxide that causes vasodilatation (3,4). These factors can have an antimitogenic effect that can in the end slow down or even stop the loss in the blood vessel compliance (5). Also, high levels of physical activity can improve retinal microcirculation both in children and adults. This happens because of the protective effect of exercise on small blood vessels (6). Some studies show that exercise causes changes in the optic nerve, macular perfusion, and there is an increase in the blood flow of the retina (7,8). All of these show how physical activity has a positive impact on the retina and that it may have a protective effect against diseases such as diabetic retinopathy and age related macular degeneration (9).

AIM

The aim of this research was to analyze differences in retinal microcirculation between subjects with different physical fitness levels.

METHODS

The subjects were divided into two groups, athletes and non-athletes. Study subjects underwent two sets of measurements. The first measurement was carried out before the acute physical exercise, and the second one immediately after it.

The mean age of athletes was 22.6±2.4 years; there were 32% of females. The mean age of non-athletes was 22.5±2.1 years of age, with 52% of females. In the first group of subjects there were 25 athletes (50 eyes), and in the second group 25 non-athletes (50 eyes) who were not previously exposed to acute physical stress. Athletes were defined as people who have been involved in some form of regular physical activity for at least 5 years in the form of training a sport, fitness, aerobic training, etc., and according to the Standardized International Physical Activity Questionnaire (IPAQ), they spent at least 3 days a week in intense physical activity (lifting weights, fast running/cycling, aerobics, etc.) or 7 days of combining intense and moderate physical activity and walking. Non-athletes were subjects who were physically inactive or at least not regularly engaged in exercise during the same period and did not meet the IPAQ criteria. The subjects were men and women between 18 and 26 years of age who did not have any cardiovascular disease, did not use drugs affecting the cardiovascular system, nor had an eye disease or a refractive error greater than spherical equivalent of +/- 3 diopters. The examination consisted of optical coherence tomography angiography (OCT-A) imaging pre- and post-workout using Zeiss Cirrus HD5000 device (Carl Zeiss Vision GmbH, Berlin, Germany). The parameters taken into account were vascular density (VD) at three different macular areas according to standard Early Treatment Diabetic Retinopathy Study (ETDRS) grid, i.e., central zone, inner zone and full area; perfusion density (PD), also at the three mentioned areas; and the area of foveal avascular zone (FAZ) in both eyes. The research also included a standardized IPAQ survey on physical activity of the subjects, which was completed before the examination. The acute physical exercise consisted of the standardized incremental cycling ergometer test (ICET), which was performed on a stationary exercise bike for 5 minutes, at a given load of 12 degrees. Subject pulse and oxygen saturation were measured using pulse oximeter (Pulse oximeter, model OXY 300, Beijing Choice Electronic Technology Co. Ltd., Beijing, China) pre- and post-workout. Numerical data were expressed as mean ± standard deviation (SD), and difference in numerical values between the groups was analyzed using Student's t-test. On statistical processing of the data obtained, SPSS for Windows (version 13.0, SPSS Inc. Chicago, Illinois, USA) software was used. The level of statistical significance was set at $p<0.05$.

RESULTS

Vascular density in basal conditions before acute exercise did not show statistically significant differences between the groups of athletes and non-athletes (Figure 1).

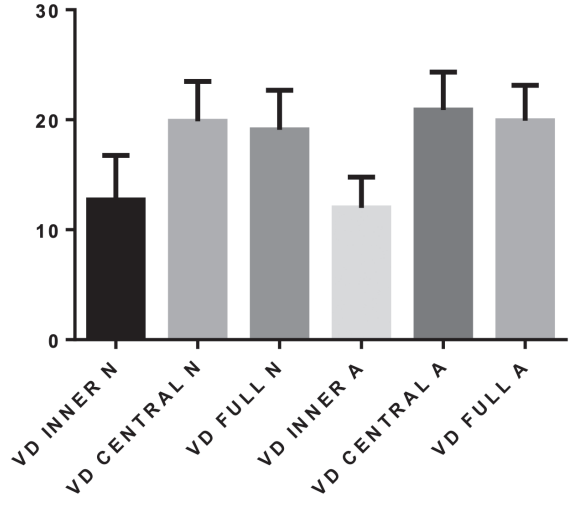

Figure 1. *Vascular density levels (arbitrary units) between the group of athletes (A) and the group of non-athletes (N) in basal conditions (pre-workout); VD INNER N – vascular density inner non-athletes, VD CENTRAL N – vascular density central non-athletes, VD FULL N – vascular density full non-athletes, VD INNER A – vascular density inner athletes, VD CENTRAL A – vascular density central athletes, VD FULL A – vascular density full athletes; data are expressed as mean ± standard deviation; n=50 per group, p>0.05, Student's t test.*

Perfusion density was similar between the groups at baseline measurements (Figure 2).

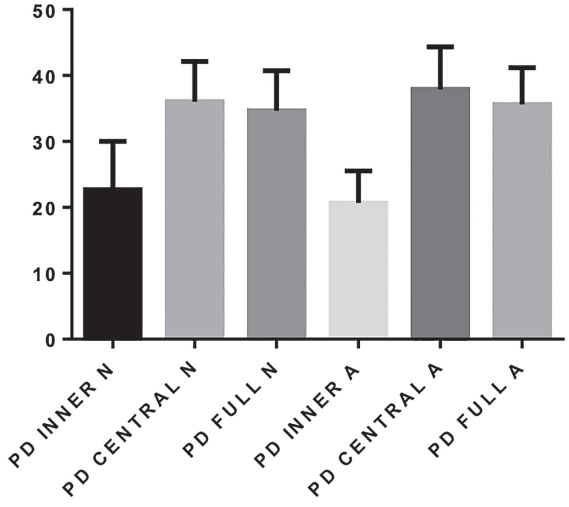

Figure 2. *Perfusion density levels (arbitrary units) between the group of athletes (A) and the group of non-athletes (N) in basal conditions (pre-workout); PD INNER N – vascular density inner non-athletes, PD CENTRAL N – vascular density central non-athletes, PD FULL N – vascular density full non-athletes, PD INNER A – vascular density inner athletes, PD CENTRAL A – vascular density central athletes, PD FULL A – vascular density full athletes. Data are expressed as mean ± standard deviation; n=50 per group, p>0.05, Student's t test.*

Athletes exhibited a larger surface of FAZ compared with non-athletes (0.17±0.01 mm^2*vs.* 0.21±0.01 mm^2, n=50 *per* group, p=0.008) (Figure 3).

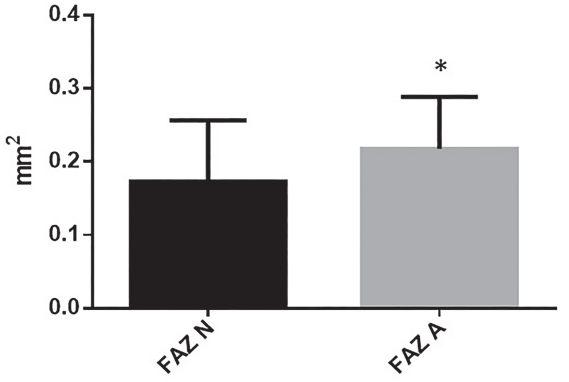

Figure 3. *Difference in surface area off oveal avascular zone (FAZ) between the group of athletes (A) and the group of non-athletes (N); data are expressed as mean ± standard deviation; n=50 per group, *p=0.008, Student's t-test.*

After exercise, non-athletes exhibited similar results as at baseline. Vascular density was significantly higher in athletes post-exercise at all measured areas, central, inner and full (11.99±0.39 *vs.* 17.55±0.92; 20.9±0.4 *vs.* 30.86±1.4; and 19.92±0.45 *vs.* 29.1±1.2, respectively, n=50 *per* group, p<0.001). Central PD was also significantly increased after exercise in the athlete group, and not in the non-athlete group (20.64±0.68 *vs.* 23.23±0.79, n=50 *per* group, p=0.01) (Figures 4 and 5).

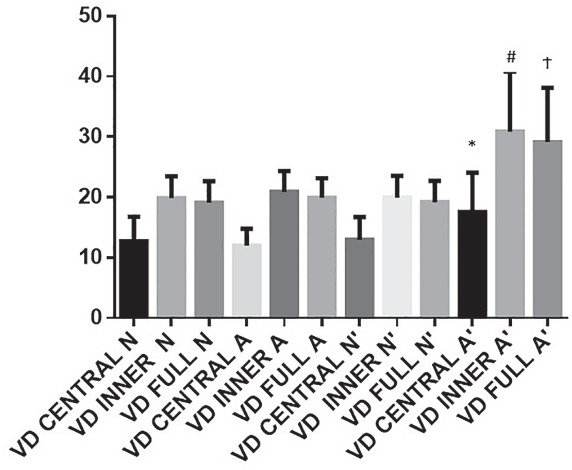

Figure 4. *Vascular density levels (arbitrary units) between the group of athletes (A) and the group of non-athletes(N) pre- and post-workout; VD INNER N – vascular density inner non-athletes, VD CENTRAL N – vascular density central non-athletes, VD FULL N – vascular density full non-athletes, PD INNER A – vascular density inner athletes, VD CENTRAL A – vascular density central athletes, VD FULL A – vascular density full athletes; post-workout marked with '; data are expressed as mean ± standard deviation; n=50 per group. *p<0.001 (VD CENTRAL A vs. VD CENTRAL A'), #p<0.001 (VD INNER A vs. VD INNER A'), #p<0.001 (VD FULL A v.s VD FULL A').*

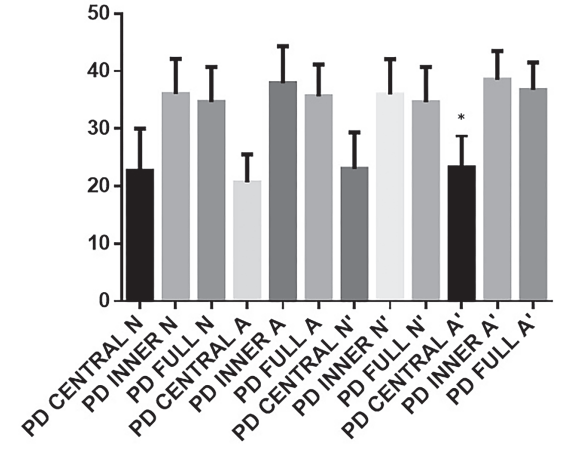

Figure 5. *Perfusion density levels (arbitrary units) between the group of athletes (A) and the group of non-athletes(N) pre- and post-workout; PD INNER N – perfusion density inner non-athletes, PD CENTRAL N – perfusion density central non-athletes, PD FULL N – perfusion density full non-athletes, PD INNER A – perfusion density inner athletes, PD CENTRAL A – perfusion density central athletes, PD FULL A – perfusion density full athletes; post-workout marked with '; data are expressed as mean ± standard deviation; n=50 per group, *p=0.01 (PD CENTRAL A vs. PD CENTRAL A').*

Foveal avascular zone demonstrated similar properties before and after exercise. The athlete group had a significantly larger surface of FAZ pre- and post-workout compared with the non-athlete group. The exercise itself did not affect FAZ surface in either group (0.17±0.01 mm^2 *vs.* 0.22±0.008 mm^2, n=50 *per* group, p=0.003) (Figure 6).

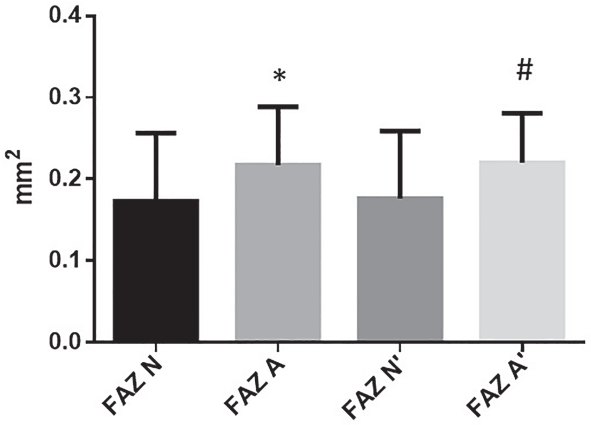

Figure 6. *Difference in surface area off oveal avascular zone (FAZ) between the group of athletes (A) and the group of non-athletes (N); post-workout marked with '; data are expressed as mean ± standard deviation; n=50 per group, *p=0.008 (FAZ N vs. FAZ A), #p=0.03 (FAZ N' vs. FAZ A').*

Athletes had a significantly lower pulse rate before and after exercise compared with non-athletes. There was no difference in the oxygen saturation rate (Table 1).

Table 1.*Pulse rate and oxygen saturation before and after acute exercise between athletes and non-athletes (n=50 per group, *Student's t-test)*

	Non-athletes		Athletes		*p**
	X	SD	X	SD	
PULSE RATE	87.04	13.12	74.68	9.39	**<0.001**
Oxygen saturation	97.24	0.83	97.24	1.01	>0.999
PULSE RATE after exercise	136.76	16.38	101.24	11.37	**<0.001**
Oxygen saturation after exercise	96.96	1.14	97.16	1.14	0.538

DISCUSSION

The study included two groups according to their fitness level, athletes and non-athletes. The research was divided into two parts when the subjects underwent two measurements, i.e., before and after physical load, and the parameters were observed in those two measurements.

According to study results, there was no difference in the VD and PD parameters in basal conditions, which might mean that regular physical activity and fitness level did not affect density of retinal blood vessels or perfusion, but a difference was observed in the FAZ between the examined groups. During the study, the VD and PD parameters were observed in the surface layer of the retina. Although no difference was found in that layer, Kim *et al.* showed a reduction of VD in the surface layer in their research (10). Nelis *et al.* report that the main finding in OCT-A measurements was change in the size of the FAZ conditioned by greater physical fitness. They used multivariate regression analysis and found that running speed at individual lactate threshold, a marker strongly associated with aerobic performance capacity, significantly contributed to differences in the FAZ area. They conclude that smaller areas of FAZ were found in athletes (11). In the present study, we did not record such findings in young healthy adults. It is possible that differences in FAZ in the present study were due to acute vasoconstriction. Although certain trends were visible, it probably takes a longer period of different lifestyle to have an effect on FAZ that could be quantified by OCT-A.

This research showed significant differences in heart rate before and after exercise in athletes and non-athletes. Athletes had a lower heart rate than non-athletes in both measurements, which was expected, since the heart of athletes has a 40% higher stroke volume on average compared to physically inactive people (12). Concerning oxygen saturation, there were no statistically significant differences in relation to exercise, which is also consisted with previously reported data.

In the second set of experiments, after the respondents were subjected to a moderate form of physical activity, statistically significant increases in VD and PD were observed in the athlete group. The research conducted by Kim *et al.* showed no significant changes in retinal circulation before and after moderate physical activity, but there were significant changes in cardiovascular parameters, i.e., pulse and blood pressure (10). The study conducted by Nelis *et al.* determined that the only significant difference after physical activity in the two groups of subjects also was only recorded in FAZ, while other variables did not show statistically significant differences. Also, in this study, significant changes were recorded in heart rate. Among athletes, the expected values were significantly lower, and it was observed that these two variables are clearly related to regular physical activity (11). A major limitation of this study was that only young healthy adults were examined. Therefore, we could not observe long-term benefits of regular exercise and its effect on retinal circulation. Also, we did not separate different forms of regular exercise, and therefore could not establish

specific benefits related to a certain sport or exercise form. Future studies are needed to further elucidate the effects of exercise on long-term retinal health.

CONCLUSIONS

In basal conditions, there was no statistically significant difference between athletes and non-athletes. People with increased physical fitness showed a larger area of FAZ in basal conditions and after exercise. Athletes demonstrated an increase in PD and VD parameters after physical activity. Significant differences in post-workout values indicate a more dynamic vascular system in physically active individuals.

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SAŽETAK

UTJECAJ VJEŽBANJA I RAZINE OSPOSOBLJENOSTI NA MIKROCIRKULACIJU RETINE

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Cilj rada: Ispitati razlike u retinalnoj mikrocirkulaciji između osoba s višim i nižim stupnjem tjelesne osposobljenosti. ***Postupci:*** Ispitanici su bili podijeljeni na sportaše i nesportaše. Obje skupine pristupile su dvama odvojenim mjerenjima. Prvi pregled obavljen je prije fizičkog opterećenja, a drugi neposredno nakon njega. Prvu skupinu činilo je 25 sportaša (50 očiju), a drugu 25 nesportaša (50 očiju) koji prethodno nisu bili izloženi akutnom fizičkom stresu. Sportaše definiramo kao osobe koje se najmanje 5 godina bave nekim oblikom redovite tjelesne aktivnosti u obliku treniranja nekog sporta, fitnesa, aerobnog treninga i sl. te ispunjavaju određene uvjete prema međunarodnom upitniku o tjelesnoj aktivnosti *(International Physical Activity Questionnaire,* IPAQ). Neki sportaši nisu bili fizički aktivni ili barem ne redovito tijekom istog razdoblja i nisu ispunjavali navedene kriterije IPAQ. Ispitanici su bili muškarci i žene u dobi između 18 i 26 godina koji nisu imali nikakvu srčanožilnu bolest, nisu uzimali lijekove koji utječu na srčanožilni sustav niti su imali ikakvu bolest očiju ili refraktivnu grešku veću od sfernog ekvivalenta +/-3 dioptrije. Prikupljanje podataka provedeno je pomoću optičke koherentne tomografske angiografije (OCT-A). Analizirani su sljedeći parametri: vaskularna gustoća (VG) u tri različita područja makule prema mreži ETDRS *(Early Treatment Diabetic Retinopathy Study)*: središnja zona, unutarnja zona i puna zona; perfuzijska gustoća (PG), također u tri navedena područja; te površina fovealne avaskularne zone (FAZ). Istraživanje je uključivalo i standardiziranu anketu o tjelesnoj aktivnosti ispitanika (IPAQ) koja se ispunjavala prije samog pregleda. Fizičko opterećenje sastojalo se od standardiziranog testa ICET (incremental cycling ergometer test), koji se izvodio na stacionarnom sobnom biciklu u trajanju od 5 minuta pri zadanom opterećenju od 12 stupnjeva, nakon čega su ispitanicima izmjereni puls i saturacija. Također, prije same vježbe ispitanicima su izmjerene srčana frekvencija i saturacija u mirovanju. Za statističku obradu dobivenih podataka primijenjen je softverski sustav SPSS for Windows (verzija 13.0, SPSS Inc., Chicago, Illinois, SAD). ***Rezultati:*** Mjerenja u bazalnim uvjetima pokazala su slične vrijednosti VGi PG između skupina. Površina FAZ bila je statistički značajno veća u skupini sportaša u usporedbi s nesportašima i u bazalnim uvjetima i nakon tjelovježbe. Nakon tjelovježbe VG i središnji PG pokazali su statistički značajno povećanje u sportaša, dok u nesportaša nije bilo razlike prije i nakon tjelovježbe. ***Zaključak:*** Rezultati dobiveni u ovoj studiji pokazali su da sportaši imaju intenzivniju vaskularnu reakciju na vježbanje. Parametri u bazalnim uvjetima nisu pokazali značajnu razliku između dviju skupina osim za FAZ, koja je bila veća u sportaša. Značajne razlike bile su prisutne nakon treninga u drugim izmjerenim vrijednostima i ukazuju na dinamičniji vaskularni sustav u fizički aktivnih pojedinaca.

Ključne riječi: fizička aktivnost, retina, krvne žile, OCT-A, ICET

USPOREDBA KOMPJUTORIZIRANOM TOMOGRAFIJOM VOĐENE PERKUTANE MIKROVALNE ABLACIJE I PARCIJALNE NEFREKTOMIJE U LIJEČENJU T1a STADIJA KARCINOMA BUBREGA

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Svrha istraživanja: Zahvaljujući većoj dostupnosti radioloških metoda, raste incidencija malih karcinoma bubrega (KCB), što dovodi do sve veće potrebe za razvojem minimalno invazivne terapije uz očuvanje bubrežne funkcije. U pacijenata sa znatnim komorbiditetima, uz parcijalnu nefrektomiju (PN), koja je zlatni standard terapije, došlo je do primjene perkutanih ablativnih metoda. Mikrovalna ablacija (MVA) bubrega, unatoč dokazanim prednostima pred drugim ablativnim metodama, još uvijek nije uvrštena u terapijske smjernice te se smatra eksperimentalnom. *Ciljevi* istraživanja bili su usporediti stopu lokalnog recidiva, ukupno preživljenje, preživljenje bez metastaza i preživljenje specifično za karcinom nakon perkutanog visokoenergetskog MVA pod kontrolom kompjutorizirane tomografije (CT) i PN-a u terapiji KCB-a stadija T1a. ***Postupci:*** U retrospektivnu studiju bilo je uključeno osamdeset pacijenata, kojima je u razdoblju od siječnja 2015. do lipnja 2018. dijagnosticiran i histološki potvrđen KCB stadija T1a. Svi su pacijenti odlukom uro-onkološkog konzilija Kliničkog bolničkog centra Sestre milosrdnice bili indicirani za perkutanu termalnu mikrovalnu ablaciju tumora bubrega ili otvoreni PN. Od pacijenata indiciranih za kiruršku resekciju izabralo se pacijente koji prema veličini tumora i kompleksnosti tumora prema klasifikaciji mRENAL odgovaraju skupini pacijenata liječenih MVA-om, kako bi se ovim usklađivanjem metodom uparivanja po skoru sklonosti došlo do što kvalitetnijih spoznaja o onkološkim ishodima. U studiju su bili uključeni pacijenti koji su radiološki i klinički praćeni najmanje 12 mjeseci nakon zahvata. Zahvat MVA izvodio se u svih pacijenata perkutanim pristupom, pod kontrolom CT-a. ***Rezultati:*** Onkološki ishodi nisu dokazali postojanje statistički značajne razlike između ovih dviju terapijskih metoda. Ukupno preživljenje nakon jedne godine iznosilo je 100 % nakon MVA i PN-a. Jednogodišnje preživljenje bez lokalnog recidiva iznosilo je 92,5 % nakon MVA i 90 % nakon PN-a. Tri su pacijenta razvila lokalni recidiv na mjestu zahvata u skupini pacijenata liječenih MVA-om i pet pacijenata nakon PN-a. U sva tri slučaja MVA recidiv je bio tretiran dodatnim zahvatom MVA unutar dva do četiri tjedna s posljedičnom sekundarnom učinkovitošću MVA od 100 %. Unatoč nešto većem ukupnom broju pacijenata s lokalnim recidivom i metastazama KCB-a u skupini pacijenata liječenih PN-om, nije zabilježena statistički značajna razlika u onkološkom ishodu. Preživljenje bez metastaza nakon godinu dana iznosilo je 97,5 % nakon MVA i 95 % nakon PN-a. Iako se prosječne vrijednosti glomerulske filtracije nisu znatno razlikovale između skupina MVA i PN prije i nakon zahvata, kada se izračunao prosječni postotak gubitka bubrežne funkcije, iznosio je –8,9 ± 6 % za skupinu MVA i –21,7 ±8,2 % za skupinu PN, što predstavlja statistički značajnu razliku ($P < 0,001$). U skupini pacijenata liječenih ablacijom zabilježen je znatno manji procijenjeni operacijski gubitak krvi nego u skupini pacijenata koji su liječeni kirurškom resekcijom (54 ±19 mL vs 225,1 ±45,7 mL, $P < 0,001$). ***Zaključak:*** Perkutana terapija KCB-a metodom MVA može biti jednako vrijedna alternativa zlatnom standardu kirurškog PN-a u pacijenata sa znatnim komorbiditetima, ali i u ostalih s malim tumorima bubrega zbog dokazanih prednosti očuvanja bubrežne funkcije.

Ključne riječi: karcinom bubrega, nefrektomija, mikrovalna ablacija, kompjutorizirana tomografija, intervencijska radiologija

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UVOD

Karcinom bubrega (KCB) sedmi je najčešće dijagnosticirani karcinom u muškaraca u Hrvatskoj (1). KCB čini 4% svih malignih tumora u svijetu (5% u muškaraca i 3% u žena) s 270 000 novootkrivenih slučajeva godišnje te 116 000 smrtnih slučajeva godišnje na globalnoj razini. Dijagnoza KCB-a posljednjih se 10 godina 1,5 do 2 puta češće postavlja u muškaraca nego u žena (2).

Histopatologija KCB-a jest heterogena te iako se otprilike jedna trećina karcinoma otkrije u fazi proširene bolesti s udaljenim metastazama, većina malih KCB-a ograničenih na bubreg ima tendenciju sporog rasta (3). Također, u posljednje vrijeme raste učestalost slučajno otkrivenih tumora bubrega pri slikovnoj radiološkoj obradi te je većinom riječ o KCB-u stadija T1 (4). Opcije liječenja kod KCB-a stadija T1a uključuju radikalnu nefrektomiju (RN), otvorenu parcijalnu nefrektomiju (OPN), laparoskopsku parcijalnu nefrektomiju (LPN), perkutanu ablaciju tumora ili aktivno praćenje. Metode parcijalne kirurške resekcije u cilju poštede i očuvanja bubrežnog parenhima preuzele su mjesto zlatnog standarda liječenja KCB-a stadija T1a zbog jednakog onkološkog ishoda uz dugoročno bolju kvalitetu života pacijenata u usporedbi s radikalnom kirurškom resekcijom (5). Iako se kirurška resekcija smatra zlatnim standardom, pojavile su se minimalno invazivne perkutane termalne ablativne metode, koje su pokazale dobru učinkovitost u terapiji malih KCB-a uz manju učestalost komplikacija, kraći oporavak i manji utjecaj na funkciju bubrega (6). Zbog postojanja većeg broja opcija u liječenju ovih tumora, konačna odluka o vrsti terapije ovisi o karakteristikama tumora. Tu spadaju pozicija unutar bubrega, veličina, odnos prema kanalnom sustavu i okolnim strukturama, ali i komorbiditeti pacijenta. S tim je ciljem razvijen sustav bodovanja kompleksnosti tumora bubrega nazvan mRENAL. U brojnim se istraživanjima pokazalo da taj sustav može predvidjeti učinkovitost ili komplikacije kirurške, ali i perkutane ablacijske metode liječenja KCB-a (7).

Iako se istraživanjima pokazalo da ablacijske tehnologije imaju znatnu prednost za pacijenta i zdravstveni sustav s obzirom na niže periproceduralne komplikacije, kraće trajanje zahvata, hospitalizacije i bolje očuvanje funkcije organa uz usporedive onkološke ishode, o njihovoj se primjeni u praksi i dalje često raspravlja. Posljednje smjernice Europskog i Američkog urološkog društva izrazito su oprezne s preporukom primjene ablativnih metoda liječenja KCB-a, s objašnjenjem kako i dalje postoji manjak adekvatnih studija i dugoročnog praćenja onkoloških ishoda (8). U tim smjernicama se kao preporučene metode spominju u prvom redu krioablacija (CA) i radiofrekventna ablacija (RFA). Razlog tome je što su navedene metode dulje prisutne i posljedično istražene u većem broju studija. Nedavna retrospektivna analiza 384 pacijenta liječenih jednom od ablativnih metoda (CA, RFA ili mikrovalna ablacija, MVA) pokazala je da nema znatne razlike u onkološkim ishodima, kao ni u učestalosti komplikacija, no MVA je imala značajno kraće trajanje zahvata, uz potrebu za manjim količinama sedacije za vrijeme zahvata (9). Uz to MVA ima neke dokazane prednosti pred ostalim ablacijskim metodama u smislu kraćeg trajanja zahvata, veće zone ablacije s manje potrebnih iglenih sondi, izostanak „*heat-sinc*“ učinka te izostanak kontraindikacije u pacijenata s ugrađenim elektrostimulatorima za srce. Iako danas postoje studije koje ukazuju na učinkovitost i sigurnost MVA-e tijekom srednje dugog i dugog razdoblja praćenja (10,11) i dalje je glavni problem nedostatak visokokvalitetnih kohortnih ili prospektivnih studija koje bi izravno uspoređivale zahvat parcijalne nefrektomije (PN) i perkutanu slikovno vođenu MVA novijom generacijom uređaja. Spoznaje iz dosadašnjih istraživanja, unatoč poznatim nedostatcima tih studija, ukazuju na mogućnost uvrštavanja MVA-e u algoritam i preporuke liječenja KCB-a, uz RFA i CA.

CILJ

Cilj našeg istraživanja bio je usporediti onkološke ishode liječenja - stopu lokalnog recidiva, preživljene bez bolesti i metastaza i ukupno preživljenje nakon perkutanog visokoenergetskog MVA pod kontrolom kompjuterizirane tomografije (CT) i PN-a u terapiji KCB-a stadija T1a za pacijente nakon uparivanja pacijenata po skoru sklonosti. Uz to smo usporedili učestalost komplikacija nakon ovih zahvata, kao i utjecaj na bubrežnu funkciju.

POSTUPCI:

U retrospektivnu studiju je uključeno 80 od 96 pacijenata kojima je u razdoblju od siječnja 2015. do lipnja 2018. dijagnosticiran i histološki potvrđen KCB stadija T1a. Svi su pacijenti odlukom uro-onkološko-radiološkog konzilija, a u skladu sa smjernicama europskog i američkog urološkog društva, bili indicirani za perkutanu termalnu ablaciju tumora bubrega ili otvoren PN. Od pacijenata liječenih kirurškom resekcijom izabralo se pacijente koji prema veličini tumora, kompleksnosti tumora po klasifikaciji mRENAL te odabranim bitnim obilježjima samih pacijenata čine odgovarajuću kontrolnu skupinu. Ta je skupina izabrana da u najvećoj mogućoj mjeri odgovara ispitivanoj skupini pacijenata liječenih MVA-om. U studiju su uključeni pacijenti sa solitarnim tumorom bubrega

stadija T1a, dimenzije do 4 cm, s patohistološkom potvrdom karcinoma, koji su radiološki i klinički praćeni minimalno 12 mjeseci nakon zahvata. Iz studije su bili isključeni pacijenti s poznatom proširenom zloćudnom bolešću bubrega u trenutku zahvata ili drugim poznatim malignim bolestima, s višestrukim tumorima na istom ili suprotnom bubregu, kao i pacijenti s genskom predispozicijom za recidivirajući ili multiple tumore bubrega.

Za sve zahvate MVA primijenjen je uređaj za ablaciju s radnom frekvencijom 2,45 MHz (Amica, *Hospital Service*, Italija), kojim se visokim postavkama energije od 80 W izvodi ablacija u vremenu izabranom prema preporukama proizvođača za određenu veličinu tumora. Upotrebljavala se iglena elektroda V4 dimenzije 16 ili 14 G s unutarnjim cirkulirajućim tekućim hlađenjem vodom. Radiološko praćenje nakon zahvata svim se pacijentima radilo prema preporukama europskih intervencijskih društava mjesec dana, četiri mjeseca i 12 mjeseci od zahvata, svakih šest mjeseci nakon toga do treće godine te svakih godinu dana nakon treće godine. Praćenje se radilo CT ili MR pregledom, multifaznim protokolom, prije i nakon primjene intravenskog kontrastnog sredstva. Za svakog se pacijenta iz arhiva evidentirala i analizirala bubrežna funkcija: serumski kreatinin i ureja te glomerulska filtracija prije zahvata, neposredno nakon zahvata i pri daljnjim standardnim kontrolama. Analizirani su prikupljeni podatci o prethodnim bolestima ili operacijama, komorbiditetima, anesteziološkom statusu ASA (prema engl. *American Society of Anaesthesiologists*), dobi, BMI-ju (prema engl. *body mass index,* indeks tjelesne mase), duljini trajanja zahvata te duljini hospitalizacije. Procijenjeni gubitak krvi pri zahvatu izračunat je iz nalaza krvne slike prije zahvata te 72 sata nakon zahvata, uzimajući u obzir procijenjenu površinu tijela, procijenjeni volumen krvi prilagođen spolu, nalaz hematokrita prije zahvata i 72 sata nakon zahvata.

Evidentiralo se eventualno postojanje ranih ili kasnih komplikacija u pacijenata, koje su klasificirane kao male ili velike i stupnjevane prema klasifikaciji Clavien-Dindo. Normalnost distribucije kontinuiranih varijabli testirana je Shapiro-Wilkovim testom. Kontinuirane varijable s normalnom distribucijom uspoređivane su Studentovim t-testom. Gdje kontinuirane varijable nisu zadovoljile test normalnosti, upotrijebljen je Mann-Whitneyjev U-test. Za kategoričke varijable upotrijebljen je hi kvadrat test, osim za učestalost manju od 5, gdje je primijenjen Fisherov egzaktni test. Uparivanje prema skoru sklonosti izgrađeno je uz pomoć modula MatchIt za programski jezik R uz upotrebu varijabli vrste primjerene terapije, dobi, spola, BMI-ja pacijenta, histološke dijagnoze, ASA klasifikacije, veličine tumora, zahvaćene strane tijela, mRENAL skora te procijenjene glomerulske filtracije prije zahvata, metodom najbližeg susjeda i uz omjer u liječenoj i kontrolnoj skupini 1 : 1. Kaplan-Meierova metoda upotrijebljena je za stvaranje krivulja preživljenja, a preživljenje je uspoređeno *log-rank* testom. Izvedene su univarijatna i multivarijatna logistička regresija za prediktore preživljenja. Pri logističkoj regresiji primijenjen je Firthov model uz pomoć paketa logistf za R.

REZULTATI

U tablici 1 prikazana su demografska obilježja pacijenata, kao i obilježja tumora. U istraživanje je uključeno 80 bolesnika s KCB-om, po 40 pacijenata liječenih postupkom MVA, odnosno PN-om, srednje životne dobi 63,9 godina. Zabilježena je statistički značajna razlika u ASA anesteziološkom statusu pacijenata liječenih s MVA i PN ($P < 0,001$, Mann-Whitneyjev U test). Pacijenti liječeni ablacijom bili su znatno lošijega početnog statusa, što je često i bila indikacija za ovu vrstu zahvata zbog rizika za pacijenta zbog opće anestezije i kirurškog zahvata.

Tablica 1. *Demografska obilježja pacijenata i obilježja tumora (N = 80)*

Obilježje	Skupina liječena mikrovalnom ablacijom (n = 40)	Skupina liječena parcijalnom nefrektomijom (n = 40)	*P*
Dob (godine)	66,6 ± 9,2	61,2 ± 6,9	0,004*
Spol n (%) Muški Žene	 20 (50) 20 (50)	 21 (52,5) 19 (47,5)	0,823†
Strana tumora n (%) Lijeva Desna	 23 (57,5) 17 (42,5)	 20 (50) 20 (50)	0,501†
Patohistološka dijagnoza n (%) Svjetlostanični KCB Papilarni KCB Kromofobni KCB	 28 (70) 11 (27,5) 1 (3,6)	 28 (70) 11 (27,5) 1 (3,6)	>0,999†
Veličina tumora (cm)	2,6 (0,8 – 4)	2,5 (1,1 – 3,8)	0,559‡
mRENAL	8,1 (4 – 11)	7,9 (5 – 10)	0,386‡
Vrijeme praćenja (mjeseci)	16,6±3,6	20,3± 5	0,001*

*Studentov t-test; †χ^2 test; ‡Mann-Whitneyev U-test
KCB, karcinom bubrega

U skupini pacijenata liječenih MVA-om prosječna veličina tumora bila je 2,6 cm, dok je nakon uparivanja u skupini pacijenata liječenih kirurški iznosila 2,5 cm, što ne predstavlja statistički značajnu razliku ($P = 0,559$, Studentov t-test). Analiza anatomske kompleksnosti tumora bubrega mRENAL pokušava standardizirati procjenu KCB-a u smislu težine zahvata kirurške resekcije ili ablacije. Osim dimenzije tumora,

uzima u obzir i dodatne čimbenike, kao što su pozicija i odnos prema kanalnom sustavu bubrega. mRENAL skor u prosjeku je bio nešto veći u pacijenata liječenih ablacijom, no to nije činilo statistički značajnu razliku (P = 0,386, Mann-Whitneyjev U-test). Minimalno vrijeme praćenja, koje je postavljeno u obje skupine kao uključni kriterij, bilo je 12 mjeseci od izvedenog zahvata. Prosječno vrijeme praćenja pacijenata iznosilo je 16,6 mjeseci (±3,6, medijan 15 mjeseci) u skupini MVA i 20,3 mjeseca (±5, medijan 20 mjeseci) u skupini PN.

Prema nalazu patologa u 66 od 96 analiziranih tumora dijagnosticiran je svjetlostanični karcinom bubrežnih stanica, što odgovara 63,3% pacijenata uključenih u studiju. U skupini pacijenata liječenih MVA-om svjetlostanični karcinom činio je 70 % tumora, a u PN skupini 67,9 %. Od preostalih tumora u skupini MVA bilo je 11 pacijenata s dijagnosticiranim papilarnim KCB-om (27,5 %) i jedan kromofobni KCB (3,6 %). U tablici 2 prikazani su rezultati perioperacijskih ishoda za praćene terapijske skupine, koji uključuju gubitak krvi, trajanje zahvata, vrijeme hospitalizacije, utjecaj na bubrežnu funkciju i komplikacije. U skupini pacijenata liječenih MVA-om zabilježen je znatno manji procijenjeni operacijski gubitak krvi nego u skupini pacijenata koji su liječeni PN-om (54 ±19 mL vs 225,1 ±45,7 mL, P < 0,001). Značajna razlika zabilježena je u trajanju samog kirurškog zahvata, sa znatno kraćim zahvatom pri MVA KCB-a u usporedbi s PN-om (48,5 ±9,6 min vs 91,9 ±13,1 min). Također je nakon ablacije zabilježeno i znatno kraće ukupno trajanje hospitalizacije pacijenta (2,3 ±0,7 dana vs 7,9 ±1,6 dana).

Tablica 2. *Perioperacijski ishodi nakon mikrovalne ablacije (N = 40) i parcijalne nefrektomije (N = 40)*

Ishod	Skupina liječena mikrovalnom ablacijom (n = 40)	Skupina liječena parcijalnom nefrektomijom (n = 40)	*P*
Procijenjeni gubitak krvi (mL)	54 ± 19	225,1 ±45,7	< 0,001*
Trajanje zahvata (min)	48,5 ± 9,6	91,9 ± 13,1	< 0,001*
Promjena procijenjene glomerulske filtracije (%)	-8,9 ± 6	-21,7 ± 8,2	< 0,001*
Komplikacije n (%) Clavien Dindo 1 Clavien Dindo 2 Clavien Dindo 3	 7 (17,5) 0 (0) 0 (0)	 10 (25) 6 (15) (2,5)	0,011†

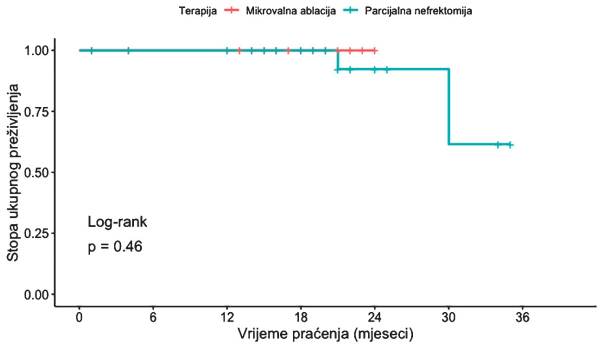
*Studentov t-test;†χ^2 test

Iako se prosječne vrijednosti glomerulske filtracije nisu znatno razlikovale u MVA i PN skupinama prije i nakon zahvata, kada se izračunao prosječni postotak gubitka bubrežne funkcije, iznosio je –8,9 ±6% za skupinu MVA i –21,7 ± 8,2% za skupinu PN, što je predstavljalo statistički značajnu razliku (P < 0,001). Ukupno je u sedam pacijenata nakon MVA zabilježeno postojanje komplikacija, od kojih su sve bile 1. stupnja. Nakon PN-a zabilježene su komplikacije u 17 pacijenata, od čega deset 1. stupnja, šest 2. stupnja i samo jedna ozbiljnija komplikacija 3. stupnja, koja je zahtijevala intervenciju zbog velikog hematoma. Usporedbom učestalosti komplikacija zabilježena je statistički značajna razlika između dviju praćenih skupina pacijenata (P = 0,011).

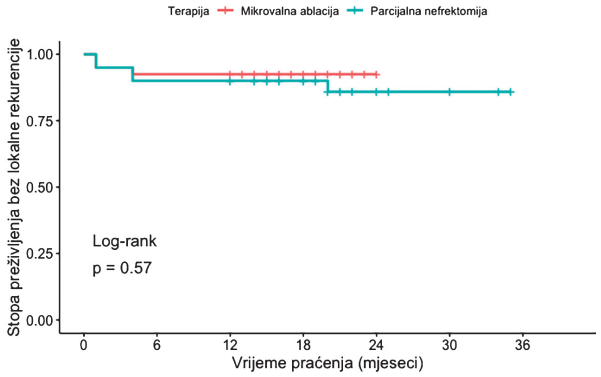
Rezultati univarijatne i multivarijatne logističke regresije pri procjeni postojanja rizičnih čimbenika za preživljenje bez bolesti nisu u skupina uparenih prema skoru sklonosti pronašle da nijedan od čimbenika – dob pacijenta, spol, veličina tumora, mRENAL skor kompleksnosti tumora ni tip zahvata – ne može statistički značajno predvidjeti preživljenje.

Onkološki ishodi nisu pokazali postojanje statistički značajne razlike između ovih dviju terapijskih metoda.

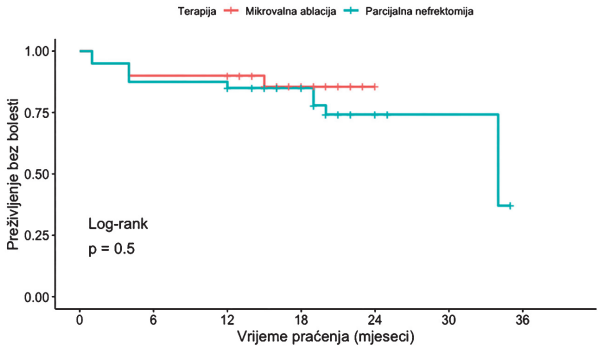
Nije zabilježena statistički značajna razlika u općem preživljenju pacijenata između dviju skupina (P = 0,45) (sl. 1). Ukupno preživljenje nakon godine dana je iznosilo 100 % nakon MVA-e, kao i nakon PN-a. Među pacijentima koji su liječeni MVA-om u razdoblju praćenja nije zabilježen nijedan smrtni slučaj. Dva su pacijenta umrla u skupini pacijenata liječenih PN-om, u drugoj i trećoj godini praćenja. Jedan od njih razvio je metastatsku bolest na drugoj kontroli te je uz kemoterapiju umro od KCB-a, dok je drugi pacijent razvio srčanu dekompenzaciju. Nije zabilježena statistički značajna razlika u općem preživljenju pacijenata između dviju skupina. Tri pacijenta razvila su lokalni recidiv na mjestu zahvata u skupini pacijenata liječenih MVA-om. Od toga su dva otkrivena na prvoj kontroli dijagnostičkim CT-om, a treći na kontroli nakon četiri mjeseca. U sva tri slučaja recidiv je bio tretiran dodatnim zahvatom mikrovalne ablacije unutar dva do četiri tjedna te na daljnjim kontrolama nije zabilježeno postojanje znakova rezidue lezije sve do kraja praćenja, što upućuje na sekundarnu učinkovitost ablacije od 100 %. U PN skupini pacijenata zabilježeno je ukupno pet lokalnih recidiva tumora od kojih su svi dodatno operirani u smislu radikalne nefrektomije. Jednogodišnje preživljenje bez lokalnog recidiva (95 % CI) (sl. 2) iznosilo je 92,5 % nakon MVA-e i 90 % nakon PN-a, što nije bilo statistički značajno različito (P = 0,57). Preživljenje bez bolesti nakon godine dana praćenja iznosilo je 90 % nakon MVA-e i 85 % nakon PN-a. Unatoč nešto većem ukupnom broju pacijenata s lokalnim recidivom ili metastazama KCB-a u skupini pacijenata liječenih PN-om, nije zabilježena statistički značajna razlika u ovom onkološkom ishodu (P = 0,96) (sl. 3).



Sl. 1. *Ukupno preživljenje nakon MVA-e i PN-a karcinoma bubrega*



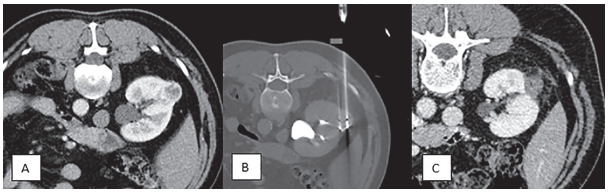
Sl. 2. *Preživljenje bez lokalnog recidiva nakon MVA-e i PN-a KCB-a*



Sl. 3. *Preživljenje bez bolesti nakon MVA-e i PN-a KCB-a*

Slika 4 prikazuje postavljanje MVA sonde u KCB-i desnog bubrega i izgled ožiljnog tkiva na mjestu ablacije pri kontrolnom praćenju pacijenta postkontrastnim CT-om.

Učinjena je analiza učinka MVA-e i stope lokalnog recidiva kod KCB-a, ovisno o dimenziji tumora. Svi liječeni KCB podijeljeni su u dvije skupine ovisno o dimenziji, na tumore manje od 3 cm i tumore najvećeg promjera 3 do 4 cm. Nije zabilježena statistički značajna razlika u učestalosti pojave recidiva s obzirom na veličinu tumora (P = 0,206).



Sl. 4. *Mikrovalna ablacija KCB-a desnog bubrega pod kontrolom CT-a. (A) Egzofitičan tumor bubrega u srednjoj trećini promjera 2,8 cm, niske kompleksnosti prema mRENAL izračunu (6) dijagnosticiran na postkontrastnim CT snimkama u arterijskoj fazi snimanja. (B) Pozicioniranje ablacijske sonde za MVA duljine 15 cm u središte tumora pod kontrolom CT-a, stražnjim međurebrenim pristupom. (C) Kontrolni CT pregled nakon četiri tjedna od ablacije u nefrogenoj fazi snimanja, s vidljivom avaskularnom zonom na mjestu ablacije, koja u potpunosti prekriva raniji KCB i zahvaća minimalni dio okolnog parenhima bubrega, uz zamućenje masnog tkiva perirenalno, bez znakova lokalnog recidiva.*

RASPRAVA

U ovom istraživanju onkološki ishodi za bolesnike s KCB-om stadija T1 liječeni postupkom perkutanog visokoenergetskog MVA pod kontrolom CT-a nisu se razlikovali od ishoda u bolesnika liječenih kirurški, postupkom PN-a. Zlatni standard liječenja bolesnika s KCB-om jest kirurška resekcija, koja ima za cilj u cijelosti odstraniti tumor sa zadovoljavajućim kirurškim rubom. Iako je RN dugo bio zlatni standard terapije svih KCB-a, napredak u tehnici PN-a posljednjih godina doveo je do toga da ima jednake onkološke ishode i stopu preživljenja kao i RN, uz manju stopu komplikacija, manji gubitak bubrežne funkcije, niže stope srčanožilnih komplikacija i manju opću stopu smrtnosti (12). Prema smjernicama Američkog (*American Urological Association*) i Europskog urološkog društva, (*European Association of Urology*) zlatni standard terapije za male KCB-e dimenzije do 4 cm, stadija T1a, jest PN, dok je RN rezerviran za pacijente koji nisu dobri kandidati za poštednu kirurško intervenciju (8). Tendencija poštede bubrežnog parenhima pri terapiji KCB-a proizlazi iz saznanja da je opsežnija bubrežna resekcija povezana sa znatno većim rizikom kronične bubrežne bolesti (KBB) i posljedično većim pobolom i smrtnosti. KBB zatajenje jest i neovisan rizični čimbenik za srčanožilne rizike i opću smrtnost. Istraživanja ukazuju da postoji znatno preklapanje rizičnih čimbenika za KBB i za nastanak KCB-a (13). Postoji velik broj analiza koje ukazuju na veliku učestalost patologije bubrežnog parenhima, posebno glomeruloskleroze, u analiziranom netumorskom tkivu parenhima bubrega nakon nefrektomije. Dio pacijenata s novootkrivenim KCB-om stadija T1 nisu kandidati za kirurgiju zbog komorbiditeta kao što su KBB, kardiomiopatija ili koagulopatija, a i zbog želje za što poštednijom te-

rapijom. U našoj studiji lošija funkcija bubrega zabilježena je u skupini MVA, što je bilo očekivano s obzirom na to da s radilo o statistički značajno starijoj skupini pacijenata prosječne dobi 66 godina, prema 61 godini u skupini PN. Također, u skupini MVA zabilježen je statistički značajno viši stupanj indeksa komorbiditeta Charlson, a pacijenti liječeni ablacijom bili su i statistički značajno lošijega početnog statusa ASA.

Pacijenti s KCB-om stadija T1, prema studijama, imaju očekivano petogodišnje preživljenje veće od 90%, zbog čega je dugoročna bubrežna funkcija važan element kvalitete života nakon liječenja. Studije su ukazale na to da nakon nefrektomije 29 % pacijenata ima smanjenje bubrežne funkcije s glomerulskom filtracijom manjom od 60 mL/min / 1,73 m^2, a 16% će razviti smanjenje glomerulske filtracije ispod 45 mL/min / 1,73 m^2 (14), što može znatno utjecati na kvalitetu života i životni vijek ovih pacijenata. U pacijenata mlađih od 65 godina s KCB-om stadija T1a RN se povezuje s kraćim općim preživljenjem (15). Zbog svih tih razloga, osim onkoloških ishoda, u izboru terapije KCB-a postaje sve važnije razmišljati i o dugoročnom utjecaju zahvata na bubrežnu funkciju, koja je najvažniji pojedinačni čimbenik na kasniju kvalitetu života pacijenata. Iako brojne studije potvrđuju opravdanost minimalno invazivnih metoda i PN-a u terapiji lokaliziranih KCB-a, posebice stadija T1a, podatci ukazuju na to da se i dalje često nedovoljno primjenjuju te metode u usporedbi s RN-om (16). U našem istraživanju zabilježen je znatno veći gubitak funkcije bubrega nakon kirurške terapije u usporedbi s ablacijom. Studija Zhao i sur. pokazala je da je kod KCB-a većih od 4 cm rizik nepotpune ablacije 40%, ako se radi pod kontrolom ultrazvuka, dok iznosi 16% ako se radi pod kontrolom CT-a (17). Upravo zbog tih podataka odlučeno je da se svi tretmani MVA-om u našoj studiji rade primarno pod navođenjem CT-a. Procedure vođene CT-om, osim prednosti dobre vidljivosti sonde i tumora u pacijenata s nepovoljnom tjelesnom konstitucijom, imaju i prednost u mogućoj kontroli učinka ablacije na kraju postupka.

Iako su istraživanja onkoloških ishoda MVA u terapiji KCB-a ograničena, MVA u teoriji uključuje sva dobra svojstva ostalih ablacijskih metoda, posebno RFA, ali ima i mnoge dokazane prednosti u odnosu na ostale vrste ablacija: postiže više temperature u tkivu (do 180 Celzijevih stupnjeva), veći volumen ablacije bez potrebe za upotrebom više sondi istodobno, kraće trajanje ablacije, učinkovitu ablaciju cističnih tumora i manji intenzitet boli za vrijeme ablacije (18,19).

Sigurnost primjene MVA u terapiji KCB-a prvi je put opisana u radu Clarka i sur. iz 2007. (10), koji opisuje kako MVA može učinkovito uništiti tumore bubrega veličine do 5,7 cm. Studija koju su proveli Castle i sur. s lošijim onkološkim ishodima znatno je pogoršala percepciju mogućnosti MVA (20), s obzirom na to da su prikazali stopu lokalnog recidiva od 38 % nakon 18 mjeseci praćenja. Međutim, nedostatak je ove studije što je rađena upravo s prvom generacijom MVA uređaja, koja je imala znatnija ograničenja u preciznosti i učinkovitosti od današnjih uređaja 3. generacije. Osim toga, čak 50 % tumora iz studije pokazivalo je znakove širenja prema kanalnom sustavu, što je značajan rizični čimbenik za nepotpunu ablaciju ili znatnu komplikaciju. Jedina prospektivna randomizirana studija koja je tijekom 32 mjeseca praćenja usporedila MVA i PN u KCB-u uključivala je 54 pacijenta liječena kirurški i 48 liječenih ablacijom (21). Trogodišnje preživljenje bez recidiva iznosilo je 91,3 % nakon MVA i 96 % nakon PN-a. MVA je bila povezana sa znatno manjim gubitkom krvi, manjom stopom komplikacija te manjom redukcijom bubrežne funkcije. Glavno je ograničenje ove studije što su zahvati ablacije bili provedeni operacijski, laparoskopski ili otvorenom operacijom i bez slikovnog navođenja.

Meta-analiza 13 studija MVA-e KCB-a od 2012. (22) pokazala je stopu lokalnog recidiva 2,1 % (95 % CI, 0,3 – 4,7), a petogodišnje preživljenje specifično za karcinom 97,8 % (95 % CI, 95 – 99,4) i opće preživljenje 81,9 % (95 % CI, 75,4 – 87,6). Također. posljednje studije koje su pratile isključivo MVA, bez usporedbe s PN-om, ukazuju na onkološke ishode koji su ekvivalentni kirurškoj resekciji te se slažu s našim rezultatima (23-25). Stopa većih komplikacija iznosila je 1,8 %, a manjih 17,5 %. Opisani bolji rezultati pripisuju se novijoj generaciji MVA uređaja i primjeni slikovnog navođenja. Onkološki ishodi našeg istraživanja pokazali su da je onkološka učinkovitost MVA jednaka onoj PN-a. Svi praćeni ishodi preživljenja nisu pokazali postojanje statistički značajne razlike između dviju skupina pacijenata. Iako je u ukupnom tijeku praćenja obih skupina ukupno bilo više zabilježenih lokalnih recidiva u skupini pacijenata liječenih kirurškom metodom, nakon uparivanja prema skoru sklonosti u razdoblju praćenja od godine dana nije zabilježena statistički značajna razlika u stopi lokalnog recidiva.

Ograničenja su ovog istraživanja relativno mali uzorak pacijenata i kratko vrijeme praćenja od minimalno 12 mjeseci. Ograničenje retrospektivnosti studije dijelom se pokušalo nadoknaditi postupkom usklađivanja pacijenata u skupinama prema skoru sklonosti. U dosadašnjim studijama nije se pri usporedbi MVA i kirurgije uzimalo u obzir, osim veličine, kompleksnost i obilježja tumora, a ti čimbenici dokazano mogu utjecati na onkološki ishod neke kirurške ili ablativne metode. Najčešće korištena ljestvica kompleksnosti KCB-a jest mRENAL za tumore T1a, koja je primijenjena i u ovom istraživanju, a temelji se na prilagodbi originalne ljestvice RENAL za KCB stadija T1a.

ZAKLJUČAK

Razvojem tehnologija za perkutanu termalnu terapiju u posljednjih 20 godina proširile su se indikacije za minimalno invazivne termalne ablativne metode liječenja u dijela onkoloških pacijenata. Naše istraživanje utvrdilo je da MVA ima usporedive onkološke ishode kao i PN uz dodatnu prednost za pacijenta i zdravstveni sustav rjeđim periproceduralnim komplikacijama, kraće trajanje zahvata i bolje očuvanje funkcije organa. Rezultati našeg istraživanja potvrđuju dosadašnje spoznaje o učinkovitosti MVA te ukazuju da se ova metoda u smjernicama ne bi trebala smatrati eksperimentalnom.

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SUMMARY

COMPARISON OF PERCUTANEOUS MICROWAVE ABLATION GUIDED BY COMPUTER TOMOGRAPHY AND PARTIAL NEPHRECTOMY IN THE TREATMENT OF T1A STAGE OF RENAL CANCER

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Purpose: Better availability of radiologic imaging leads to an increase in the incidence of small renal cell carcinoma (RCC), which in turn gives rise to the need for developing minimally invasive and nephron sparing therapy. Along with partial nephrectomy (PN), as the gold standard therapy, percutaneous ablative methods have been introduced in patients with severe comorbidities. Despite its advantages when compared to other ablative methods, microwave ablation (MWA) has not been introduced into therapy guidelines and is still considered to be an experimental method. *The aim* of the study was to compare local recurrence rates, overall survival, metastasis-free survival and cancer specific survival after percutaneous computer tomography (CT) guided MWA and PN in the therapy of T1a stage of RCC. *Methods:* The retrospective study involved 80 patients, who were diagnosed and histologically confirmed with T1a stage RCC from January 2015 to June 2018. All patients were candidates for MWA or open PN, depending on the decision of the multidisciplinary team at the University Hospital Center Sestre milosrdnice. Surgical patients were chosen, according to their tumour size and complexity, to match the patients treated with MWA in size and complexity of the tumour using propensity score matching. All included patients were under radiological and clinical follow-up for a period of at least 12 months. MWA procedures were performed via percutaneous approach under CT guidance. *Results:* Oncological outcomes did not show any statistically significant difference between MWA and PN. Overall survival was 100% after one year in both groups. One-year recurrence-free survival was 92,5% after MWA and 90% after PN, with 3 patients showing evidence of local recurrence after MWA and 5 patients after PN. All patients with local recurrence were retreated with MWA after 2-4 weeks with a secondary-efficacy of MWA being 100%. Despite a higher number of patients showing local recurrence or metastasis in the PN group, there was no significant difference found in our study. Metastasis-free survival was 97,5% after MWA and 95% after PN. Even though average glomerular filtration rates were not significantly different between the MWA and PN group before and after the procedure, the percentage decrease in the glomerular filtration rate was significantly lower after MWA, -8.9 ± 6 % vs -21.7 ± 8.2 % (P<0,001). The ablation group was associated with significantly lower estimated blood loss (54,0 ± 19,0 mL vs 225,1 ± 45,7 mL, P<0,001). *Conclusion:* It can be concluded that percutaneous MWA can be used as an equally successful therapeutic tool in small RCC, when compared to the golden standard of PN, in patients with severe comorbidities, but also in other patients due to its nephron sparing qualities.

Key words: kidney cancer, nephrectomy, microwave ablation, computorized tomography, interventional radiology

AN OVERVIEW OF FEMALE SEXUAL FUNCTION WITH PRESENTATION OF THE CROATIAN TRANSLATION OF THE FEMALE SEXUAL FUNCTION INDEX

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Introduction: The Female Sexual Function Index (FSFI) is a questionnaire used to assess female sexual function and diagnose sexual dysfunction (SD). ***Aim:*** to provide a Croatian translation of the FSFI. ***Methods:*** The translation procedure consisted of creating two independent forward translations, merging them into a single forward translation, creating a back translation, comparing the back translation with the original, and deciding on a final translation. ***Results:*** no semantic differences were found when comparing the back translation with the original. Therefore, minimal changes were made to the earlier translation when the final translation was created. ***Conclusions:*** The Croatian translation of the FSFI is now available for assessing the widespread problem of female SD in the Croatian-speaking population.

Key words: sexual dysfunction, women, Female Sexual Function Index, Croatia

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INTRODUCTION

Sexual function plays an important role in every woman's life (1). Penile-vaginal intercourse and orgasm have numerous positive effects on women's quality of life, such as improved hormonal balance, reduced menopausal symptoms, lower risk of breast cancer, and lower prevalence of depressive disorders (2-4). Human sexuality consists of various aspects: anatomical, endocrine, psychological, previous experience of human relationships, sociocultural and religious aspects (5). In 1975, the World Health Organization defined sexual health as "the integration of the somatic, emotional, intellectual, and social aspects of sexual being in a manner that is positively enriching and promotes personality, communication, and love" (6). The sexual response cycle is traditionally divided into four phases, i.e., desire, arousal, orgasm, and resolution (7). The anatomical center for sexual desire in the central nervous system is located in the hypothalamus and surrounding limbic structures. It is physiologically stimulated by testosterone in both males and females (8), but dopaminergic and serotonergic systems also play important roles in various factors of sexual response. Complex integrative activities lead to autonomic and voluntary responses that are processed in the central nervous system through the 'sexual pleasure cycle'. Key components of the sexual pleasure cycle are sex drive and pleasure perception, which depend on the interaction between the dopaminergic neurons of the reward system, located mainly in the midbrain, and the opioid-endocannabinoid system. Sexual behavior requires implicit sensory stimuli to forebrain limbic structures such as the hypothalamus, amygdala, hip-

pocampus, and septal region nuclei, which integrate stimuli in an unconscious manner and trigger typical autonomic responses. However, the entire human sexual cycle involves complex consciousness, indicating the important role of the cerebral cortex (9).

Sexual desire refers to sexual thoughts, ideas, or desires that arise spontaneously or during a relationship with a partner and may be conditioned by psychological understanding of sexuality and partnership. Sexual arousal is the subjective perception of sexual pleasure (10) accompanied by physiological changes such as vasocongestion of the genitals and chest, vaginal lubrication, tachycardia, tachypnea, and increased blood pressure (11). Orgasm refers to the climax of sexual pleasure accompanied by rhythmic contractions in the genital and pelvic areas (10). Resolution is the final phase of the sexual response cycle, following orgasm or the plateau of arousal when orgasm has not occurred, and is characterized by general relaxation and satisfaction (10). Note that these four phases overlap and their order may vary (12). In addition, some of the phases may be absent from an individual woman's sexual response cycle. For example, subjective satisfaction with a sexual act need not always involve achieving orgasm. Instead, it may consist of reaching a plateau of a satisfactory level of arousal (10). Sexual dysfunction (SD) is a major public health problem (13), common in all age groups of women, with a prevalence ranging from 19% to 63% (14-17). The prevalence is even higher in women with various comorbidities. For example, the prevalence of SD in women with chronic kidney disease (CKD) is up to 70% (18). It is characterized by a disturbance in sexual desire, arousal, orgasm, or mental satisfaction, or by the occurrence of pain associated with the sexual act (19). SD is a persistent or recurrent problem that causes personal or interpersonal distress (20) and can lead to emotional disturbance or broken relationships (21). For this reason, it is concerning that SD remains an underdiagnosed and undertreated disorder (18). Therefore, there is a real need of tools to diagnose SD. The Female Sexual Function Index (FSFI) was developed by the International Consensus Development Conference on Female Sexual Dysfunctions to assess female sexual function in the following six domains: sexual desire, arousal, orgasm, pain, lubrication, and satisfaction (22). The questionnaire has proven to be a sound instrument in various translations (23-36). FSFI questionnaire translations were made in Portuguese, Urdu, Persian, Dutch, Korean, Malay, Chinese, Japanese, Italian, German, Swedish, Hungarian, Turkish, Spanish, etc. (23-35).Those translations regularly showed sufficiently high values of the Cronbach's alpha coefficient. The values of Cronbach's alpha coefficients were 0.92-0.97 for the Italian (31), >0.84 for the Japanese (30), and 0.72-0.9 for the Persian version (25). All three translations were created using the forward-backward translation method, similar to the one that was used to create the Croatian version and is shown in Figure 1.

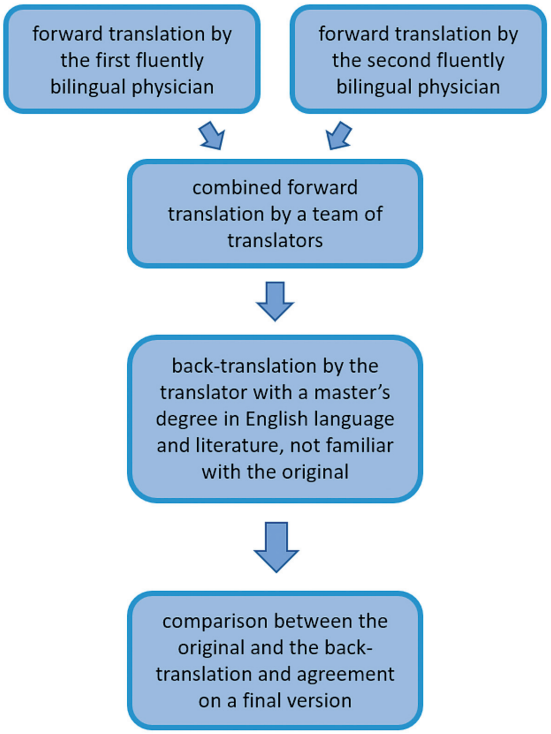

Figure 1. *The algorithm of the translation protocol applied in this study.*

Studies assessing the prevalence of SD in Croatia were performed by Stulhofer *et al.* (36,37). Since a Croatian translation did not seem to exist, our research team conducted the translation procedure recommended by various authors (39-42) and used the translated version on a sample of healthy women and patients with CKD (1,18). It is our hope that this will prove valuable for the Croatian-speaking population, knowing that determining the prevalence of SD and the associated risk factors is important in order to plan the prevention and treatment of SD (1). The Croatian version of the FSFI can be found in the supplement (Appendix 1) and may be freely used.

Appendix 1. The final version of the Croatian Female Sexual Function Index

Indeks ženske seksualne funkcije

Upute: Sljedeća pitanja ispituju o Vašim seksualnim osjećajima i odgovorima u posljednja 4 tjedna. Molimo odgovorite na sljedeća pitanja što je moguće iskrenije i jasnije. Vaši će se odgovori čuvati potpuno povjerljivo. Kod odgovaranja vrijedi sljedeće:

Seksualna aktivnost može uključivati maženje, predigru, masturbaciju i vaginalni spolni odnos.

Spolni se odnos definira kao penetracija (ulazak) penisa u vaginu.

Seksualna stimulacija uključuje situacije kao što su predigra s partnerom, samo-stimulacija (masturbacija) ili seksualne maštarije.

OZNAČITE SAMO JEDNU KUĆICU PO PITANJU.

Spolna želja ili zanimanje je osjećaj koji uključuje želju za spolnim iskustvom, osjećaj pristajanja na partnerovu seksualnu inicijativu i mišljenje ili maštanje o spolnom odnosu.

1. Tijekom posljednja 4 tjedna, koliko ste često osjetili seksualnu želju ili zanimanje?
1) Gotovo uvijek ili uvijek
2) Većinu vremena (više od polovice vremena)
3) Ponekad (otprilike polovicu vremena)
4) Nekoliko puta (manje od polovice vremena)
5) Gotovo nikad ili nikad

2. Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) svoje seksualne želje ili interesa?
1) Vrlo visoko
2) Visoko
3) Umjereno
4) Nisko
5) Vrlo nisko ili nimalo

Seksualna napetost je osjećaj koji uključuje i fizičke i psihičke aspekte seksualnog uzbuđenja. Može uključivati osjećaje topline ili trnaca u genitalijama, lubrikaciju (vlaženje) ili mišićne kontrakcije.

3. Tijekom posljednja 4 tjedna, koliko ste često bili seksualno napeti ("uzbuđeni") tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

4. Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) vaše seksualne napetosti ("uzbuđenosti") tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Vrlo visoko
3) Visoko
4) Umjereno
5) Nisko
6) Vrlo nisko ili nimalo

5. Tijekom posljednja 4 tjedna, koliko ste bili sigurni da ćete biti seksualno uzbuđeni tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Vrlo visoka sigurnost
3) Visoka sigurnost
4) Umjerena sigurnost
5) Niska sigurnost
6) Vrlo niska sigurnost ili nedostatak sigurnosti

6. Tijekom posljednja 4 tjedna, koliko ste često bili zadovoljni svojom seksualnom napetosti (uzbuđenjem) tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

7. Tijekom posljednja 4 tjedna, koliko ste često bili lubricirani ("navlaženi") tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

8. Tijekom posljednja 4 tjedna, koliko Vam je bilo teško postati lubricirani ("navlaženi") tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Izuzetno teško ili nemoguće
3) Vrlo teško
4) Teško
5) Pomalo teško
6) Bez poteškoća

9. Tijekom posljednja 4 tjedna, koliko ste često održali svoju lubrikaciju ("navlaženost") do kraja seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

10. Tijekom posljednja 4 tjedna, koliko Vam je bilo teško održati svoju lubrikaciju ("navlaženost") do kraja seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Izuzetno teško ili nemoguće
3) Vrlo teško
4) Teško
5) Pomalo teško
6) Bez teškoća

11. Tijekom posljednja 4 tjedna, kad ste imali seksualnu stimulaciju ili spolni odnos, koliko ste često postigli orgazam (vrhunac)?
1) Nisam imala seksualnu aktivnost
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

12. Tijekom posljednja 4 tjedna, kad ste imali seksualnu stimulaciju ili spolni odnos, koliko Vam je bilo teško postići orgazam (vrhunac)?
1) Nisam imala seksualnu aktivnost
2) Izuzetno teško ili nemoguće
3) Vrlo teško
4) Teško
5) Pomalo teško
6) Bez teškoća

13. Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni svojom sposobnosti postizanja orgazma (vrhunca) tijekom seksualne aktivnosti ili spolnog odnosa?
1) Nisam imala seksualnu aktivnost
2) Vrlo zadovoljna
3) Umjereno zadovoljna
4) Podjednako zadovoljna i nezadovoljna
5) Umjereno nezadovoljna
6) Vrlo nezadovoljna

14. Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni količinom emotivne bliskosti tijekom seksualne aktivnosti između Vas i Vašeg partnera?
1) Nisam imala seksualnu aktivnost
2) Vrlo zadovoljna
3) Umjereno zadovoljna
4) Podjednako zadovoljna i nezadovoljna
5) Umjereno nezadovoljna
6) Vrlo nezadovoljna

15. Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni seksualnom vezom sa svojim partnerom?
1) Vrlo zadovoljna
2) Umjereno zadovoljna
3) Podjednako zadovoljna i nezadovoljna
4) Umjereno nezadovoljna
5) Vrlo nezadovoljna

16. Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni Vašim ukupnim spolnim životom?
1) Vrlo zadovoljna
2) Umjereno zadovoljna
3) Podjednako zadovoljna i nezadovoljna
4) Umjereno nezadovoljna
5) Vrlo nezadovoljna

17. Tijekom posljednja 4 tjedna, koliko ste često osjetili nelagodu ili bol tijekom penetracije u vaginu?
1) Nisam pokušala imati spolni odnos
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

18. Tijekom posljednja 4 tjedna, koliko ste često osjetili nelagodu ili bol nakon penetracije u vaginu?
1) Nisam pokušala imati spolni odnos
2) Gotovo uvijek ili uvijek
3) Većinu vremena (više od polovice vremena)
4) Ponekad (otprilike polovicu vremena)
5) Nekoliko puta (manje od polovice vremena)
6) Gotovo nikad ili nikad

19. Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) nelagode ili boli tijekom ili nakon penetracije u vaginu?
1) Nisam pokušala imati spolni odnos
2) Vrlo visoka
3) Visoka
4) Umjerena
5) Niska
6) Vrlo niska ili nepostojeća

METHODS

The Female Sexual Function Index

The FSFI is a 19-item questionnaire that assesses a woman's sexual function within the four weeks preceding the examination. As we mentioned earlier, it quantifies the following six domains of female sexual function: desire, arousal, orgasm, pain, lubrication, and satisfaction. The response options range from 0 or 1 to 5, with 0 indicating no sexual activity. The individual scores related to each of the six domains above are calculated as the sum of the scores of the questions related to a single domain multiplied by the factor determined by the authors of the scale. For example, the score for the arousal domain is calculated as the sum of the scores of the four questionnaire items related to the arousal domain multiplied by the specified factor. The sum of all six individual scores for the domain equals the total FSFI. The total FSFI ranges from 2 to 36, with higher scores indicating better sexual function, and *vice versa*. A cutoff value of 26.55 is suggested by Wiegel*et al.*; subjects with total FSFI below the cutoff are likely to have SD (43). The FSFI questionnaire showed excellent internal consistency (Cronbach's alpha >0.9) (43-45). The questionnaire is also suitable for internet use and has shown good psychometric results (46-48).

Procedure

Two fluent bilingual physicians independently prepared two forward translations of the original English version into Croatian. The two forward translations were then combined into a single translation by a team of translators. Subsequently, the combined forward translation was back-translated into English by a native Croatian translator with a master's degree in English language and literature, who was neither involved in the preparation of the forward translations nor familiar with the original English version. Finally, a team of translators compared the back translation with the original version, found the translation process satisfactory, and agreed on a final version. The algorithm of the procedure is shown in Figure 1. This algorithm was selected after consulting suggestions from various sources (40,42,49-53).

Results

The original version, the combined forward and backward translation can be seen in Table 1. Comparison of the two independent forward translations is shown in Table 2. The aim of the translation was to create a Croatian version of the questionnaire that would not have significant differences in the meaning of the questions compared to the original version. When comparing the back-translation with the original, no semantic differences were found. The only differences were in the use of different forms of the same meaning (e.g., question 2 in the original reads "Over the past 4 weeks, how would you rate your level (degree) of sexual desire or interest?" and in the back translation it reads "During the last 4 weeks, how would you grade the level (degree) of your sexual desire or interest?").

Table 1. *The translation process of the FSFI*

Original	Combined translation	Back-translaton
1. Over the past 4 weeks, how often did you feel sexual desire or interest?	Tijekom posljednja 4 tjedna koliko ste često osjetili seksualnu želju ili zanimanje?	During the last 4 weeks, how often did you feel sexual desire or interest?
2. Over the past 4 weeks, how would you rate your level (degree) of sexual desire or interest?	Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) vaše seksualne želje ili interesa?	During the last 4 weeks, how would you grade the level (degree) of your sexual desire or interest?
3. Over the past 4 weeks, how often did you feel sexually aroused ("turned on") during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko ste često bili seksualno napeti ("uzbuđeni") tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how many times have you been sexually tense (aroused) during sexual activity or intercourse?
4. Over the past 4 weeks, how would you rate your level of sexual arousal ("turn on") during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) vaše seksualne napetosti ("uzbuđenosti") tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how would you grade the level (degree) of your sexual tension (arousal) during sexual activity or intercourse?
5. Over the past 4 weeks, how confident were you about becoming sexually aroused during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko ste bili sigurni da ćete biti seksualno uzbuđeni tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how certain were you that you will be sexually aroused during sexual activity or intercourse?
6. Over the past 4 weeks, how often have you been satisfied with your arousal (excitement) during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko ste često bili zadovoljni svojom seksualnom napetosti (uzbuđenjem) tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how often were you satisfied with your sexual tension (arousal) during sexual activity or intercourse?
7. Over the past 4 weeks, how often did you become lubricated ("wet") during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko ste često bili lubricirani ("navlaženi") tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how often were you lubricated (wet) during sexual activity or intercourse?
8. Over the past 4 weeks, how difficult was it to become lubricated ("wet") during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko Vam je bilo teško postati lubricirani ("navlaženi") tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how difficult was it for you to get lubricated (wet) during sexual activity or intercourse?
9. Over the past 4 weeks, how often did you maintain your lubrication ("wetness") until completion of sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko ste često održali svoju lubrikaciju ("navlaženost") do kraja seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how often did you maintain your lubrication (wetness) until the end of the sexual activity or intercourse?
10. Over the past 4 weeks, how difficult was it to maintain your lubrication ("wetness") until completion of sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko Vam je bilo teško održati svoju lubrikaciju ("navlaženost") do kraja seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how difficult was it for you to maintain your lubrication (wetness) until the end of the sexual activity or intercourse?
11. Over the past 4 weeks, when you had sexual stimulation or intercourse, how often did you reach orgasm (climax)?	Tijekom posljednja 4 tjedna, kad ste imali seksualnu stimulaciju ili spolni odnos, koliko ste često postigli orgazam (vrhunac)?	During the last 4 weeks, when you had sexual stimulation or intercourse, how often did you reach orgasm (climax)?
12. Over the past 4 weeks, when you had sexual stimulation or intercourse, how difficult was it for you to reach orgasm (climax)?	Tijekom posljednja 4 tjedna, kad ste imali seksualnu stimulaciju ili spolni odnos, koliko Vam je bilo teško postići orgazam (vrhunac)?	During the last 4 weeks, when you had sexual stimulation or intercourse, how difficult was it for you to reach orgasm (climax)?
13. Over the past 4 weeks, how satisfied were you with your ability to reach orgasm (climax) during sexual activity or intercourse?	Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni sa svojom sposobnosti postizanja orgazma (vrhunca) tijekom seksualne aktivnosti ili spolnog odnosa?	During the last 4 weeks, how satisfied were you with your ability to reach orgasm (climax) during sexual activity or intercourse?

14. Over the past 4 weeks, how satisfied have you been with the amount of emotional closeness during sexual activity between you and your partner?	Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni s količinom emotivne bliskosti tijekom seksualne aktivnosti između Vas i Vašeg partnera?	During the last 4 weeks, how satisfied were you with the amount of emotional closeness during a sexual activity between you and your partner?
15. Over the past 4 weeks, how satisfied have you been with your sexual relationship with your partner?	Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni sa seksualnom vezom sa svojim partnerom?	During the last 4 weeks, how satisfied were you with the sexual relationship with your partner?
16. Over the past 4 weeks, how satisfied have you been with your overall sexual life?	Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni s Vašim ukupnim spolnim životom?	During the last 4 weeks, how satisfied were you with your overall sex life?
17. Over the past 4 weeks, how often did you experience discomfort or pain during vaginal penetration?	Tijekom posljednja 4 tjedna, koliko ste često osjetili nelagodu ili bol tijekom penetracije u vaginu?	During the last 4 weeks, how often did you feel discomfort or pain during the penetration into the vagina?
18. Over the past 4 weeks, how often did you experience discomfort or pain following vaginal penetration?	Tijekom posljednja 4 tjedna, koliko ste često osjetili nelagodu ili bol nakon penetracije u vaginu?	During the last 4 weeks, how often did you feel discomfort or pain after the penetration into the vagina?
19. Over the past 4 weeks, how would you rate your level (degree) of discomfort or pain during or following vaginal penetration?	Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) nelagode ili boli tijekom ili nakon penetracije u vaginu?	During the last 4 weeks, how would you grade the level (degree) of discomfort or pain during or after the penetration into the vagina?

Table 2. *The comparison of two forward translations of the FSFI*

Forward translation 1	**Forward translation 2**
Tijekom posljednja 4 tjedna, koliko ste često osjetili seksualnu želju ili zanimanje?	Tijekom protekla 4 tjedna, koliko često ste osjetili seksualnu želju ili interes?
Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) svoje seksualne želje ili interesa?	Kako biste ocijenili razinu svoje seksualne želje ili interesa tijekom protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste često bili seksualno napeti (“napaljeni”) tijekom seksualne aktivnosti ili spolnog odnosa?	Tijekom protekla 4 tjedna, koliko često ste osjećali seksualnu napetost tijekom seksualne aktivnosti ili spolnog odnosa?
Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) vaše seksualne napetosti (“napaljenosti”) tijekom seksualne aktivnosti ili spolnog odnosa?	Kako biste ocijenili razinu seksualne napetosti tijekom seksualne aktivnosti ili spolnog odnosa u protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste bili sigurni da ćete biti seksualno napeti tijekom seksualne aktivnosti ili spolnog odnosa?	Koliko ste bili samouvjereni u vezi seksualne napetosti tijekom seksualne aktivnosti ili spolnog odnosa u protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste često bili zadovoljni svojom seksualnom napetosti (uzbuđenjem) tijekom seksualne aktivnosti ili spolnog odnosa?	Koliko često ste bili zadovoljni svojim seksualnim uzbuđenjem tijekom seksualne aktivnosti ili odnosa u protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste često bili lubricirani (“navlaženi”) tijekom seksualne aktivnosti ili spolnog odnosa?	U protekla 4 tjedna, koliko često ste mogli postići lubrikaciju (vlažnost) tijekom seksualne aktivnosti ili odnosa?
Tijekom posljednja 4 tjedna, koliko Vam je bilo teško postati lubricirani (“navlaženi”) tijekom seksualne aktivnosti ili spolnog odnosa?	U protekla 4 tjedna, koliko je teško bilo postići lubrikaciju (vlažnost) tijekom seksualne aktivnosti ili odnosa?
Tijekom posljednja 4 tjedna, koliko ste često održali svoju lubrikaciju (“navlaženost”) do kraja seksualne aktivnosti ili spolnog odnosa?	U protekla 4 tjedna, koliko često ste mogli održati lubrikaciju (vlažnost) do kraja seksualne aktivnosti ili odnosa?
Tijekom posljednja 4 tjedna, koliko vam je bilo teško održati svoju lubrikaciju (“navlaženost”) do kraja seksualne aktivnosti ili spolnog odnosa?	U protekla 4 tjedna, koliko naporno je bilo održati lubrikaciju (vlažnost) do kraja seksualne aktivnosti ili odnosa?
Tijekom posljednja 4 tjedna, kad ste imali seksualnu stimulaciju ili spolni odnos, koliko ste često postigli orgazam (vrhunac)?	U protekla 4 tjedna, kada biste imali seksualnu stimulaciju ili odnos, koliko često biste postigli orgazam?
Tijekom posljednja 4 tjedna, kad ste imali seksualnu stimulaciju ili spolni odnos, koliko vam je bilo teško postići orgazam (vrhunac)?	U protekla 4 tjedna, kada biste imali seksualnu stimulaciju ili odnos, koliko teško je bilo postići orgazam?
Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni sa svojom sposobnosti postizanja orgazma (vrhunca) tijekom seksualne aktivnosti ili spolnog odnosa?	Koliko ste bili zadovoljni svojom mogućnošću postizanja orgazma tijekom seksualne stimulacije ili odnosa u protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni količinom emotivne bliskosti tijekom seksualne aktivnosti između Vas i Vašeg partnera?	Tijekom protekla 4 tjedna, koliko ste bili zadovoljni emocionalnom bliskošću između Vas i Vašeg partnera tijekom seksualne aktivnosti?
Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni sa seksualnom vezom sa svojim partnerom?	Koliko ste bili zadovoljni seksualnom vezom sa svojim partnerom tijekom protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste bili zadovoljni s vašim ukupnim spolnim životom?	Tijekom protekla 4 tjedna, koliko ste bili zadovoljni svojim cjelokupnim seksualnim životom?
Tijekom posljednja 4 tjedna, koliko ste često osjetili nelagodu ili bol tijekom penetracije u vaginu?	Koliko često ste osjećali neugodu ili bol tijekom vaginalne penetracije u protekla 4 tjedna?
Tijekom posljednja 4 tjedna, koliko ste često osjetili nelagodu ili bol nakon penetracije u vaginu?	Koliko često ste osjećali neugodu ili bol nakon vaginalne penetracije u protekla 4 tjedna?
Tijekom posljednja 4 tjedna, kako biste ocijenili razinu (stupanj) nelagode ili boli tijekom ili nakon penetracije u vaginu?	Kako biste ocijenili razinu neugode ili boli tijekom ili nakon spolnog odnosa u protekla 4 tjedna?

DISCUSSION

Our translation of FSFI into the translation language showed appropriate choice as confirmed by back-translation. We believe that the translation algorithm enabled the best Croatian version of the questionnaire, as it was experienced in other languages (24-32,34,35). Research on women's sexual function has increased considerably recently, and a number of questionnaires have been developed to facilitate sexual history taking and assessment (44). According to the systematic literature review by Lim-Watson *et al*., the most commonly used questionnaires are FSFI, Sexual Satisfying Events (SSE), and Female Sexual Distress Scale-Revised (FSDS-R) (54), possibly due to the 2016 Food and Drug Administration (FDA) guidance on clinical trials for female SD. Among these, the FSFI remains the gold standard, adapted and validated in more than 20 languages and most commonly used for screening and outcome measurement of female sexual function (38).

However, the FDA has raised concerns about the psychometric properties of the FSFI, particularly regarding content validity. First, it was developed primarily for sexually active women, and the inclusion of sexually inactive women could bias the results. Women who are not sexually active for various reasons could select a zero response even though they do not have sexual dysfunction (47). In addition, there is a discrepancy in the definition of sexual desire, which is defined as spontaneous in the FSFI model, whereas research suggests that female sexual desire can often be triggered. The overlap between subjective arousal and desire has been ignored in the creation of a separate domain for desire. Another shortcoming is that the complexity of sexual desire can hardly be captured by the experience over four weeks (55). The final concern is the lack of assessment of sexual distress, which is currently considered a necessary condition, along with sexual functioning, for the diagnosis of SD. This is particularly important because sexual distress is associated with greater motivation to discuss sexually related problems with professionals and seek treatment (56).

However, Croatian translation of the FSFI is now available instrument for measuring female sexual function and diagnosing SD. The authors have developed the Croatian translation in the hope that it will prove useful and reliable over time for dealing with the widespread problem of female SD in the Croatian-speaking population.

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S A Ž E T A K

PREGLED ŽENSKE SPOLNE FUNKCIJE S PRIKAZOM HRVATSKOG PRIJEVODA INDEKSA ŽENSKE SPOLNE FUNKCIJE

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Uvod: Indeks ženske seksualne funkcije (*engl. Female Sexual Function Index,* FSFI) je upitnik koji se rabi za procjenu ženske seksualne funkcije i dijagnosticiranje seksualne disfunkcije (SD). ***Cilj:*** prikazati prijevod upitnika FSFI na hrvatski jezik. ***Postupci:*** Postupak prevođenja sastojao se od stvaranja dvaju neovisnih prijevoda upitnika s engleskog na hrvatski jezik, njihovog spajanja u jedan prijevod, ponovnog prevođenja stvorene hrvatske inačice upitnika na engleski jezik te usporedbe konačne inačice prijevoda s originalnim upitnikom i konačnih prilagodba finalne verzije prijevoda. ***Rezultati:*** Nisu pronađene semantičke razlike pri usporedbi hrvatskog prijevoda s originalnom verzijom upitnika. Stoga su napravljene minimalne promjene u prvoj verziji prijevoda pri stvaranju konačnog prijevoda. ***Zaključak:*** Hrvatski prijevod upitnika FSFI je sada na raspolaganju za ispitivanje raširenog problema SD u ženskoj populaciji hrvatskog govornog područja.

Ključne riječi: seksualna disfunkcija, žene, Indeks ženske seksualne funkcije, Hrvatska

OBILJEŽJA, AUTOPROTUTIJELA I LIJEČENJE BOLESNIKA S AUTOIMUNOSNOM HEMOLITIČKOM ANEMIJOM

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Uvod: Autoimunosna hemolitička anemija (AIHA) je vrlo rijetka autoimunosna bolest uzrokovana autoprotutijelima usmjerenima na bolesnikove eritrocite koja se dokazuje kliničkom slikom, biokemijskim pokazateljima hemolize uz isključenje ostalih uzroka hemolize i pozitivnim direktnim antiglobulinskim testom (DAT), osim kod DAT negativnih AIHA. Pozitivan DAT mogu imati i zdrave osobe i bolesnici bez AIHA-e. Liječenje ovisi o vrsti AIHA-e i osnovnoj bolesti, a sastoji se od više linija terapije. Transfuzijsko liječenje je rizik zbog moguće prisutnih aloprotutijela prikrivenih autoprotutijelima koja mogu uzrokovati hemolitičku transfuzijsku reakciju (HTR). ***Cilj*** rada bio je istražiti obilježja bolesnika, serološka obilježja autoprotutijela, hematološke pokazatelje anemije, biokemijske pokazatelje hemolize i liječenje bolesnika s AIHA-om. ***Postupci:*** U ovo retrospektivno istraživanje uključeno je 27 bolesnika kojima je od 1.siječnja 2018. do 31.prosinca 2020. godine dijagnosticirana AIHA u Kliničkom bolničkom centru Zagreb. Za dijagnostiku su provedena imunohematološka ispitivanja i laboratorijski pokazatelji hemolize. Učinkovitost transfuzijskog liječena definirana je kao porast vrijednosti hemoglobina (Hb) ≥5 g/L po dozi koncentrata eritrocita. ***Rezultati:*** Najviše je bolesnika dijagnosticirano s toplom AIHA-om (70,4 %), zatim s bolesti hladnih aglutinina (14,8 %) i miješanom AIHA-om (7,4 %), a najmanje s paroksizmalnom hladnom hemoglobinurijom (PCH, engl. *Paroxysmal cold hemoglobinuria*) i DAT-negativnom AIHA-om (po 3,7 % svaka). Prva linija terapije sastojala se od primjene kortikosteroida, a u drugoj i trećoj liniji 37 % bolesnika je primilo rituksimab. U 7,4 % bolesnika otkrivena su aloprotutijela. Transfuzijsko liječenje primilo je 81,5 % bolesnika, bez prijavljene transfuzijske reakcije. Učinkovitost transfuzijskog liječenja zabilježena je u 76,8 % slučajeva, s medijanom porasta Hb od 7,5 g/L po dozi krvi. ***Zaključak:*** Najviše je bilo dijagnosticirano toplih AIHA, a najmanje PCH i DAT-negativnih AIHA. U prvoj liniji terapije primjenjivani su kortikosteroidi, dok je u drugoj i trećoj liniji terapije najčešće primijenjen rituksimab uz imunosupresivnu terapiju. Transfuzijsko liječenje bilo je uspješno u većine bolesnika, bez prijavljenih transfuzijskih reakcija.

Ključne riječi: autoimunosna hemolitička anemija, autoprotutijela, aloprotutijela, liječenje, transfuzijsko liječenje

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UVOD

Autoimunosna hemolitička anemija (AIHA) je stečena autoimunosna bolest obilježena stvaranjem autoprotutijela imunoglobulina (Ig) G, IgM i/ili IgA usmjerenih na antigene koji se nalaze na površini vlastitih eritrocita, uz aktivaciju ili bez aktivacije komponenata komplementa, što uzrokuje hemolizu i anemiju (1). AIHA je relativno rijetka bolest za koju se procjenjuje da se javlja u 1 do 3 slučaja na 100 000 stanovnika godišnje (2). Zahvaća sve uzraste, s time da se u više od 70 % slučajeva javlja u bolesnika starijih od 40 godina, s najvećom incidencijom između 60 i 70 godina (3). AIHA se pojavljuje češće u žena nego u muškaraca, s omjerom 6:4 (3). Klinički se AIHA dijeli na primarnu i sekundarnu, ovisno o tome je li AIHA osnovna bolest, ili se nalazi uz neku drugu osnovnu bolest, najčešće autoimunosnu bolest (26 %), limfoproliferativnu bolest (24 %), imunodeficijenciju, tumor i infekciju (4).

Dijagnoza bolesti se postavlja na temelju kliničke slike i laboratorijskih pokazatelja anemije i hemolize, te

testova koji ukazuju na prisutnost autoprotutijela i/ili aktiviranih komponenti komplementa vezanih za eritrocite bolesnika. Laboratorijski pokazatelji anemije su smanjena serumska koncentracija hemoglobina i vrijednost hematokrita, a hemolize povećana serumska koncentracija bilirubina (posebice nekonjugiranoga) i enzima laktat dehidrogenaze (LDH) te smanjena vrijednost haptoglobina. Kao posljedica kompenzacijskog mehanizma koštane srži na anemiju, broj retikulocita u krvi je povećan (5). Osnovni dijagnostički test je direktni antiglobulinski test (DAT). Polispecifičnim DAT-om se dokazuju anti-IgG autoprotutijela i/ili komponente komplementa vezane za eritrocite bolesnika. Monospecifičnim DAT-om se dokazuju specifična autoprotutijela IgG, IgA, IgM te komponente komplementaC3c i C3d. Prije postavljanja dijagnoze potrebno je isključiti ostale bolesti ili stanja povezana s hemolizom, npr. nasljedne te stečene imunosne (uzrokovane aloprotutijelima, lijekovima ili transplantacijom krvotvornih matičnih stanica i organa) i neimunosne hemolitičke anemije (6).

Prema serološkim obilježjima autoprotutijela,AIHA-e se dijele na tople (60-70 %), hladne (20 – 25 %) i miješane (5 – 10 %) (7). Najčešće hladne AIHA-e su bolest hladnih aglutinina (CAD, od engl. *cold agglutinin disease*), ili ako je prisutna i osnovna bolest, sindrom hladnih aglutinina (CAS, od engl. *cold agglutinin syndrome*) (8), a vrlo rijetko se radi o paroksizmalnoj hladnoj hemoglobinuriji (PCH, od engl. *paroxysmal cold hemoglobinuria*). Topla AIHA obilježena je autoprotutijelima IgG (rjeđe IgA ili IgM), optimalno reaktivnima na 37° C. Kod hladnih AIHA, CAD je uzrokovan autoprotutijelima IgM, optimalno reaktivnima na 4° C,u titru ≥64, a PCH bifazičnim autoprotutijelima IgG. U miješanoj AIHA-i prisutna su topla autoprotutijela IgG i hladna IgM, širokog temperaturnog raspona djelovanja ≥30° C (9). U rijetkim slučajevima bolesti DAT može biti negativan. DAT-negativna AIHA uzrokovana je autoprotutijelima IgG ispod praga osjetljivosti testa ili rjeđe autoprotutijelima IgM ili IgA koja se ne otkrivaju rutinskim testiranjem (10).

Liječenje ovisi o vrsti AIHA-e i osnovnoj bolesti. Tople AIHA-e liječe se kortikosteroidima, a u težim slučajevima i rituksimabom. Poseban izazov u liječenju predstavljaju bolesnici s Evansovim sindromom koji je obilježen istodobnom pojavom AIHA-e i imunosne trombocitopenije (ITP), bez poznate etiologije. Radi se o bolesnicima s nepredvidljivim tijekom bolesti,praćenim razdobljima remisije i egzacerbacije u kojih se odgovor na terapiju i trajna remisija bolesti postiže primjenom rituksimaba (11). U kasnijim linijama terapije moguće je primijeniti imunosupresivne lijekove, kao npr. azatioprin, ciklosporin, mikofenolat, ili splenektomiju. Liječenje kod CAD-a je potrebno samo u težim slučajevima, a sastoji se od primjene rituksimaba, uz primjenu ili bez primjene bendamustina, dok je kod PCH rijetko potrebno. Obvezno je utopljavanje bolesnika i izbjegavanje hladnoće. U liječenju bolesnika s miješanom AIHA-om preporučuje se primjena kortikosteroida u kombinaciji s rituksimabom. U životno ugroženih bolesnika može se kao pomoćna terapija primijeniti intravenski imunoglobulin (IVIG) i provesti izmjena plazme postupcima plazmafereze (4). Bolesnici sa simptomatskom anemijom, najčešće umorom, vrtoglavicom, zaduhom i tahikardijom, primaju potporno transfuzijsko liječenje eritrocitnim pripravcima. Aloprotutijela prikrivena autoprotutijelima mogu uzorkovati HTR nakon transfuzije nepodudarnih eritrocitnih pripravaka i dodatno pogoršati stanje bolesnika (12).

CILJ ISTRAŽIVANJA

Cilj ovog rada bio je istražiti obilježja bolesnika, serološka obilježja autoprotutijela, hematološke pokazatelje anemije, biokemijske pokazatelje hemolize i liječenje bolesnika s AIHA-om.

POSTUPCI

Ovo jednocentrično retrospektivno istraživanje provedeno je u razdoblju od 1.1.2018. do 31.12.2020. godine u Kliničkom zavodu za transfuzijsku medicinu i transplantacijsku biologiju Kliničkog bolničkog centra (KBC) Zagreb. Istraživanje je odobrilo Etičko povjerenstvo KBC-a Zagreb i provedeno je u skladu s etičkim standardima Helsinške deklaracije iz 1975. godine, revidirane 2000. godine.

Dijagnoza AIHA-e je postavljena ako je u bolesnika s anemijom bio pozitivan DAT, uz prisutan barem jedan pokazatelj hemolize (serumska koncentracijabilirubina i laktatdehidrogenaze - LDH, povećana ahaptoglobina smanjena), a prethodno su isključeni ostali uzroci hemolize. Transfuzijsko liječenje definirano je kao transfuzijski događaj u kojemu su bolesnici primili određen broj doza koncentrata eritrocita (KE). Učinkovitost transfuzijskog liječenja zabilježena je ako je vrijednost hemoglobina (Hb) porasla za ≥5 g/l po dozi KE.

Eritrocitni antigeni bolesnika, barem antigeni D, C, c, E, e, K, Jk^a, Jk^b, određeni su primjenom monoklonskih reagensa (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA) prema uputi proizvođača. U slučaju prethodnog transfuzijskog liječenja (<3 mjeseca) ili ako nije bilo moguće određivanje eritrocitnih antigena reagensima kojima se testiranje izvodi u indirektnom antiglobulinskom testu (IAT) zbog autoreaktivnosti

nakon dodavanja antihumanog globulina, učinjena je genotipizacija. Genotip je određen postupkom reakcije lančane polimeraze u stvarnom vremenu, s pomoćukompleta RBC-Fluogene vERYfy eXtend (Inno-Train Diagnostik GmbH, Kronberg, Njemačka).

Polispecifični DAT rađen je u mikrokartici Polyspecific anti-IgG, anti-C3d(Bio-Rad Laboratories, Inc., CA, USA), a monospecifični DAT u mikrokartici Monospecific anti-IgG, anti-IgA, anti-IgM, anti-C3c i anti-C3d (Bio-Rad Laboratories, Inc., CA, USA) prema uputama proizvođača.U slučaju otkrivanja za eritrocite vezanih autoprotutijela razreda IgG rađena je kemijska elucija pomoću kompleta za eluciju DiaCidel (Bio-Rad Laboratories, Inc., CA, USA) prema uputi proizvođača. Za pretraživanje iregularnih antieritrocitnih autoprotutijela korištene su polispecifične mikrokartice Ortho BioVue Poly mikrokartica (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA) i komercijalni 0,8%-tni trostanični panel test eritrocita Surgiscreen(Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA). U slučaju pozitivnog rezultata IAT-a određena je specifičnost protutijela u polispecifičnim mikrokarticama polyspecific anti-IgG, anti-C3d (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA) i mikrokarticama neutral (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA) s komercijalnim 0,8 %-tnim jedanaeststaničnim panelom test eritrocita obrađenih i neobrađenih enzimom Panel C (Ortho-Clinical Diagnostics, Inc., Raritan, NJ, USA). U svrhu otkrivanja prisutnih aloprotutijela korišteni su postupci adsorpcije za uklanjenje autopotutijela iz plazme bolesnika. Ukoliko bolesnik prethodno nije transfuzijski liječen, primjenjivani su eritrociti bolesnika za autolognu adsorpciju koji su prethodno obrađeni reagensom ZZAP. Reagens ZZAP je mješavina papaina i 0,2 M ditioteritola (DTT) koja služi za uklanjanje autoprotutijela vezanih na eritrocite bolesnika. Ako je bolesnik prethodno transfuzijski liječen (<3 mjeseca), za adsorpciju su primijenjeni alogeni eritrociti u kombinacijama tri različita Rhesus fenotipa krvne skupine O (R_1R_1, R_2R_2, rr), od čega su barem jedni bili Jk^a, a drugi Jk^b negativni.

Titar hladnih autoprotutijela rađen je uz primjenu mješavine odraslih eritrocita, eritrocita novorođenčeta i vlastitih eritrocita na 4°C. Kao rezultat titra uzet je pozitivan rezultat s najvećim razrjeđenjem seruma. Temperaturni raspon rađen je iz serumskog razrjeđenja na temperaturama 4°C, 20°C, 30°C i 37°C. Na svakoj temperaturi koristio se novi set epruveta kako bi se izbjeglo prenošenje aglutinata. Za test je korištena mješavina odraslih eritrocita krvne skupine O. Donath-Landsteinerov test rađen je inkubacijom plazme bolesnika s P_1 eritrocitima krvne skupine O na 0°C, pa potom na 37°C. Kao izvor komplementa korišten je svježi serum darovatelja. Rezultat je bio pozitivan ako je nakon inkubacije na 37°C opažena hemoliza.

Podaci o dijagnostici i liječenju bolesnika dobiveni su iz transfuzijskog informacijskog sustava i bolničkog informacijskog sustava. Za analizu podataka korišteni su postupci deskriptivne statistike.

REZULTATI

Osnovna obilježja bolesnika s AIHA-om

U istraživanom razdoblju AIHA je dijagnosticirana u 27 bolesnika (medijan dobi 64 godine, od 24 do 90). Ukupno je 23 (85,2 %) bolesnika bilo starije od 40 godina. Bolesnici su najčešće bile žene (66,7%). Manje od polovice bolesnika imalo je primarnu AIHA-u. Od sekundarnih AIHA, najviše bolesnika bolovalo je od zloćudnih bolesti, najčešće limfoproliferativnih bolesti, a manje od stanja nakon alogenične transplantacije krvotvornih matičnih stanica, imunosnih bolesti i infekcija (Tablica 1).

Tablica 1. *Osnovna obilježja bolesnika s autoimunosnom hemolitičkom anemijom (N=27)*

Obilježja bolesnika	**N (%)**
Spol (ženski:muški)	18:9 (66,7:33,3)
Dob godine (medijan, raspon)	65 (24-90)
<40 godina	4 (14,8)
40 do 60 godina	8 (29,6)
>60 godina	15 (55,6)
Klinička podjela AIHA-e	
Primarna	11 (40,7)
Sekundarna	16 (59,3)
Osnovna bolest	
Zloćudne bolesti	12 (44,4)
Stanje nakon alogenične transplantacije krvotvornih matičnih stanica	2 (7,4)
Imunosne bolesti	1 (3,7)
Infekcije	1 (3,7)
Serološka podjela AIHA-e	
Topla AIHA	19 (70,4)
CAD	4 (14,8)
Miješana AIHA	2 (7,4)
PCH	1 (3,7)
DAT-negativna AIHA	1 (3,7)

AIHA, autoimunosna hemolitička anemija; CAD, bolest hladnih aglutinina; DAT, direktni antiglobulinski test; PCH, paroksizmalna hladna hemoglobinurija

Prema serološkim obilježjima autoprotutijela većina bolesnika imala je toplu AIHA-u (70,4 %), zatim CAD (14,8%) i potom miješanu AIHA-u (7,4 %), a po jedan bolesnik PCH i DAT-negativnu AIHA-u (3,7 %) (Tablica 1). Uz prisutna slobodna autoprotutijela u 2 (7,4 %) bolesnika otkrivena su i aloprotutijela specifičnosti anti-k, odnosno anti-S.

Laboratorijski pokazatelji anemije i hemolize

Laboratorijski pokazatelji anemije i hemolize pri dijagnostičkoj obradi bolesnika prikazani su u tablici 2 (broj učinjenih laboratorijskih pokazatelja prikazan je u legendi tablice)**.** Svi bolesnici imali su smanjenu serumsku koncentraciju Hb. Više od polovice bolesnika, 15 (55,6 %), imalo je početni Hb niži od 60 g/l. Broj retikulocita u krvi bio je snižen u 5 %, a povišen u 75 % slučajeva. Svi bolesnici imali su povećanu serumsku koncentraciju LDH, 88 % bolesnika imalo je povećanu serumsku koncentraciju ukupnog bilirubina, 93,8 % konjugiranog bilirubina, a 76,5 % smanjenu serumsku koncentraciju haptoglobina.

Tablica 2. *Laboratorijski krvni pokazatelji anemije i hemolize (N=27)**

Laboratorijski pokazatelji (mjerne jedinice)	Medijan (minimum - maksimum)	Referentne vrijednosti
Hb (g/l)	55,0 (37 - 86)	M 138 - 175; Ž 119 - 157
Htc (l/l)	0,159 (0,114 - 0,244)	M 0,415 - 0,530; Ž 0,356 - 0,470
Rtc (/1000)	103,9 (4,8 - 452,9)	22 - 97
Rtc ($x10^9$)	202 (10 - 667)	5 - 21,6
T-Bil (µmol/l)	40 (9 - 169)	3 - 20
D-Bil (µmol/l)	11 (6 - 37)	< 5
LDH (U/l)	461 (261 - 1596)	< 241
HP (g/l)	0,1 (0,08 - 4,13)	0,3 - 2

Hb, hemoglobin; Htc, hematokrit; Rtc, retikulociti; T-Bil, ukupni (T od engl. total) bilirubin; D-Bil, konjugirani (D od direktni) bilirubin; LDH, laktat dehidrogenaza; HP, haptoglobin

*Dostupni su podaci za sve bolesnike za Hb i Htc, N=27; za Rtc N=20; za T-Bil N=25; za D-Bil N=16; za LDH N=26, za HP N=17

Liječenje AIHA-e

Bolesnici su liječeni s tri linije terapije. Prvu liniju terapije primilo je 26 (96,3 %) bolesnika, drugu liniju 9 (33,3 %), a treću 2 (7,4 %) bolesnika. Prva linija terapije sastojala se od primjene kortikosteroida. Uz kortikosteroide u životno ugroženih bolesnika u prvoj liniji terapije primijenje nisu IVIG u 11 (40,7 %) slučajeva ili je učinjen postupak plazmafereze u 2 (7,4 %) slučaja. U jednog bolesnika liječenog kortikosteroidima i IVIG-om liječenje kortikosteroidima je prekinuto zbog komplikacija liječenja, a bolesnik je u nastavku liječen azatioprinom. Druga linija terapije sastojala se od primjene rituksimaba, samostalno u 3 (11,1 %), ili u sklopu citostatičke terapije u 5 (18,5 %) bolesnika. U jednom slučaju bolesnik je u drugoj liniji terapije bio liječen splenektomijom. Treća linija terapije sastojala se od primjene rituksimaba u 2 (7,4 %) bolesnika, od kojih se u jednom slučaju radilo o bolesniku koji je prethodno splenektomiran izvan naše ustanove.

Tablica 3. *Liječenje autoimunosne hemolitičke anemije (N=27)*

Linija terapije	Terapija	N (%)
Prva linija	Kortikosteroidi	13 (48,1)
	Kortikosteroidi uz intravenske imunoglobuline	11 (40,7)
	Kortikosteroidi uz plazmaferezu	2 (7,4)
	Nije primijenjena prva linija terapije*	1 (3,7)
	Ukupno	**27 (100)**
Druga linija	Rituksimab	3 (11,1)
	Rituksimab u sklopu citostatičke terapije	5 (18,5)
	Splenektomija	1 (3,7)
	Ukupno	**9 (33,3)**
Treća linija	Rituksimab	2 (7,4)
	Ukupno	**2 (7,4)**

*za jednog bolesnika s bolešću hladnih aglutinina nije primijenjeno liječenje

Transfuzijsko liječenje eritrocitnim pripravcima

Od ukupno 27 bolesnika, transfuzijsko liječenje eritrocitnim pripravcima primila su 22 (81,5 %) bolesnika. Dva (7,4 %) bolesnika su uz autoprotutijela razvila aloprotutijela specifičnosti anti-k, odnosno anti-S, te su im za transfuzijsko liječenje primijenjeni specifični antigen negativni eritrocitni pripravci.

Bolesnici su primili ukupno 98 (1-21) doza KE tijekom 65 transfuzijskih događaja, što je činilo 1,5 doza po bolesniku u svakoj transfuziji. Od 59 transfuzijskih događaja za koje su bili poznati podaci za Hb i poslije transfuzijskog liječenja, bilo je 46 (78 %) učinkovitih transfuzija (porast Hb ≥5 g/l).Nakon transfuzijskog liječenja nije zabilježena niti jedna transfuzijska reakcija, a medijan porasta Hb iznosio je 7,5 g/l po dozi krvi.

RASPRAVA

U ovom radu najviše bolesnika imalo je toplu AIHA-u, zatim CAD, potom miješanu, a najmanje PCH i DAT-negativnu AIHA-u. Od svih vrsta AIHA, jedino je CAD s udjelom od 14,8 % bio nešto niži od 20 – 25 % prijavljenih u literaturi (7,13), što se može protuma-

čiti teškom kliničkom slikom bolesnika s AIHA-om liječenih u KBC-u Zagreb. Obilježja analiziranih bolesnika s AIHA-om odgovarala su podacima iz literature (2). Prema spolu, najčešće su bile zastupljene žene, a s obzirom na dob, bolesnici stariji od 40 godina. Više od polovice bolesnika imalo je sekundarnu AIHA-u, najčešće uz zloćudnu bolest.

Udio bolesnika s teškom anemijom, gdje je Hb bio manji od 60 g/l imalo preko polovice bolesnika tijekom dijagnostičke obrade. U 75 % bolesnika s retikulocitozom postignut je dobar kompenzacijski odgovor na anemiju, iako je više od polovice bolesnika u podlozi AIHA-e imalo neku drugu osnovnu bolest. Retikulocitopenija je rijetka pojava u AIHA-i, a posljedica je nedovoljne proizvodnje eritrocita u koštanoj srži, npr. zbog infiltracije koštane srži, nedostatka željeza, vitamina, infekcija ili autoimunosne reakcije usmjerene na eritroidne prekursorske stanice (14). U ovom radu retikulocitopenija je zabilježena samo u jednom slučaju bolesnika s kroničnom limfocitnom leukemijom (KLL)i sekundarnom toplom AIHA-om koji prethodno nije primao terapiju za KLL. Bolesnik je liječen kortikosteroidima nakon čega je postignuta parcijalna remisija, a potom je započeto liječenje KLL-a prema protokolu bendamustin-rituksimab.

Gotovo svi bolesnici primili su prvu liniju terapije koja se sastojala od kortikosteroida, osim bolesnika s CAD-om koji nije primio nikakvu terapiju jer je imao blaži tijek bolesti. U jednom slučaju liječenje kortikosteroidom je prekinuto zbog komplikacija te je nastavljeno azatioprinom. Uz kortikosteroide 40,7 % životno ugroženih bolesnika primilo je IVIG, a dvoje ih je bilo podvrgnuto postupcima plazmafereze, što također ide u prilog teškoj kliničkoj slici bolesnika liječenih u KBC-u Zagreb.

Rituksimab je primijenjen u 37 % bolesnika, u drugoj (28,3 %), odnosno trećoj liniji terapije (7,7 %). U jednom slučaju kirurški je odstranjena slezena u drugoj liniji terapije, u bolesnika koji je u to vrijeme liječen izvan naše ustanove. U svom istraživanju Roumier i sur. su imali veći udio bolesnika liječenih rituksimabom (45 %) i splenektomijom (15 %), dok je udio sekundarnih AIHA bio neznatno veći (65 %) nego u našem radu (15). Barcellini i sur. su na istraživanju bolesnika samo s primarnim AIHA-ma prikazali primjenu rituksimaba u 25 % slučajeva pretežito u drugoj liniji terapije, s boljim odgovorom na niske doze, nego na standardne doze terapije kod tople AIHA-e, osim u slučajevima CAD-a gdje se boljom pokazala primjena standardne doze terapije (16). Sukladno smjernicama (4,17) u ovom radu u liječenju teških bolesnika primijenjen je najčešće rituksimab, dok se splenektomija izbjegavalazbog komplikacija, posebno u bolesnika s AIHA-om u kombinaciji s drugim autoimunosnim bolestima.

Većina bolesnika je primila transfuziju eritrocitnih pripravaka, a liječenje je bilo učinkovito u 76,8 % bolesnika. Nakon transfuzijskog liječenja zabilježen je medijan porasta Hb od 7,5 g/l po dozi KE. Prema smjernicama za transfuzijsko liječenje, očekivani porast Hb nakon primijenjene jedne doze KEu odraslog klinički stabilnog bolesnika (bez krvarenja u tijeku) bez AIHA-e, iznosi otprilike 10 g/l (18). Das i sur. su u bolesnika s AIHA-om nakon transfuzijskog liječenja prijavili medijan porasta vrijednosti Hb od 8,8 g/l (19).

Ako tijekom prijetransfuzijskog ispitivanja aloprotutijela ostanu neotkrivena zbog prisutnih polispecifičnih autoprotutijela u krvi bolesnika, ona mogu uzrokovati HTR (20). Stoga je za njihovo otkrivanje potrebno primijeniti specifične postupke koji uključuju adsorpciju autoprotutijela. Prema svjetskoj literaturi, aloprotutijela su prisutna u otprilike jedne trećine bolesnika s AIHA-om (21). Učestalost aloprotutijela u ovom radu iznosila je 7,4 %, što je manje od 11,1 % koji su prijavili Barros i sur (22). Za razliku od često prijavljenih aloprotutijela uslučajevima samo serološki dokazanih autoprotutijela, u njihovom radu aloprotutijela su utvrđena na klinički potvrđenim slučajevima AIHA-e (22), što je također bio slučaj i u ovom radu. Manji udio aloprotutijela u ovom radu, može se također tumačiti i manjom stopom aloimunizacije zbog preventivnog transfuzijskog liječenja antigen podudarnim eritrocitnim krvnim pripravcima po Rhesus, Kell i Kidd sustavima krvnih skupina svih bolesnika s AIHA-om.

Chen i sur. su analizirali transfuzijsko liječenje u 269 od 450 hospitaliziranih bolesnika s AIHA-om u 885 transfuzijskih događaja za koje su podaci bili dostupni i prijavili su učestalost transfuzijskih reakcija u 14 (1,6%) bolesnika: 13 febrilnih nehemolitičkih transfuzijskih reakcija, jedna alergijska reakcija i jedna nespecifična reakcija obilježena glavoboljom i povišenim krvnim tlakom, bez prijavljenog HTR-a (23). Yürek S i sur. nisu opazili transfuzijske reakcije uzrokovane auto- i/ili aloprotutijelima u32 bolesnika s AIHA-om koja su transfuzijski liječena (12). U ovom radu nije bilo prijavljenih transfuzijskih reakcija, dok su svi bolesnici prije transfuzijskog liječenja primili premedikaciju. Rezultati prethodnog istraživanja nisu pokazali razliku u transfuzijskim reakcijama s obzirom na primijenjenu medikaciju (12).

ZAKLJUČAK

Najviše bolesnika imalo je toplu AIHA-u, zatim CAD i miješanu AIHA-u, a najmanje PCH i DAT-negativnu AIHA-u. Gotovo svi bolesnici primili su prvu liniju terapije kortikosteroidima, dok je u drugoj i trećoj liniji terapije najčešće primijenjen rituksimab, sili bez primjene imunosupresivne terapije. Splenektomija je

izvedena samo u jednom slučaju. Transfuzijsko liječenje je bilo uspješno u većine bolesnika, bez prijavljenih transfuzijskih reakcija, te se sa sigurnošću može primijeniti u bolesnika s AIHA-om. Ograničenje ovog istraživanja bio je mali broj bolesnika s AIHA-om.

ZAHVALA

Zahvala svim liječnicima Kliničkog zavoda za transfuzijsku medicinu i transplantacijsku biologiju i Zavoda za hematologiju Klinike za unutarnje bolesti KBC-a Zagreb koji su sudjelovali u dijagnostici i liječenju bolesnika s autoimunosnom hemolitičkom anemijom.

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SUMMARY

CHARACTERISTICS, AUTOANTIBODIES AND TREATMENT OF PATIENTS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA

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Introduction: Autoimmune haemolytic anaemia (AIHA) is a very rare autoimmune disease caused by autoantibodies directed at patient's red blood cells as evidenced by a clinical picture, biochemical indicators of hemolysis, excluding other causes of hemolysis, and a positive direct antiglobulin test (DAT), except for DAT-negative AIHA. Both healthy people and patients without AIHA can have a positive DAT. Treatment depends on the type of AIHA and the underlying disease, and consists of multiple lines of therapy. Blood transfusion poses a risk due to the possible presence of alloantibodies masked by autoantibodies, that may cause a haemolytic transfusion reaction (HTR). ***The aim:*** To analyse patient's characteristics, serological characteristics of autoantibodies, laboratory parameters for anaemia, biochemical parameters for haemolysis and treatment of patients with AIHA. ***Methods:*** This retrospective study included 27 patients who were diagnosed with AIHA from1 January 2018 to 31 December 2020 in Clinical Hospital Centre Zagreb. For diagnosis, immunohematological tests and laboratory parameters of haemolysis were performed. The efficacy of transfusion was defined as haemoglobin (Hb) value increase of ≥5g/L per unit of blood. ***Results:*** Most patients were diagnosed with warm AIHA (70.4%), then with cold agglutinin disease (14.8%) and mixed AIHA (7.4%), and the least number of patients with paroxysmal cold haemoglobinuria (PCH) and DAT-negative AIHA (3.7% each). The first line of therapy consisted of corticosteroids, and in the second and third lines 37% of patients received rituximab. In 7.4% of patients alloantibodies were detected. Transfusion was administrated in 81.5% of patients, with no reactions reported. Efficacy of transfusion was noted in 76.8% of the cases, with median increase of Hb of 7.5 g/L per unit of blood. ***Conclusion:*** Warm AIHA was diagnosed the most and PCH and DAT-negative AIHA the least frequently. Corticosteroids were used in the fi rst line of therapy, while rituximab was the most commonly used in combination with immunosuppressive therapy in the second and third lines of therapy. Transfusion was successful in most patients, with no transfusion reactions reported.

Key words: autoimmune haemolytic anaemia, autoantibodies, alloantibodies, treatment, blood transfusion

ENTEROBIUS VERMICULARIS FAMILY INFECTION DETECTED BY URINE SEDIMENT SCREENING

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Introduction: Pinworm *Enterobius vermicularis* is transmitted through the anal-oral route, by swallowing eggs with larvae that become invasive in already four hours. The larvae hatch in the small intestine and migrate to the large intestine where they mature into adults in 2 to 6 weeks. Males impregnate females that come out in the anal area at night and lay the characteristic asymmetric eggs. The main symptom is persistent itching of the anal skin. *Case report:* A female patient aged 35 years experienced itching and burning sensation in the urogenital area. A week ago, similar symptoms have appeared in her 3-years old daughter for which her physician prescribed antimicrobial and antifungal therapy. The patient brought her first voiding morning urine sample for urine culture. Additionally, ten milliliters of urine was centrifuged, supernatant removed and the sediment examined microscopically. Examination of urine sediment at 10x magnification revealed many epithelial cells and calcium carbonate crystals, rare polymorphonuclear leukocytes, rare bacteria and many round and oval shapes. Examination of the same sample at 40x magnification confirmed that those regular shapes were eggs of *Enterobius vermicularis.* Larvae were visible both within the eggs and free out of the eggs, including those that were just about to leave the thin egg shell. Family history was taken and perianal specimens (tape test) from all family members were obtained. *Enterobius vermicularis* eggs were found in the patient's (the mother) perianal tape sample, the 3-year old daughter and a 10-year old son, while her husband's (the father) and the 14-years old son's specimens were negative. *Conclusion:* Perianal tape test is the method of choice for enterobiasis. The presented case showed that urine sediment microbiological examination could provide a worthy information on family infestation with small baby worm, as well. Urinary sediment could serve for valuable examination in unclear situations and if the reliable data were not available, as in small children.

Key words: Enterobius vermicularis, urine sediment, schotch tape, mebendazole

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BACKGROUND

Enterobius vermicularis (*E. vermicularis*) is distributed worldwide and primarily is a parasite in young children. *Enterobius* eggs are elongated and are approximately 50-60 μm long and 20-40 μm wide. The eggs develop rapidly and become infective within 4-6 h, at which time eggs contain larvae (1). Such fast development of eggs to the infective stage and their ability to persist in external environment leads to rapid dissemination of the infection from child to child and to adults. Infection is very common in institutional settings and in families with young children.

Adult *E. vermicularis* females migrate out of the intestinal tract and lay their eggs on the perianal surface, from where they adhere to the skin, hair, or bed clothing. Such infected surfaces may be a source of infection or reinfection in others. Large worm burdens cause pruritus and may cause loss of sleep, especially in young children. Occasionally, adult females enter the vagina, uterus, or fallopian tubes, where they die. Disintegration of dead worms and liberation of the eggs contained in the uterus results in inflammatory response and granuloma formation (1). Ectopic migration and involvement of urinary tract may lead to recurrent urinary tract infection (UTI) and inva-

sion of other unusual areas of the human body with consequent infection (2,3). According to one study, annual incidence of *E. vermicularis* in acute appendicitis specimen from a pediatric cohort was 7% (4). *E. vermicularis* eggs can be seen in the vaginal smear because of contamination, but also as a cause of vulvovaginitis with a lot of acute inflammatory cells (5).

Scotch tape or cellulose tape method is the most widely used procedure for the diagnosis of pinworm infection (1). Scotch tape technique should be done in the morning with a clear tape before bathing or defecation, so that the eggs laid during the night by the migrating females can be picked up. Transparent Scotch tape is applied directly to the perianal area, and then placed on the microscopic slide for examination.

Microscopic examination of the urine sediment provides useful information about the health condition of the patient. It is particularly important for the diagnosis of urinary tract diseases. The presence of a large number of bacteria, white blood cells and red blood cells can indicate kidney or bladder disease. Several parasites may be recovered and identified from urine,- such as *Trichomonas vaginalis* and *Schistosoma haematobium* (1).

CASE REPORT

A healthy young woman presented with unpleasant sensations in the urogenital region, pricking and itching, which led her to believe she had an UTI. She brought her first morning midstream urine to the laboratory for microbiological analysis. The urine collection was performed following all the urine culture collection instructions (6).

The urine was centrifuged at 3000 g for 10 minutes and the supernatant was decanted. The sediment was analyzed under the light microscope at x10 and x40 magnification. The analysis at x10 magnification identified a lot of squamous epithelial cells and calcium carbonate crystals, rare polymorphonuclear leucocytes, bacteria, and a lot of round formations resembling eggs. The examination at x40 magnification confirmed *E. vermicularis* eggs with visible larvae inside and outside the thin shell (Figure 1).

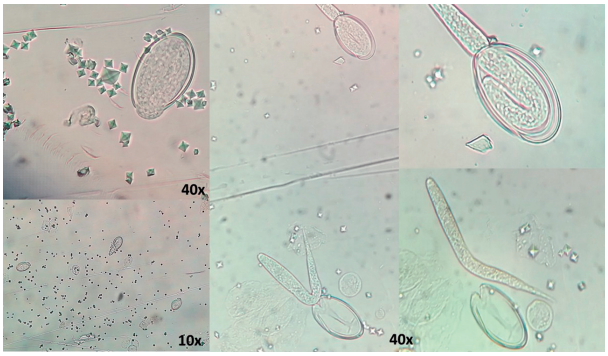

Figure 1. *Enterobius vermicularis eggs in urine sediment with larvae inside and outside shell (10x and 40x magnification), Batarilo*

A week prior to the patient's first symptoms, her 3-year-old daughter, not going to kindergarten, presented with extreme urogenital itching and burning sensations, particularly during the night. The general practitioner performed a urine dipstick test, which indicated the presence of polymorphonuclear leukocytes in the tested urine. Antibiotic therapy was initiated, assuming the child had an UTI. Three days later, since the symptoms did not disappear, therapy was shifted, and an antifungal ointment was administered.

Other family members, the husband and two sons, had no symptoms indicating the presence of *Enterobius*. Scotch tape specimens from all family members were taken and examined. *E. vermicularis* eggs were found in the Scotch tape specimens collected from the mother, the 3-year-old daughter and the 10-year-old son (Figure 2), whereas specimens from the husband (the father) and 14-year-old son were negative.

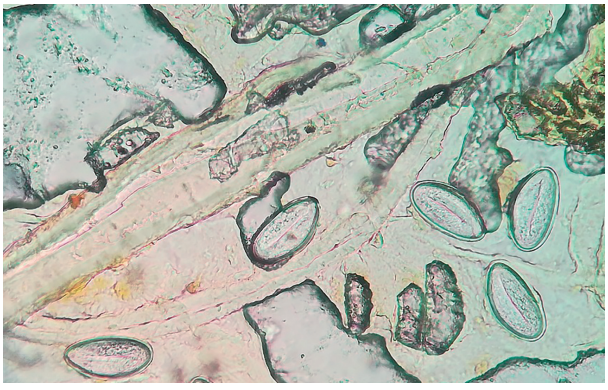

Figure 2. *Enterobius vermicularis eggs in Scotch tape (40x magnification), Batarilo*

All family members were treated with anthelmintic therapy, mebendazole, twice daily for 3 days. Therapy was repeated after 2 weeks. The follow-up Scotch tape examination of all family members' specimens proved negative, confirming that therapy was effective.

DISCUSSION

Microscopic examination of urine sediment may be a valuable diagnostic tool in patients with unusual urinary tract symptoms, which are not attributed to bacterial bladder infections. Accidental findings of *E. vermicularis* eggs in the mother's urine sample helped resolve the 3-year-old girl's infestation with *E. vermicularis*, which was misdiagnosed as an UTI due to the similarity of symptoms. The child was misdiagnosed also because of her inability to express her symptoms as adults do, which led to misuse of antibiotic and antifungal drugs. Microscopic examination of urine sediment also helped detect and resolve family infestation with *E. vermicularis.*

CONCLUSION

Scotch tape specimens are the method of choice for intestinal enterobiasis diagnosis. This report shows that urine sediment microscopy provided valuable information indicating family *E. vermicularis* infestation. Urine sediment microscopy, although inexpensive and quite simple, is often a neglected procedure. Unfortunately, the interest seems to be lost and the utility forgotten, although it can provide very valuable information. This case report emphasizes the importance of urine sediment microscopy, especially when there is a challenge of acquiring adequate data, for example in small children.

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SAŽETAK

OBITELJSKA INFESTACIJA DJEČJOM GLISTOM *ENTEROBIUS VERMICULARIS* OTKRIVENA PREGLEDOM SEDIMENTA MOKRAĆE

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Uvod: ***Enterobius vermicularis*** prenosi se analno-oralnim putom, gutanjem jajašaca s ličinkom koje postaju invazivne već nakon 4 sata. Ličinke se izlegu u tankom crijevu i migriraju u debelo crijevo gdje sazrijevaju u odrasle jedinke za 2 do 6 tjedana. Mužjaci oplode ženke koje noću izlaze u analno područje i odlažu karakteristična asimetrična jajašca. Glavni simptom je uporan svrbež kože tog područja. ***Prikaz slučaja:*** Pacijentica u dobi od 35 godina imala je svrbež i osjećaj pečenja u urogenitalnom području. Tjedan dana prije pojave simptoma slične simptome imala je njezina trogodišnja kći kojoj je liječnik ordinirao antibiotičku i antifungalnu terapiju. Pacijentica je donijela uzorak prve jutarnje mokraće i napravljena je urinokultura. Dodatno je 10 mL mokraće centrifugirano, supernatant je uklonjen i sediment mikroskopski pregledan. Pri povećanju 10x utvrđeno je mnogo pločastih epitelnih stanica i kristala kalcijeva karbonata, rijetki polimorfonuklearni leukociti, rijetke bakterije i puno okruglih i ovalnih oblika. Pregledom istog uzorka sedimenta uz povećanje 40 x potvrđeno je da su ti pravilni oblici jajašca ***Enterobius vermicularis.*** Ličinke su bile vidljive unutar jajašca, ali bilo ih je i slobodnih izvan jajašaca, kao i onih koje su se upravo oslobađale iz tanke ljuske jajeta. Prikupljena je ciljana obiteljska anamneza i dobiveni su uzorci perianalnih otisaka svih članova obitelji. Jaja ***Enterobius vermicularis*** pronađena su u uzorku perianalnog otiska pacijentice (majke), trogodišnje kćeri i 10-godišnjeg sina. Uzorci perianalnog otiska supruga (oca) i 14-godišnjeg sina bili su negativni. ***Zaključak:*** Perianalni otisak ljepljivom vrpcom je postupak izbora za otkrivanje crijevne enterobijaze. U prikazanom slučaju mikroskopski pregled sedimenta mokraće dao je vrlo vrijedne informacije, odnosno otkrivena je obiteljska infestacija malom dječjom glistom. Pregled sedimenta mokraće može biti osobito vrijedna pretraga u nejasnim situacijama i kada se ne mogu dobiti odgovarajući podatci, primjerice od male djece.

Ključne riječi: ***Enterobius vermicularis,*** sediment mokraće, samoljepljiva traka, mebendazol

MOGUĆNOSTI LIJEČENJA DEPRESIJE U PERIMENOPAUZALNOJ DOBI S OBZIROM NA RAZLIČITE ETIOLOŠKE ČIMBENIKE

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Depresija je poremećaj s tisuću lica koji se češće pojavljuje u žena u određenim životnim razdobljima. Jedno od razdoblja posebne ranjivosti i osjetljivosti žena je perimenopauza kada su podložne različitim promjenama. Sve to utječe na kvalitetu života pacijenata i njihovo funkcioniranje u različitim životnim sferama. Zbog toga je važno na vrijeme prepoznati simptome bolesti, moguće preventivne i etiološke čimbenike i započeti sveobuhvatno i prilagođeno liječenje. Sve češće istraživan vitamin D je u negativnoj korelaciji s depresijom u perimenopauzalnoj dobi zbog regulacijskih i zaštitnih učinaka na dopaminski sustav. Prikazujemo pacijenticu kojoj je dijagnosticirana depresija u perimenopauzalnoj dobi, njen tijek liječenja i funkcionalnog oporavka. Ovim bismo prikazom htjeli skrenuti pozornost na potrebu za budućim dobro kontroliranim istraživanjima etioloških čimbenika te potrebnog razvoja bolje suradljivosti u liječenju i prevenciji duševnih poremećaja.

Ključne riječi: depresija, perimenopauza, vitamin D, socioterapija

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UVOD

Perimenopauza je iznimno osjetljivo razdoblje u životu žena te vrijeme različitih somatskih, društvenih i psiholoških promjena. Zbog toga je to razdoblje prozor ranjivosti za razvoj duševnih poremećaja, osobito depresivnih simptoma izazvanih različitim hormonskim i reproduktivnim promjenama. U različitim istraživanjima pokazana je povezanost između perimenopauze i depresije (1). Depresija je jedna od najčešćih bolesti u svijetu koja je u globalnom porastu, a svoje štetne učinke može imati na različite životne sfere i značajno utjecati na kvalitetu života pojedinca. Utječe na međuljudske odnose pojedinca, njegovu sposobnost za rad, financijski status, a nažalost i na rizike od samoozljeđivanja i samoubojstava. Osim toga depresija može imati ozbiljne posljedice i na tjelesno zdravlje pa su tako smrtnost i pobol povezani s karcinomom i ishemijskom bolesti srca povećani u osoba kojima je dijagnosticirana depresija (1-3). U kombinaciji sa sve starijim stanovništvom u našim krajevima i u mnogim drugim zemljama važno je utjecati na moguće preventivne čimbenike, na vrijeme prepoznati depresiju i započeti valjano liječenje. Sve je to velik izazov u dijagnosticiranju i liječenju ovog poremećaja te zahtijeva sveobuhvatni pristup radi poboljšanja cjelokupnog funkcioniranja bolesnika i njegove rehabilitacije, resocijalizacije i reintegracije u zajednici (4). Zbog svega navedenog cilj je otkriti čimbenike na koje možemo utjecati, a u posljednje vrijeme sve je češće istraživan vitamin D u negativnoj korelaciji s depresijom. Vitamin D je iznimno popularan i izdvaja se od ostalih hormona zbog utjecaja na važne fiziološke procese, uključenosti u rast i diferencijaciju stanica, imunomodulaciju, neurotransmisiju, protuupalne čimbenike. Različiti čimbenici mogu modulirati razinu vitamina D, a uključuju okolišne čimbenike, životni stil, prehrambene navike i neravnotežu hranjivih tvari, ali i neke genetičke čimbenike. Većina vitamina D u ljudskom tijelu se sintetizira endogeno, čak 90 %, i složenim mehanizmom

se pretvara iz provitamina D (7-dehidrokolesterol) u aktivni metabolit vitamina D (1,25-dihidroksivitamin D), u čemu ulogu imaju koža, jetra i bubrezi. Zbog duljeg poluživota (oko 3 tjedna) u usporedbi s aktivnim metabolitom (oko 6 sati) status vitamina D u tijelu se procjenjuje preko metabolita koji nastaje u jetri prvom hidroksilacijom (25-OH vitamin D) (5). Provitamin D (odnosno 7-dehidrokolesterol) je neposredni prekursor kolesterola, lipida koji je nezamjenjiv i neophodan za sve stanice, osobito za normalan razvoj i funkcioniranje mozga. Zbog toga poremećaj biosinteze kolesterola može rezultirati različitim intelektualnim i razvojnim teškoćama, a neki često propisivani lijekovi (antihipertenzivi, antiaritmici, a i određeni antipsihotici i antidepresivi) mogu ometati njegovu biosintezu, inhibirajući posljednji korak u njegovoj sintezi zbog čega dolazi do nakupljanja izrazito nestabilnih i toksičnih metabolita. Uz to pacijenti često zbog određenih komorbiditeta u redovitoj terapiji imaju propisane lijekove koji utječu na metabolizam lipida (statini) pa je u takvih potreban dodatan oprez pri kombinaciji lijekova radi poboljšanja sigurnosti pacijenta i prilagođavanja lijekova genotipu i životnom stadiju (6). Time isto naglašavamo važnost individualnog planiranja liječenja i uspostavljanja odnosa terapijskog saveza i povjerenja, što je glavna vodilja u različitim kliničkim smjernicama za liječenje depresivnog poremećaja. U smjernicama je vidljivo kako postoje različite mogućnosti liječenja ovog poremećaja kao što su primjena psihofarmaka, psihoterapijsko liječenje, metode samopomoći, psihosocijalne metode i neurostimulativne biološke metode liječenja (7).

Prikazujemo pacijenticu kojoj je dijagnosticirana teška depresivna epizoda bez simptoma psihoze u perimenopauzalnoj dobi, tijek liječenja i funkcionalnog oporavka.

PRIKAZ BOLESNICE

Žena u dobi od 48 godina primljena je Zavod za integrativnu psihijatriju putem hitne psihijatrijske ambulante zbog pogoršanja psihičkog stanja u obliku depresivne dekompenzacije. Iz anamnestičkih podataka: bolesnica je udana, majka dvaju sinova, mlađii sin preminuo prije pet godina, po zanimanju njegovateljica, zaposlena, živi sama. Iz obiteljske anamneze negativnog psihijatrijskog herediteta. Do sada nije pregledavana psihijatrijski, ovo joj je prvo hospitalno psihijatrijsko liječenje. Na tjelesnom planu boluje od hipertenzije i hiperlipidemije. Od internističke terapije uzima ramipril 2,5 mg, atorvastatin 20 mg, ibuprofen 600 mg prema potrebi. Alergična je na penicilin. Puši cigarete, alkohol ne konzumira. Pri primitku pacijentica opisuje pogoršanje stanja unatrag tri mjeseca u obliku sniženog raspoloženja, bezvoljnosti, tjeskobe, napetosti, smanjene razine energije, otežanog obavljanja kućanskih aktivnosti, otežanog funkcioniranja na radnom mjestu. Žali se na narušenu dinamiku spavanja, uz otežano usnivanje i prosnivanje, učestale glavobolje, napade vrućine, preznojavanje, osjećaj usamljenosti, bespomoćnosti, nemoći, narušeno samopouzdanje, socijalnu disfunkcionalnost. Opisuje narušene obiteljske odnose, suprug radi u inozemstvu, a sa sinom nema kontakt. Navodi povremene suicidalne ideje, moli za pomoć. Pri pregledu je bila uredne svijesti, adekvatna u kontaktu, uredno orijentirana u svim pravcima, psihomotorno napeta, tjeskobna, depresivnog raspoloženja, afektivno labilna, plačljiva, oskudnog misaonog duktusa po formi, u sadržaju depresivne ideje, obiteljska dinamika, kognitivne teškoće, sniženih voljno- nagonskih dinamizama, bez sumanutosti, bez obmana osjetila, verbalizira povremene autodestruktivne ideje, nije agresivna. Afebrilna je, eupnoična, kardiopulmonalno kompenzirana, dobrog općeg stanja, urednog neurološkog statusa, bez žarišnih ispada i lateralizacija.… Učinjena je laboratorijska obrada krvi i mokraće, utvrđena je hiperkolesterolemija, hipovitaminoza D, blago povišen C reaktivni protein (CRP), a ostale laboratorijske pretrage su bile u granicama normale. Učinjena je i elektroencefalografija (EEG) koja je bila uredna te psihologijsko testiranje koje je potvrdilo inicijalnu dijagnozu depresije. Nakon primitka je pacijentica uvedena u terapiju psihofarmacima (antidepresiv uz anksiolitik) te je uključena u sve socioterapijske aktivnosti na Zavodu i provođenje individualnih psihoterapijskih razgovora. Budući da se žalila na nuspojave prvotno uvedenog antidepresiva sertralina, isključili smo ga iz terapije i s obzirom na tadašnju kliničku sliku uveli fluvoksamin navečer. Naknadno je pacijentici određena koncentracija antidepresiva u krvi koja je bila snižena. Pacijentica nakon mjesec dana hospitalnog liječenja u našem Zavodu opisuje poboljšanje psihičkog stanja u smislu pozitivnog pomaka na planu raspoloženja i voljno-nagonskih dinamizama uz urednije reguliranu dinamiku spavanja te potpuno distanciranje od suicidalnih ideja, misli i pulzija.

RASPRAVA

Danas, u moderno doba medicine sve se više naglašava važnost individualnog i sveobuhvatnog pristupa svakom pacijentu pa je tako unatrag nekoliko desetljeća poraslo zanimanje i za duševno zdravlje žena, specifičnost njihove psihopatologije, različit tijek bolesti i različito reagiranje na terapijske intervencije. Poznato je da depresija prožima cijelu osobu od njene srži do najviših očitovanja duše, a žene imaju višu stopu velikih depresivnih epizoda i podložnije su razvoju depresije u određenim životnim razdobljima (8). Prije početka obrade i liječenja te uvođenja u terapiju naše pacijentice u obzir smo uzeli sve navedene informa-

cije i moguće etiološke čimbenike. Danas postoje različite etiološke hipoteze o razvoju depresije: biološke, psihosocijalne i psihoanalitičke, a razvojem medicine dokazana je neurohormonska, neurokemijska i neuroimuloška pozadina depresije koje na određeni način potvrđuju klasične psihoanalitičke teorije. Psihoanalitičar Freud započeo je psihoanalitičke teorije te je depresiju objašnjavao kao patološki analog žalovanja koja proizlazi iz gubitka objekta koji može biti i nestvaran. Kasnije su autori depresiju opisivali kao krik za ljubavi, agresiju prema selfu, konflikt ega, fiksaciju na prethodna iskustva bespomoćnosti, pad libidinalnog ulaganja u self reprezentaciju… Poznata je i važna varijabla depresije prema Becku takozvana kognitivna trijada, a uključuje negativnu percepciju sebe, svijeta i budućnosti (9,10). Tijekom primitka naše pacijentice i dobivanja anamnestičkih podataka s obzirom na simptome i gore navedene čimbenike odlučili smo se za liječenje pacijentice na Zavodu za integrativnu psihijatriju radi mogućeg ostvarivanja specifičnog liječenja i uključivanja u različite socioterapijske aktivnosti tog Zavoda, a radi potrebne rehabilitacije. Prije početka liječenja našoj pacijentici učinili smo potrebnu obradu somatskog stanja, laboratorijsku obradu krvi i urina te EEG. Utvrđena je hipovitaminoza D, hiperkolesterolemija te blago povišen pokazatelj upalne reakcije CRP. Prema nekim istraživanjima pokazano je kako upravo CRP predviđa daljnji razvoj depresije i otpornost prema terapiji antidepresivima. Naime, poznato je kako imunološki sustav ima ulogu i u patofiziologiji depresije pa tako povećana koncentracija CRP-a u plazmi korelira s ozbiljnošću simptoma depresije (9,11). Pacijentica je već otprije imala dokazanu hiperkolesterolemiju zbog čega je u terapiji statinima koji djeluju na metabolizam lipida. Imajući to na umu pri izboru antidepresiva, s obzirom na opisane simptome i nesanicu koja je među najčešće opisivanim simptomima u perimenopauzi, kao lijek izbora činio nam se trazodon, ali ga nismo primijenili zbog opisanog djelovanja na metabolizam lipida i preporuke nekombiniranja lijekova koji djeluju u tom putu (6). Zbog svega navedenog a nakon prvotnog nepodnošenja sertralina odlučili smo se za primjenu fluvoksamina. Radi što bolje titracije pacijentici je izmjerena koncentracija lijeka u serumu, koja je bila ispod preporučenih vrijednosti. Bez obzira na to, pacijentica je opisivala poboljšanje stanja i nije bila zainteresirana za prilagodbu doze lijeka navodeći kako su joj druge metode liječenja odnosno rehabilitacije na Zavodu dodatno pomogle i koristile. Osim toga, pacijentici je bila snižena i koncentracija vitamina D što je često i opisivano u različitim istraživanjima. Pri izboru lijekova potrebno je voditi računa i o tome s obzirom da je i endogena sinteza vitamina D ovisna o metabolizmu lipida (5,6). Rehabilitacija je potrebna svakoj osobi sa psihičkim smetnjama, jer je sveobuhvatan i složen proces te joj je cilj vođenje zdravog života unatoč prisutnim teškoćama i simptomima bolesti. Sastoji se od primjene psihofarmaka, psihoterapije i socioterapije koji imaju za cilj poboljšati funkcioniranje bolesnika i ojačati sposobnosti socijalne prilagodbe. Velik udio psihijatrijskih bolesnika nakon hospitalnog liječenja bude neadekvatno adaptiran na okolinu u koju se vraća zbog čega veliku važnost imaju socioterapijske metode kojima je temeljni cilj motiviranje i uključivanje bolesnika u vlastito liječenje. Postoje različiti socioterapijski postupci, a obuhvaćaju aktivnosti s kojima se pojedinac svakodnevno susreće kao što su rekreacija, terapijska zajednica, radna i okupacijska terapija, terapija glazbom, art-terapija, ples, filmoterapija, ekspresivno pisanje (4,12). Uspješno provedena socioterapija, odnosno rekonstruktivna terapijska metoda, očituje se zadovoljavajućim radnim i obiteljskim funkcioniranjem uz uredno funkcioniranje u široj zajednici i vođenjem zdravog života. Brojna istraživanja pokazuju kako se dodatnu snagu i mogućnost za oporavak i nadrastanje izazova može dobiti uključivanjem u različite kreativne aktivnosti, posebice na umjetničkim poljima radi podizanja sposobnosti suočavanja s teškim životnim iskustvima i situacijama. Terapijske koristi kreativnih aktivnosti objašnjavaju se pomoću nekoliko različitih mehanizma kao što su angažman i tijek, katarza, distrakcija i pozitivne emocije i pridavanje značenja. Angažmanom i uključivanjem u kreativne aktivnosti i tijekom koji je obilježen koncentracijom i fokusom, djelovanjem, pronalaskom vremena i nagrade objašnjava se kako kreativnost može utjecati na pozitivne emocije, osobne kompetencije, osjećaj postignuća i smisla života. Katarzom koju je još opisivao Aristotel postiže se emocionalno opuštanje i pročišćenje negativnih misli i emocija te tako kreativne aktivnosti mogu osobama služiti kao sredstvo za izražavanje tih bolnih emocija. Odvlačenjem pozornosti na druge aktivnosti tijekom obavljanja kreativnih aktivnosti promovira se izražavanje pozitivnih emocija i stvara novi način kako se osoba nosi sa svojim psihičkim simptomima. Izravan rezultat kreativnih aktivnosti je stvaranje značenja i utjecaja na razmišljanje i dodatno naglašava sve dobrobiti i koristi od kreativnih aktivnosti (12). Pacijenticu smo po otpustu nastavili pratiti putem ambulantnih kontrolnih pregleda te je opisivano daljnje poboljšanje stanja i zadovoljavajuće funkcioniranje. Navela je kako se potaknuta aktivnostima na Zavodu odlučila ponovno baviti hobijima te se uključiti u novu aktivnost – ples, koja je dodatno pridonijela pozitivnim emocijama. Isto tako navodila je i poboljšanje odnosa u obitelji, bolje odnose sa suprugom i sinom, uredno funkcioniranje na radnom mjestu. Promijenila je i prehrambene navike, uzimala namirnice bogate vitaminom D (riba, plodovi mora, namirnice koje su dodatno obogaćene vitaminom D) te je više boravila u prirodi na suncu. Nakon nekoliko mjeseci učinjena je kontrolna laboratorijska obrada krvi koja je bila u granicama referentnih vrijednosti, uključujući i vitamin D.

ZAKLJUČAK

I u ovom slučaju je pokazano kako je depresija poremećaj s tisuću lica i s različitim etiološkim čimbenicima koje je važno prepoznati. Vidljivo je kako su žene posebno osjetljive u određenim životnim razdobljima kada je dodatno potrebno utjecati na preventivne čimbenike, odnosno na vrijeme prepoznati bolest i započeti valjano liječenje. Osim sveobuhvatnog pristupa pacijentici potrebna je i prilagodba lijekova prema spolu, odnosno životnom stadiju i prisutnim komorbiditetima. Uključivanjem pacijentice u vlastito liječenje i u socioterapijske postupke i kreativne aktivnosti postiže se rehabilitacija i poboljšava funkcionalnost i prilagođenost u različitim životnim sferama. Naglašava se i važnost edukacije i komunikacije s pacijenticama, psihoedukacije, edukacije o prehrani, metodama samopomoći, mogućim relaksacijama…

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SUMMARY

POSSIBILITIES OF DEPRESSION TREATMENT IN PERIMENOPAUSAL AGE CONCERNING VARIOUS ETIOLOGIC FACTORS

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Depression is a disorder with a thousand faces that appears more often in women in certain periods of life. One of the periods of special vulnerability and sensitivity of women is perimenopause when they are subject to various changes. All this affects the quality of life of patients and their functioning in different spheres of life. For this reason, it is important to recognize the symptoms of the disease on time and to start a comprehensive and adapted treatment. Therefore, it is necessary to recognize possible preventive and etiological factors. The increasingly researched vitamin D is negatively correlated with depression in the perimenopausal age due to its regulatory and protective effects on the dopamine system. In this paper, we present a patient diagnosed with perimenopausal depression, her course of treatment and functional recovery. With this presentation, we wanted to draw attention to the need for future well-controlled research into etiological factors and the necessary development of better cooperation in the treatment and prevention of mental disorders.

Key words: depression, perimenopause, vitamin D, sociotherapy

AMANITA PHALLOIDES POISONING, EARLY ACTIVATED CHARCOAL PLUS N-ACETYL CYSTEINE TREATMENT: CASE REPORT ANDA BRIEF LITERATURE REVIEW

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Consumption of wild poison mushrooms is one of the serious poisonings which may end in death. The present case report and recent literature review describe *Amanita phalloides* mushroom poisoning and possible treatment for this emergency state. A 59-year-old male presented in the Emergency Unit of the Foggia University Hospital, Italy, with clinical signs of extreme dizziness, nausea, vomiting, and diarrhea, 12 h after consuming one ovule of a wild mushroom that was mistaken for an edible ovule of the *Boletus edulis* mushroom. The suspected poison mushrooms were collected in the forest near the city of Foggia, Italy. Urgent examination of urine showed the presence of α-amanitin. After 6 days of intensive and supportive treatment with activated charcoal and N-acetyl cysteine, the patient was transferred to the internal medicine department and discharged without organ complications 10 days after mushroom ingestion. Early recognition of mushroom poisoning and immediate intensive treatment with supportive care give the patients a better chance for survival after this fatal poisoning.

Key words: **activated charcoal, *Amanita phalloides,* amatoxins, mushrooms, poisoning, N-acetyl cysteine**

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INTRODUCTION

Out of thousands of mushroom species found in the world, just up to 100 are toxic to humans; most of them are *Amanita* species. Two toxins produced by amanita species, phallotoxins and amatoxins, are responsible for phalloidin syndrome. Amatoxin-containing mushrooms (*Amanita, Lepiota,* and *Galerina*) are responsible for 90% of mushroom ingestion-related deaths (1). Although it is more dangerous in children, the amount that can be found in species like Amanita is sufficient to poison an adult person causing liver and renal damage (2, 3). The human lethal dose, LD50, is 0.1 mg/kg body weight (4). Amanita mushroom poisoning is one of the most serious food poisonings in the world characterized by a long incubation phase, gastrointestinal and liver phase, eventual coma, and death. *Amanita* species such as *A. phalloides*, *A. verna*, *A. virosa, A. amerivirosa,* and *A. vidua* sp. *nov.,* are responsible for almost all mushroom poisoning in Europe (5), but this health problem is present worldwide (6-9).

Amatoxins are heat- and frozen-stable toxic cyclic peptides (10-12). Two potent amatoxins are α- and β-amanitins (AMA). They irreversibly inhibit ribonucleic acid polymerase (RNA) II in the liver and kidneys, blocking the synthesis of proteins including intracellular enzymes (13-15). Studies on animal models suggest that α-AMA causes hepatocyte necrosis and apoptotic cell death (16,17).

The fatal course of mushroom poisoning can be avoided with early diagnosis, but despite intensive treatment, antidotes, and antioxidative therapies, mortality rate in this type of poisoning remains high (18-22). Our case report describes mushroom *Amanita phalloides* poisoning in one of our patients (Figure 1).

Figure 1. *The Amanita phalloides mushroom (original photo). This photo shows the second mushroom ovule that was not ingested by our patient.*

METHODS

Using the PubMed, Medline, Ovid, Google, Google Scholar, and Cochrane databases, the keywords (activated charcoal, *Amanita phalloides*, amatoxins, mushrooms, poisoning, N-acetyl cysteine [NAC]) as the medical subject headings were searched with results limited to the past 20 years (2002-2022), including only peer-reviewed scientific articles on amatoxin-containing mushroom poisonings and new treatment guidelines over the study period to meet the main objectives of this report. Attention was mainly on the therapies used in case reports, case series, short communications, and letters to the editor in the study period.

CASE REPORT

Our patient was a 59-year-old male with suspected *Amanita phalloides* poisoning. The patient was received to the Emergency Unit with a red code, about twelve hours after the suspected ingestion of a poison mushroom. The circumstances under which the poisoning occurred were unintentional and accidental. He was conscious, oriented, and eupneic. He reported symptoms of dizziness, nausea, uncontrollable vomiting, diarrhea, and severe abdominal pain. The Apulian Regional Poison Center was contacted and emergency blood chemistry and gas tests, blood pressure, electrocardiogram, and echocardiogram were performed. The patient was transferred to the Intensive Care Unit (ICU) for continuous intensive care and monitoring.

In ICU, after cardio-circulatory monitoring, a nasogastric tube was placed and activated charcoal administered every 6 hours in doses of 20 g. The patient was hydrated with Ringer lactate intravenously (IV) at 63 mL/h; it was administered in a bolus of NAC 150 mg/kg body weight in 60 minutes, then 300 mg/kg body weight (2 mL/h[1] in glucose 5% 500 mL) in continuous infusion. Biological samples were sent for detection of AMA toxins (with confirmation of the presence of the α-AMA toxins in the urine sample). We contacted immediately the University Hospital Polyclinic of Bari for a possible liver transplant. We began monitoring hepatic, renal, and coagulation function and hemachrome every six hours (Table 1, Figure 2). On day 6 of ICU stay and after normalization of all examinations, the patient was transferred to the internal medicine department.

Table 1. *Laboratory parameters over time (T) in hours in our patient*

Recovery time (T) (hours)	PT (%)	PTT (sec)	INR (Ratio)	AST (U/L)	ALT (U/L)	LDH (U/L)	DB (mg/dL)	TB (mg/dL)	PLT (mm^3)	Hb (g/dL)	Cr (mg/dL)	BUN (mg/dL)
Normal range	70-120 %	30-40 sec	0.8-1.2	15-41 U/L	17-63 U/L	140-280 U/L	≤0.3 mg/dL	0.2-1.2 mg/dL	150-450 $x10^3$	M13.8-17.2; F12.1-15.1 g/dL	0.8-1.2 mg/dL	7-20 mg/dL
T0	93	39.7	1.04	32	32	507	0.1	0.7	171	14.9	0.82	43
T6	88	32.6	1.09	51	56	898	0.1	1,1	185	14.4	0.85	39
T12	81	33.6	1.14	147	169	923	0.2	1	168	14.3	0.84	39
T18	81	33	1.14	647	991	1156	0.2	1.5	175	14.3	0.75	37
T24	85	34.5	1.1	680	839	1762	0.3	1,9	160	13.6	0.81	37
T30	75	33.4	1.1	455	973	1091	0.3	1.9	164	13.8	0.85	34
T36	71	33.3	1.25	412	887	945	0.3	1.9	170	14.6	0.69	30
T42	71	31.4	1.25	315	836	1091	0.3	1.8	159	13.9	0.71	29
T48	65	32.4	1.34	295	789	698	0.3	1.8	169	14.3	0.8	29
T54	65	32.7	1.34	246	761	523	0.25	1.6	158	14	0.73	27
T60	63	32.6	1.35	152	566	498	0.2	1.3	157	13.3	0.74	23
T66	62	32.5	1.36	76	381	452	0.3	1.8	157	13.3	0.85	23
T72	60	32	1.37	60	326	380	0.2	0.8	156	12.4	0.79	20
T78	62	31	1.35	45	278	379	0.2	0.8	145	12.1	0.81	19
T84	60	29	1.41	34	227	424	0.2	0.8	139	12	0.75	20
T90	62	31.5	1.05	32	198	365	0.2	0.8	135	11.8	0.8	22
T96	50	32.3	1.57	22	151	344	0.2	0.8	130	11.6	0.83	19

PT = prothrombin time; PTT = partial thromboplastin time; INR = International Normalized Ratio; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactate dehydrogenase; DB = direct bilirubin; TB = total bilirubin; PLT = platelet; Hb = hemoglobin; Cr = creatinine; BUN = blood urea nitrogen; M=male; F=female

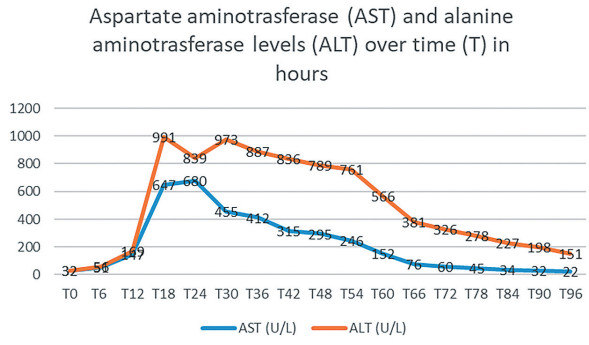

Figure 2. *Liver aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in our poisoned patient over time (T) in hours during hospitalization. Serum ALT and AST have been used as sensitive indicators of liver injury caused by amatoxins.*

DISCUSSION AND CONCLUSION

Amanita phalloides is a toxic mushroom that can cause serious health problems in adults and in children, such as acute renal and liver injury, and even death(1-3). Angioi *et al.* report unusual end-stage kidney disease in a patient intoxicated with *A. phalloides* (2). Amanita poisoning mostly occurs worldwide as an un intentional poisoning due to false distinction between toxic and edible mushroom species.

The lethal dose (LD) for humans of AMA is 0.1 mg/kg body weight (4). This LD can be present in a single mushroom and can generate irreversible liver damage within 48 hours of ingestion (23).

The two toxins of Amanita species are phallotoxins and amatoxins. Phallotoxins are not absorbable in gastrointestinal tract and lead to irritation of gastric mucosa; as a result, their toxicity is limited to only gastrointestinal manifestations within the first 24 hours of ingestion. On the contrary, a massive amount of amatoxin α-AMA reaches the liver after gastrointestinal absorption. The two transporters of the liver cellular membrane that transport these toxins into cells are organic anion-transporting polypeptides (OATP1B3) and bile acid transporter Na(+)/taurocholate transport protein (NTCP) (14,28). OATPs mediate the uptake of a wide variety of organic compounds and also amatoxins into the liver cells. The presence of the OATP1B3 hepatic entry transporter, which mediates a rapid uptake of amatoxins, on hepatic cells, makes the liver a privileged target organ in case of amatoxins poisoning (14).

The toxic mechanism of AMAs is attributed to the non-covalent nuclear inhibition of RNA polymerase II in renal tubules, hepatocytes, and lymphocytes. The messenger RNA transcription process is suppressed, thus toxin-inhibited protein synthesis leads to cellular hepatic necrosis and apoptosis (1,4, 11-15).The hepatic centrilobular and periportal hemorrhagic hepatic necrosis brings to the rapid rise in hepatic damage biomarkers, principally serum transaminases, and coagulopathies result from deficiencies in the synthesis of hepatic clotting factors. As the liver function fails, tubulointerstitial nephropathy follows and precipitates the hepatorenal syndrome that may be rapidly fatal if liver transplantation is not possible or available as a treatment option (22,31,35,36).

Moreover, in the first 72 h of intoxication, amatoxins are excreted unchanged in the urine and their concentration rises from 6 to 90 times higher than in the liver (24). This explains the nephrotoxicity of those toxins, while dehydration may also contribute to kidney injury in these patients. Amatoxins can be found for 30 h in plasma or serum, and up to 72 h in urine (25).

The first gastrointestinal symptoms can be seen only 6 to 12 hours after ingesting the mushrooms (1, 11-15, 21). Sometimes the symptoms may appear after a long latency period of 40 hours, and can negatively influence medical intervention because cellular apoptosis and severe hepatic necrosis with fulminant hepatic failure occurs (1,4,5). Bonacini *et al.* also found that women and older patients were more likely to have a poor outcome than men and younger patients (4). Rapid analysis of amatoxins and phallotoxins that are found in body fluids is essential for vital organ recovery (26,27).

Actually, there are no guidelines or current strategies for amatoxin poisoning, and therapies have not been subjected to rigorous efficacy testing in randomized controlled trials because of ethical reasons. Around 10%-20% of cases of *Amanita phalloides* poisoning have fatal outcome or finish with liver transplantation, despite the use of antidotes (11).

The new literature recommendations indicate that prompt contact with regional poison centers is an important factor to provide the best clinical approach in cases of poisoning. Furthermore, supportive therapy for vital functions, including volume replacement and electrolyte and bicarbonate correction is highly important. The rationale of this intervention is to avoid hypovolemic shock and reduce the risk of tubular necrosis (2,29,30).

The next step is toxin binding and decrease in the concentration of amatoxins. The intervention consists of gastrointestinal decontamination with gastric lavage that removes the contaminated meal and the amatoxin concentration from the stomach. Together with

activated charcoal, it has a synergistic effect to avoid the absorption process of toxins from the enteric tract. This intervention interrupts the enterohepatic reabsorption of amatoxins (31-39).

Regarding α-AMA antidote therapy, there are three medications available, i.e., NAC which is an antioxidant, and penicillin G and silibinin, which act by inhibiting the hepatocyte uptake of α-AMA. Several studies recommend the administration of two antidotes with different mechanisms of action to achieve maximum benefit (2,11,13,14,29-38,40-42) (Table 2). NAC has antioxidant and glutathione-regenerating effects. Thus, penicillin G and silibinin inhibit the uptake of amatoxins by hepatocytes, which are mediated by the OATP1B3 transporter (43).

Table 2. *Principal articles available in the literature in the last 20 years on Amanita phalloides poisoning with short description of their main characteristics and relevant intervention and experimental therapies*

Paper ID	Year	Type of article	Patient(s)	Mushroom	Intervention therapy
Enjalbert *et al.* **(11)**	2002	Retrospective study	2108 patients	*A. phalloides*	Silibinin, NAC, benzylpenicillin
Letschert *et al.* **(14)**	2006	Experimental study	*In vitro* study	*A. phalloides* toxin uptake	Rifampicin, antamadine, MK571, silibinin, dihemisuccinate, cyclosporine A
Ennecker-Jans *et al.* **(40)**	2007	Case report	54-year-old man, 51-year-old woman, 55-year-old woman	*A. phalloides*	High-dose penicillin G, silibinin, and NAC
Thaler *et al.* **(33)**	2008	Letter to the editor	72-year-old man	*A. phalloides*	Activated charcoal, NAC, silibinin
Magdalan *et al.* **(22)**	2010	Comparative study	*in vitro* study on cultured human hepatocytes	*A. phalloides* α-amanitin	Benzylpenicillin, NAC,silibinin
Grabhorn *et al.* **(34)**	2013	Retrospective study	5 children	*A. phalloides*	Intravenous (IV) silibinin, NAC, active charcoal, liver transplantation
Ward *et al.* **(36)**	2013	Case reports	72-year-old woman, 45-year-old son	*Amanita Ocreata*	Activated charcoal , NAC, penicillin (1,000,000 units intramuscular), silibinin, and transfer to a center with liver transplant capabilities
Vanooteghem *et al.* **(42)**	2014	Case series	4 women	*A. phalloides*	NAC, silibinin, liver transplantation
Varvenne *et al.* **(30)**	2015	Illustrative case	9-year-old boy, 11-years-old girl,mother	*Lepiota brunneoincarnata*	IV rehydration, analgesics, oral activated charcoal, IV silibinin, penicillin G, NAC
Chibishev *et al.* **(38)**	2015	Case series	8 patients	*A. verna*	Charcoal, NAC, vitamin therapy, penicillin, H2 blockers, ornicetil
Yilmaz *et al.* **(37)**	2015	Case report	61-year-old man	*A. Phalloides*	Activated charcoal, penicillin G
Garcia *et al.* **(49)**	2015	*In silico* study on animals	intoxicated animals (n=20 male CD-1 mice)	α-amanitin	Polymyxin B
Vo *et al.* **(29)**	2017	Morbidity and Mortality Weekly Report, California Department of Public Health	14cases	*Amatoxin exposure*	Aggressive IV fluid hydration, IV octreotide, IV silibinin, liver transplantation
Bonacini *et al.* **(32)**	2017	Brief Communication	27 patients	*A. phalloides*	Activated charcoal and NAC, penicillin G, silymarin, orthotopic liver transplantation
Li *et al.* **(41)**	2018	Case report	41 and 48- years-old woman	*Amanita fuliginea*	Silibinin, penicillin G, and plasma exchange
Sun *et al.* **(47)**	2019	Case reports	55-year-old male	*Lepiota brunneoincarnata*	Naso-biliary drainage, Legalon silibinin
Wang *et al.* **(35)**	2019	Case series	56-years-old male, 58-years-old female, 58-years-old female	*Amanita fuliginea*	Silibinin, activated charcoal, glutathione, and magnesium isoglycyrrhizinate, fibrinogen and platelets
Garcia *et al.* **(50)**	2019	Short-term study (24 h) and a survival study (30 days) on animals	intoxicated animals (n=40+40 male CD-1 mice)	α-amanitin	Polymyxin B and methylprednisolone
Wennig *et al.* **(39)**	2020	review article	4441 cases in Germany	90% of 32 death was caused by *A. phalloides*	Activated charcoal, silibinin, NAC, hemodialysis/ albumin dialysis, liver transplant as a lifesaving measure
Zuker-Herman *et al.* **(51)**	2021	Short communication	79-year-old man, 72-year-old woman	*A. phalloides*	IV rifampicin, activated charcoal, NAC
Le Daré *et al.* **(48)**	2021	Short communication	*in vitro* study	*A. phalloides*	HEMO2life®

Angioi *et al.* **(2)**	2021	Case report	a 79-year-old man and his wife	*A. phalloides*	NAC, SLEDD-f, hemodialysis
Smędra *et al.* **(10)**	2022	Case report	28-year-old man	*A. phalloides*	Liver transplantation

SLEDD-f= sustained low-efficiency daily diafiltration; MK571 [(3-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-dimethylamino-3-oxopropyl) thio)methyl)thiopropanoic acid];NAC= N-acetylcysteine; HEMO2life®=extracellular hemoglobin commercialized by Hemarina, Morlaix, France

Treatment for liver failure is the same as for the liver failure of other etiologies. The Clichy criteria (44) and Munich criteria (45) provide a suitable basis for decision-making on whether a liver transplant is indicated. Liver transplantation is limited mainly by the availability of donors.

Impairment of coagulation factor production caused by liver damage may necessitate administration of fresh frozen plasma, and forced diuresis may be indicated in the first 5 days as α-AMA is mainly excreted in the urine.

Extracorporeal purification techniques such as hemodialysis, hemoperfusion, or plasmapheresis have been described, although their effectiveness remains limited (11). In the case of liver transplantation, treatment with high-volume plasma exchange has been shown to increase liver transplant-free survival (46).

Interestingly, Sun *et al.* showed that using biliary drainage to interrupt enterohepatic cycling of amatoxins reduced the intestinal amatoxin reuptake (47).

Some of new and promising therapies for amatoxin poisoning include extracellular hemoglobin called M101 extracted from the marine worm *Arenicola marina*. It has intrinsic Cu/Zn-SOD-like (SOD, superoxide dismutase) activity and can be used as an oxygen carrier. Le Darè *et al.* suggest that M101 might effectively reduce AMA-induced hepatotoxicity and may have the potential for further investigation and therapeutic development (48) (Table 2).

Furthermore, some *in vitro* studies with polymyxin B may reveal it as a promising antidote for the future. It reduces liver necrosis caused by α-AMA and improves survival in intoxicated animals (49,50) (Table 2). It still needs to be approved by clinical trials on humans as an effective treatment for amatoxin poisoning. Also, rifampicin may be effective in the management of α-AMA toxicity (51).

This clinical case of severe poisoning with α-AMA confirmed by laboratory tests underlines the clinical importance of early diagnosis. Close collaboration between the Emergency Unit, ICU, and Apulian Regional Poison Center allowed the patient to recover, especially liver and kidney function compromised by poisoning, and to avoid liver transplantation (Figure 2, Table 1). It is crucial to start rapidly with correct medical procedures when amatoxin poisoning is suspected. The poisoned person must be transported to the hospital, perform toxicologic tests, and start appropriate treatment. The time of admission to the hospital is crucial for treatment options including gastric lavage, large doses of activated charcoal, moderately enhanced diuresis, silibinin, penicillin G, and NAC as single or combined therapy.

Nowadays, for aging and eating wild mushrooms is still popular all over the world, so variable prevention modalities can have an important role in reducing the incidence of this kind of poisoning. Public education, particularly in schools, organizing licensed mushroom collectors, and the sites where the collectors can control the collected mushrooms before ingestion are very important. Nevertheless, institutional information campaigns targeting inexperienced mushroom collectors should invest more effort to make them aware of the presence of such lethal mushrooms.

History of poisoning: Two poisonous mushroom ovules were collected in the forest near Foggia, Italy, by the patient's brother-in-law, an expert licensed mushroom collector. All the mushrooms collected were the edible mushroom *Boletus edulis* consumed by two families on the previous day. The two mushroom ovules mimicked *Boletus edulis* ovules and were present together at the same locus. The fact to be stressed is the high polymorphism of this mushroom, and for this reason, the popular names of the 'angel of death' and 'bastard egg' are justified.

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SAŽETAK

OTROVANJE GLJIVOM *AMANITA PHALLOIDES* – RANO LIJEČENJE AKTIVNIM UGLJENOM I N-ACETIL CISTEINOM: PRIKAZ BOLESNIKA I KRATAK PREGLED LITERATURE

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Jedenje divljih otrovnih gljiva može dovesti do ozbiljnog otrovanja i smrti. Prikaz bolesnika i pregled najnovije literature opisuje otrovanje gljivama *Amanita phalloides* te moguće načine liječenja ovoga hitnog stanja. Poslije 12 sati nakon što je pojeo jajašce otrovne gljive, koje je zabunom zamijenio za jajašce jestive gljive vrganj *(Boletus edulis)*, 54-godišnji muškarac je došao na hitni prijam Kliničke bolnice u Foggiai, Italija, s kliničkom slikom velike slabosti, mučninom, povraćanjem i proljevom. Prikupljene gljive bile su ubrane u šumi blizu grada Foggia. Hitan pregled mokraće je pokazao prisutnost otrova alfa-amanitina. Nakon šest dana liječenja na intenzivnoj njezi aktivnim ugljenom i N-acetil cisteinom pacijent je bio prebačen na odjel interne medicine bez komplikacija na organima te je nakon 10 dana bio otpušten iz bolnice. Rano prepoznavanje otrovanja gljivama i rano intenzivno liječenje s potporom životnih funkcija daje dobre izglede za preživljavanje ovoga opasnog otrovanja.

Ključne riječi: **aktivni ugljen, *Amanita phalloides*, amatoksini, gljive, N-acetil cistein**

EUTHANASIA COMBINED WITH ORGAN DONATION: CURRENT STATUS AND CONCERNS

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DEAR EDITOR,

In parallel with the increasing number of countries legalizing euthanasia, we can observe an increase in the practice of combining euthanasia with organ donation, including donors euthanized for psychiatric disease. Euthanasia combined with organ donation has been practised in the Netherlands, Belgium, Canada and Spain (1).

Professional and public awareness of this evolving practice are limited. Understanding the complexity of pre- and postmortal interventions on donors is becoming a challenge even for medical professionals, especially when complex perimortal procedures (like normothermic regional perfusion) are introduced to improve organ oxygenation and allow heart procurement, along with other organs (2).

A scoping review of practice and challenges for organ donation after euthanasia (=medical assistance in dying) was published recently, summarizing that an increasing percentage of patients have received organs from euthanized patients. 286 euthanized patients donated organs, and 873 recipients have received organs from euthanized patients, including in 2021. This represented 14% of all recipients from DCDD (donors after circulatory determination of death) in countries where euthanasia is legal (1).

Recently, lung transplantations from 22 euthanized donors were reported. Nine donors were euthanized for neuromuscular disease, 8 for non-specified psychiatric disease (including a 28-year-old female), and 5 due to unbearable pain (presumably not from malignant disease, which is a contraindication for donation) (3). Transplanting lungs from a female euthanized for mental disease was reported in 2011 (4).

Euthanasia for psychiatric disease or mental suffering is controversial in itself, and even more so if combined with organ donation. Mental disorders were accepted for granting euthanasia or assisted suicide in 6/8 countries, in 4/8 even for minors, as reported by Mehlum et al. in 2020 (5) in a paper reviewing euthanasia and assisted suicide in patients with personality disorders. Based on their findings, the authors concluded that the current legislation and practice of euthanasia and assisted suicide for people with personality disorders were based on an inadequate understanding of the underlying psychopathology and a lack of awareness about contemporary treatment literature. Moreover, they asserted that this practice has neglected the individual's potential for having a life worth living (5).

In the context of organ donation combined with euthanasia, one should not overlook the fact that terminally ill, very old patients or those with malignant disease (the primary candidates for euthanasia) are not suitable for organ donation, either because of suboptimal organ quality or disease transmissibility. The most »desirable«organ donors (as concerns organ quality and disease transmissibility) may be relatively young patients euthanized for psychiatric disease or mental suffering.

The proponents of combining euthanasia with organ donation have several arguments: 1) the patient wishes to combine euthanasia with organ donation, if, in realizing their wish, we respect the patient's autonomy; 2) euthanasia combined with organ donation gives meaning to death; 3) euthanasia combined with organ donation is a chance for a second life (of the donor and of the recipients); 4) euthanasia and organ donation is an act of (extreme) altruism (6); 5) decision for euthanasia and decision for organ donation are completely separated- only after euthanasia is granted can the to-

pic of organ donationbe discussed; 6) the procedures of euthanasia and organ donation should be kept as separated as possible.

However, separating a decision for euthanasia from one for organ donation may not (always) be possible. As argued by Buturović Z, "patients proceeding through the euthanasia pipeline already face substantial situational pressure, and adding organ donation on top of it can make the whole process work as a commitment device. By allowing euthanasia patients to donate their organs, we are giving them additional reason to end their lives, thus creating an unbreakable connection between the two." (7).

It is not possible to overlook the slippery slope in real life after legalizing euthanasia. Euthanasia was primarily introduced to end suffering of "the sickest of the sick". This was the basis for public support. But soon after its legalization (and sooner in every new country), euthanasia is expanded to include mental suffering, children and organ procurement. Organs are procured first from patients euthanized for neuromuscular disease, then for psychiatric disease.

Transplantation medicine is one of the major achievements of the 20th century. Together with artificial organ replacement, it was animportant field of medicine contributing to the birth of modern bioethics. Transplantation medicine is deeply involved in our understanding and definition of death. However, in parallel with all the achievements, one should never overlook the limitations and ethical concerns related to transplantation medicine. We should never forget to protect the weak and the vulnerable. And be careful not to cross the red lines, regardless of the most noble aim.

Organ procurement from euthanized patients, especially if the basis for euthanasia was psychiatric disease, causes deep concern and requires deep reflection. As underlined by the fathers of modern bioethics 40 years after publishing their ground-breaking book, "Principles of Biomedical Ethics", all four bioethics principles are of equal importance (respect for autonomy, non-maleficence, beneficence and justice) (8). In the concluding paragraph of a paper published in N Engl J Med 2005, Eric J Cassel wrote that "the biggest thief of autonomyis sickness". We should not neglectthe power of authority in medicine when obtaining informed consent (9), and should be aware that consent may be signed out of obedience and not from an authentic, autonomous decision.

The challenges in obtaining informed consent from patients requiring euthanasia combined withan organ donation procedure are substantial. Can patients understand the process of determining death and all the pre- and postmortem interventions and procedures involved when combining euthanasia and organ donation? Recently, postmortal extracorporeal oxygenation was introduced after euthanasia (to enable procurement of the heart), making the entire procedure all the more complex (2). Can patients withdraw their consent for euthanasia and organ donation at any time, despite being under the pressure of organ recipients and medical teams waiting for their organs? Rosenbaum reported on the case(s) of patient who admitted that she still wanted to live after requesting euthanasia, but were afraid to admit it in front of her family (6).

To conclude, transplantation is based on trust in medicine and doctors. Owing to ethical controversies (like transplanting organs from patients euthanized for psychiatric disease), we may, as a consequence, have less and not more organs available for transplantation in future.

Increasing professional and public awareness of the present status, evolution and perspective of euthanasia combined with organ donation is necessary, as well as critical debate on the slippery slope of this practice and related ethical concerns. Euthanasia combined with organ donation should be included as an important part of the general debate on euthanasia.

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