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PERCUTANEOUS BALLOON AORTIC VALVULOPLASTY IN CHILDHOOD

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We assessed the effectiveness of aortic balloon valvuloplasty (AoVP) in 34 children who were admitted for aortic valve balloon dilatation over 7 years (Feb 1997-Feb 2004) in two institutions (Stuttgart and Zagreb). There was a prevalence of male children (28/6; p<0.01), mean age at dilatation 35.55±55.59 months (mean ± SD, min 1 day, max 14.2 years) and mean body weight 13.1±15.9 kg (min 2640 g, max 57 kg). Patients were divided into two groups as follows: group 1 including neonates and small infants younger than 2 months at dilatation with criteria for critical aortic stenosis (n=18); and group 2 including infants older than 2 months at dilatation (n=16). Sixteen (47%) of all patients had no clinical symptoms, 12 (35.3%) were dyspneic at rest and sweating at feeding (NYHA III), and 6 (17.6%) had severe heart failure (NYHA IV). All NYHA IV patients were in group 1 (n=6) *versus* 0 in group 2 (p<0.05). According to ECHO estimation, left ventricular (LV) function was normal in 16 (47%), moderately limited in 12 (35.3%) and severely impaired in 6 (17.6%) patients. All patients with severely impaired LV function belonged to group 1 (n=6) *versus* 0 in group 2 (p<0.05). Balloon dilatation was performed retrogradely *via* the percutaneous femoral artery approach in all except one patient in which the balloon catheter was introduced anterogradely *via* the mitral valve (MV). Indexed aortic valve-annulus/body surface area (BSA) (mm/m^2) was 30.97±10.02) (max 47.5, min 12.02) for overall study sample, 37.60±5.99 in group 1 and 23.03±7.86 in group 2 (group 1 *vs.* group 2, p<0.05). Ao/Ba ratio (mm) was 0.85±0.09 for overall study sample, 0.81±0.11 for group 1 and 0.89±0.05 for group (group 2 *vs.* group 1, p<0.01). Immediately after dilatation, the mean systolic pressure gradient across the aortic valve decreased from 70.62±20.78 (max 120, min 45 mm Hg) to 20.03±13.7 (max 65, min 0 mm Hg) in the whole study group (p<0.05), from 73.23±21.57 (max 120, min. 50 mm Hg) to 15.25±11.09 (max 40, min 0 mm Hg) in group 1 (p<0.05), and from 67.78±20.21 (max 111, min 45 mm Hg) to 24.81±14.71 (max 65, min 10 mm Hg) in group 2 (p<0.05) (catheter measurement). Follow-up results were studied in 31 (91%) patients at 3.5-84 months (20.91±22.19) after AoVP and revealed continuously increasing residual aortic valve gradient (31.35±12.01, max 50, min 15 mm Hg), still being significantly lower (p<0.001) than before valvuloplasty. The overall actuarial survival rate after 7 years was 91%. Freedom of three categories (any reintervention, surgical reintervention, and re-dilatation) was 77, 74, 61; 87, 84, 77; and 90, 90, 83 at 2, 4 and 7 years for the total number of patients, respectively. The actuarial freedom for the same categories in group 1 *vs.* group 2 was 72, 67, 56 *vs.* 87, 87, 75 (p<0.05); 89, 83, 78 *vs.* 87, 87, 75 (NS); 83, 83, 78 *vs.* 100, 100, 94 (p<0.05) at 2, 4 and 7 years. The degree of aortic regurgitation immediately after catheterization did not significantly increase; only 1 patient developed moderate aortic regurgitation, which was treated with surgical valve reconstruction on day 1 after intervention. At follow-up, aortic regurgitation increased to grade 3 in 3 (10%) and to grade 2 in 7 (23.3%) patients. All three patients with high grade of aortic insufficiency were from group 1 *vs.* 0 in group 2 (p<0.05). Eight (26%) patients required reintervention, 4 (13%) balloon valvuloplasty plus surgery, and 4 surgery only. Of 8 patients requiring surgery, 4 (13%) were operated on during a period of 27-78 months and 4 within one month after dilatation. One patient died one week after dilatation, re-dilatation and surgery due to fibroelastosis (confirmation by histology). *Conclusion:* Percutaneous balloon valvotomy provides an effective palliative interventional method in the treatment of infants and children with aortic valve stenosis. The majority of problems in the early and late period after dilatation appear in the group of patients with critical aortic stenosis.

Key words: aortic stenosis, interventional catheterization, balloon aortic valvuloplasty, neonates, infants, children, outcome, immediate and mid-term follow-up

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INTRODUCTION

Since the first description in 1983 (1), aortic balloon valvuloplasty has been accepted as a form of first-choice treatment of aortic stenosis in children. The effectiveness of the method in gradient reduction was published for children with congenital aortic stenosis as short-term (2,3), mid-term (3,4) and long-term results (5,6). Dilatation of critical aortic stenosis was performed for the first time in 1986 (7) and since has been established as a palliative method of choice for newborns and young infants (8,9) despite controversial discussion in the literature about surgery and percutaneous dilatation (10). Patients with critical aortic stenosis that require balloon dilatation very early have a high rate of early re-intervention and have a poorer outcome than those requiring the procedure later (8,11,12). Our study aimed to evaluate the mid-term results of percutaneous balloon valvuloplasty in 34 children during a 7-year period, including the subgroup of children with critical aortic stenosis.

PATIENTS AND METHODS

Patients and criteria

This retrospective study reviewed 34 patients who admitted for valve balloon dilatation during a 7-year period (from February to February 2004) at two institutions (Stuttgart and Zagreb). Indication for the procedure was based on clinical symptoms, patient presenting with syncope and left ventricular (LV) strain on electrocardiography (ECG) independent of transvalvular gradient. Early balloon dilatation was indicated in a subgroup of neonates and small infants younger than 2 months (group 1). They had clinical criteria (presence of low cardiac output, cardiogenic shock, heart failure, need of inotropic support or mechanical ventilation, prostaglandin dependency) and echocardiographic criteria (morphological evidence of LV hypertrophy, depression of LV function, see the criteria below*)* for critical aortic stenosis, irrespective of transvalvular gradient. This group included age-matched patients with (and/or) a Doppler gradient >70 mm Hg and those with preserved LV function. Group 2 included all patients older than 2 months at dilatation.

Cardiovascular condition was assessed by *the New York Heart Association (NYHA)* criteria, i.e., NYHA III (defined as dyspnea at rest and sweat at feeding) and NYHA IV (as presence of low cardiac output, severe cardiac failure) for neonates. Ventricular function before dilatation was estimated by *echocardiogram (echo*); fractional shortening (FS) by more than 30% was estimated as normal (despite LV hypertrophy), FS 20%-29% as moderately limited, and FS <20% as severely restricted. Annulus diameter was measured on echocardiography and angiocardiography with a smaller size chosen as reference. Balloon diameter/aortic valve size were indexed to body surface area (Figure 1, Table 1).

Valvoplasty

All procedures were performed in analgosedation or general anesthesia *via* the percutaneous femoral artery approach except for one patient in whom the balloon

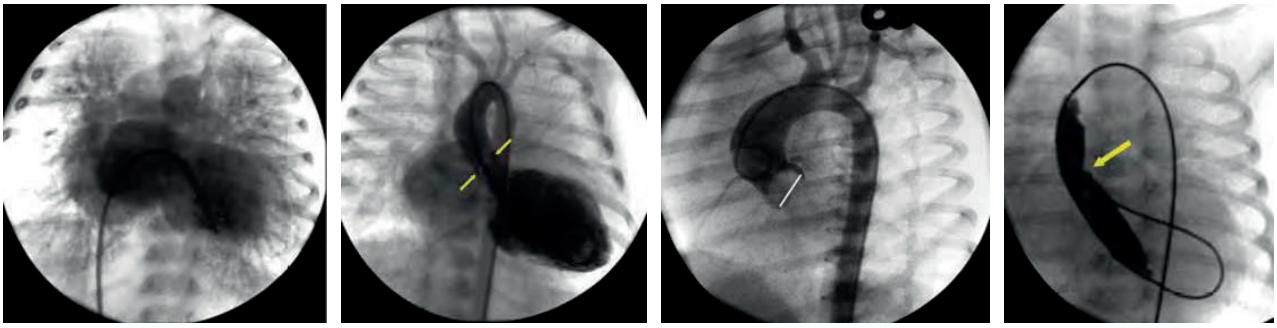

Figure 1. *Critical aortic stenosis*

A*. Angiographic presentation (pre-dilatation); congestive heart failure, pulmonary congestion, enlargement of LA and LV, massive mitral insufficiency, need for inotropic support, pulmonary arterial hypertension, PGE1 dependency* ***B****. Immediately after dilatation; mild mitral insufficiency, LA enlargement, LV hypertrophy (and enlargement), enlargement of ascending aorta (bicuspid aortic valve).* ***C.*** *Difficulties with introduce of balloon catheter into the LV; special guidewires, special catheters, using „washout" phenomen, retrograde femoral artery approach, carotid artery approach or anterograde (via PFO-LA-MV-LV-AO). Importance of aortic ring measurement and balloon selection and calculation of balloon/aortic rig ratio (0,81-0.89).* ***D.*** *Balloon inflation technique; pressure 4-6 bar, quickly (< 5 sec), cum Adenosine use, general anesthesia and analgosedation, ductus dependency (PGE_1 use) – incisura on the level of aortic valve during inflation of balloon (yellow arrow).*

Table 1. *Clinical and echocardiographic features in the two patient groups*

Variable (±SD)	Overall	Group 1 <2 months	Group 2 ³2 months	p value
Median age at dilatation (months)	35.55 (55.59)	0.64 months (19 days)	74.78 (60.39)	
Median weight (kg)	13.1 (15.9)	3 666 g (4.280)	24.44 (18.09)	
Median BSA (m2)	0.51 (0.44)	0.25 (0.02)	0.85 (0.46)	
No clinical symptoms	16	7	9	NS
NYHA III	12	6	6	NS
NYHY IV	6	6	0	P<0.01
Normal LV function	16	7	9	NS
Moderately limited LV function	12	6	7	NS
Severely limited LV function	6	6	0	P<0.01
Serious procedure complications	7	5	2	NS
Early death	1	1	0	NS
Severe AO insufficiency on follow-up (Gr ≥ 3)	3	3	0	P<0.01
Severe limitation of LV on follow-up	10	6	4	?

Table 2. *Immediate and follow-up mid-term results of dilatation*

Variable: mean ± SD	Overall (N=34)	Group 1 (N=18)	Group 2 (N=16)	p value
Gradient before dil. (mm Hg)	70.62 (20.78)	73.23 (21.57)	67.8 (20.21)	NS (t=0.74)
Gradient after dil. (mm Hg)	20.03 (13.7)	15.25 (11.09)	24.81 (14.71)	p<0.05 (t=2.7)
Ao/Ba Ratio	0.85 (0.09)	0.81 (0.11)	0.89 (0.05)	p<0.01 (t=2.96)
Follow-up (months)	20.91 (22.19)	19.98 (21.89)	16.50 (16.79)	NS
Gradient follow-up	31.35 (12.01)	29.4 (10.75)	33.92 (13.49)	NS (t=1.02)
Fluoroscopy time (min)	23.54 (16.31)	29.19 (19.42)	18.94 (12.02)	NS (t=1.74)
Indexed ao. annulus (mm/m^2)	30.97 (10.02)	37.60 (5.99)	23.03 (7.86)	p<0.01 (t=6.04)

catheter was introduced antegradely *via* mitral valve. The chosen balloon diameter was preferably slightly smaller. We did not use any balloon larger than aortic valve annulus (ratio balloon/annulus less than 1.0). The inflation pressure was 4-6 bar. The resulting balloon to annulus diameter ratio ranged from 0.60 to 1.00 (mean 0.85±0.09) in all study patients. The aortiv annulus/body surface area (BSA) index ranged from 12.02 to 47.5 (mean 30.97±10.02). Fluoroscopy time was 23.54±16.31, range 6.7-86.6 minutes. All important differences between the two patient groups were statistically tested (Tables 1 and 2).

Echocardiography

Aortic valve gradient and development of aortic insufficiency on follow-up was estimated by continuous and pulsed Doppler from the subcostal, jugular and supraclavicular approach. The highest gradient measured was accepted. Aortic insufficiency was assessed by color flow mapping and pulsed Doppler and scored on a four-grade scale according to Moore *et al.* (4).

Follow-up

The follow-up period ranged from 3 to 84 (median 21±22) months in survivors. One patient who died was not included and two patients were lost for follow-up. All other patients had complete clinical and echocardiographic evaluation in our outpatient clinic at different intervals after intervention or operation. Follow-up data were available for 91% of patients.

Statistical analysis

Data were tested for normal distribution and displayed in ranges and as mean ± standard deviation (SD). Groups were compared by unpaired t-test. Difference in the proportion were tested by χ^2-test. Difference between the two groups was considered significant at p<0.05. The Kaplan-Maier estimate was used to calculate actuarial probabilities, followed by a log rank test.

RESULTS

From February 1997 to February 2004 (7 years), 34 patients underwent balloon valvuloplasty. The prevalence of male patients was significant (25/9, 73.5%; p<0.05). The patients were divided into two groups according to the criteria for dilatation. Group 1 consisted of 18 (53%) patients with the criteria for critical aortic stenosis, whereas group 2 of 16 (47%) patients had indications for balloon dilatation following the criteria described above. Clinical and echocardiographic data before dilatation are summarized in Table 1. The age at dilatation was 35.55±55.59 months (mean ± SD, min 1 day, max 14.2 years), body weight (BW) 13.1±15.9 kg (min 2 640 g, max 57 kg), BSA 0.51±0.44 (min 0.18, max 1.58 m^2).

Sixteen (47%) patients did not have obvious clinical symptoms before dilatation (group 1:group 2=7:9), 12 (35%) were in NYHA III (group 1:group 2=6:7), and 6 (18%) patients in NYHA IV, all in group 1 (p<0.01). Following echocardiographic and Doppler assessment, 11 (32%) patients had aortic regurgitation grade I before dilatation without difference between the two groups (5/6, p>0.05). LV function as estimated by echocardiography was normal in 16 patients (group 1:group 2=7:9), 12 had moderately limited function (group 1:group 2=6:7), while all 6 patients with severe limitation of LV belonged to group 1 (p<0.01). We think that severe limitation of LV function causes a very high level of clinical symptoms. We conclude from this analysis that our selection criteria for critical aortic stenosis were correct.

On patient died in group 1 one week after dilatation, re-dilatation and surgery due to fibroelastosis (confirmed histologically). There were no late deaths on follow-up. Survival rate for all patients was 97%. The degree of aortic regurgitation immediately after catheterization did not significantly increase; only 1 patient developed moderate aortic regurgitation which was treated with surgical valve reconstruction on the day of intervention. Two patients developed severe aortic regurgitation on follow-up and both underwent Ross operation at 27/78 months after initial dilatation. These three patients belonged to group 1 (p<0.01). A lower degree of aortic regurgitation was observed in all (group 1/group 2) patients, as follows: competent valve in 5 (4/1), aortic regurgitation grade I in 15 (7/8) and grade II in 7 (3/4) patients. Using the criteria described above, we estimated LV function by ECHO for all (group 1/group 2) patients, as follows: normal in 17 (10/7), moderately limited in 7 (3/4), and severely limited in 6 (6/4) patients. We did not find any statistically significant difference between the two groups with respect to LV function on follow-up. Complications during and immediately after dilatation procedure were seen in 5 patients, all in group 1. These complications were serious and included: 1) left ventricular perforation by exchange guidewire without development of tamponade; 2) severe cardiac impairment during the procedure, which required inotropic support, re-dilatation, urgent Kaye-Damus-Stansel (K-D-S) operation and central AO-PA shunt; this patient died a week later (fibroelastosis, histologically confirmed); 3) supraventricular tachycardia that required medical treatment; 4) insufficiency of aortic valve grade IV caused by dilatation, surgical reconstruction of aortic valve on the same day; and 5) insufficiency of aortic valve grade II, early Ross procedure because of increasing insufficiency over the next months. In group 2, complications were recorded in two patients, including: 1) arrhythmias, early surgical commissurotomy and later aortic stenosis; and 2) serious arrhythmias that required urgent drug treatment.

Balloon dilatation was performed retrogradely *via* the percutaneous femoral artery approach in all except for one patient with critical aortic stenosis (gradient 70 mm Hg, BW 3,400 g, BSA 0.22 m^2). In this patient, balloon catheter was introduced anterogradely *via* mitral valve. There were no complications except for arrhythmias during intervention. In three newborns, we had transitional pulse loss, no surgical interventions and no permanent pulse loss. Immediate results of balloon dilatation, follow-up results and some other parameters are summarized in Table 2. Immediately after dilatation, invasive mean systolic pressure gradient across the aortic valve decreased from 70.62±20.78 (max 120, min 45 mm Hg) to 20.03±13.7 (max 65, min 0 mm Hg) in the overall group (p<0.05); from 73.23±21.57 (max 120, min 50 mm Hg) to 15.25±11.09 (max 40, min 0 mm Hg in group 1 (p<0.05); and from 67.78±20.21 (max 111, min 45 mm Hg) to 24.81±14.71 (max 65, min 10 mm Hg) in group 2 (p<0.05). Follow-up term results were studied in 31 (91%) survivor patients at 3.5-84 months before valvuloplasty. Although there was no difference in aortic valve gradient after dilatation between group 1 and group 2, success in gradient reduction was favorable in group 1 as compared with group 2 (p<0.05). There were no significant differences between the two groups in follow-up time, increasing gradient on follow-up, and fluoroscopy time (Figures 1 and 2).

We found statistically significant differences in the ratio of aortic annulus/balloon

(group 1 *vs*. group 2=0.81±0.11 *vs*. 0.89±0.05; p<0.01) and indexed ao-annulus to BSA (group 1 *vs*. group 2=37.60±5.99 *vs*. 23.03±7.86; p<0.01), but this relationship was extra studied

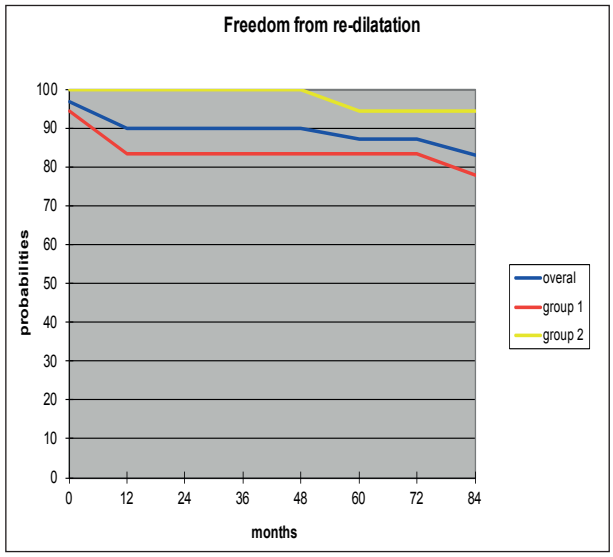

Figure 2. *Graphically presentation of immediate results of aortic valvule dilatation in two different groups. (G1 and G2).*

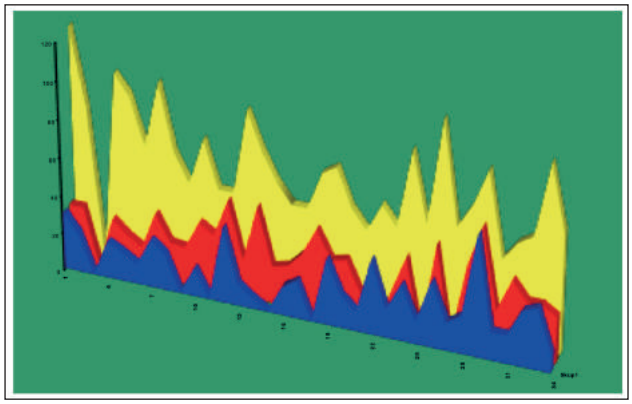

Figure 3. *Graphic presentation of valvular gradient before dilatation (yellow), immediately after dilatation(blue) and intermediate follow-up (red) for all of patients.*

Actuarial freedom analysis

The overall actuarial survival rate after 7 years was 96.6%. Three freedom categories (any re-intervention, surgical re-intervention and re-dilatation) are summarized in Table 3.

Thirty-one of 34 patients could be followed-up, 23 (75%) needed any reintervention, and 8 (26%) needed different re-interventions (4 re-dilatation and surgery, 4 surgery only). Five of them belonged to group 1 and three to group 2 (Table 2). Three of the five patients from group 1 had re-dilatation at 1, 5 and 6 months after first dilatation. All of them were finally treated surgically, i.e., one by Ross procedure 27 months after first dilatation (because of progressive development of aortic insufficiency), one by K-D-S operation with central shunt and atrial septectomy (this patient died from fibroelastosis in the second week of life), and one by commissurotomy 5 months after dilatation and by Ross procedure 78 months after dilatation (because of the progressive development of aortic stenosis and insufficiency). Two of the five patients from group 1 were treated surgically only, i.e., one by reconstruction of the aortic valve because of insufficiency grade IV after dilatation (on the same day), and one by Ross procedure 60 months after dilatation because of the development of aortic stenosis and insufficiency. One of the three patients from group 2 with re-intervention had unsuccessful re-dilatation 52 months after first dilatation and Ross procedure five months later (because of progressive aortic stenosis), one had commissurotomy 36 days after dilatation, and one had commissurotomy 1 month after dilatation and aortic homograft implantation three weeks later (Table 4).

Actuarial freedom from reintervention at two, four and seven years was 77%, 74% and 71% for overall pa-

Table 3. *Actuarial freedom from any re-intervention, re-dilatation and surgery intervention for overall, Gr1 and Gr2 at 2, 4, and 7 years.*

Reintervention	Group	2y	4y	7y	p-value
No	Overall	77	74	61	
	1	72	67	56	
	2	87	87	75	P<0.01
Re-dilatation	Overall	90	90	83	
	1	83	83	78	
	2	100	100	94	P<0.01
Surgery	overall	87	84	77	
	1	89	83	78	NS
	2	87	87	75	

Table 4. *Reintervention in 8 patients (26%)*

4 surgery (surg.) only
1. G1. AoI – gr. IV (caused by dilatation)-reconstruction on the same day
2. G1. AoS (50 mm Hg) + gr. AoI-III - Ross 60 mo. later
3. G2. surgery commissurotomy 4 months later
4. G2. surgery commissurotomy I mo. later, and Ao homograft 2 mo. later
4 redilatation (re-d.) and surgery (surg.)
1. G1. Re-d. 6 months later, Ross 27 mo. later (AOI gr III, Schone)
2. G1. Re-d. 5 days later, K-D-S 8 days later - died (fibroelastosis)
3. G1. Re-d. 3 days later, surg. commissurotomy 5 mo. later, Ross 6 ys. later (AoI, AoS)
4. G2. Re-d. after 52 months, Ross after 58 months (AoS, AoI)

AoI – aortic insufficiency, AoS – aortic stenosis

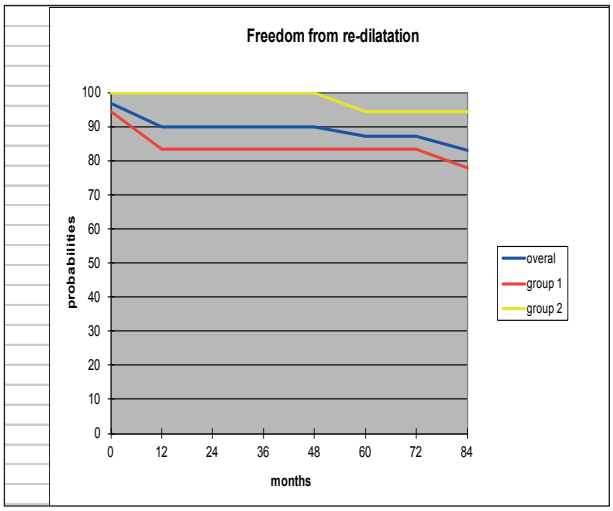

Figure 4. *Kaplan-Meier freedom curves for any reintervention for overall (77.4%, 74.2%, 61.3%), Gr.1 (72,3%, 67.7%, 55,6%) and Gr.2 (87.5%, 87.5%, 75%) for 2, 4 and 7 years*

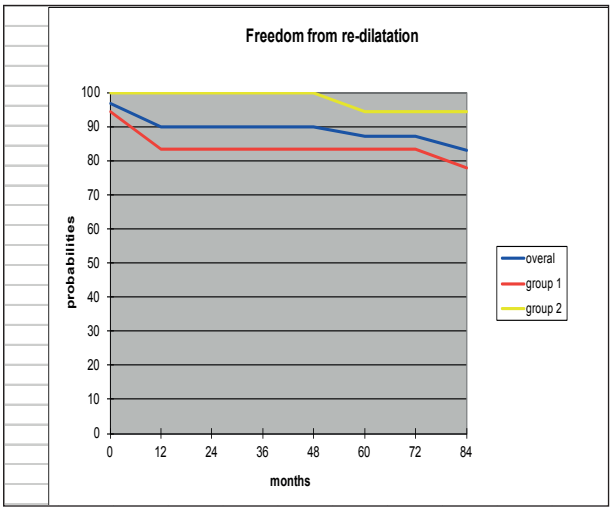

Figure 5. *Kaplan-Meier freedom curves from re-dilatation for overall (90%, 90%, 83%), Gr. 1 (83.3%, 83,3%, 77,8%) and Gr.2 (100%, 100%, 94,5%) for 2, 4 ad 7 years*

tient population, 72%, 67% and 56% for group 1, and 87%, 87% and 75% for group 2. Following Kaplan-Meier equation, there were obvious differences for re-intervention between group 1 and group 2. Group 1 patients needed re-intervention more often than group 2 patients ($p<0.01$) (Figure 4).

Actuarial freedom from re-dilatation at two, four and seven years was 90%, 90% and 83% for overall patient population, 83%, 83% and 78% for group 1, and 100%, 100% and 94% for group 2. Group 1 patients needed re-dilatation more often than group 2 patients ($p<0.01$) (Figure 5).

According to Kaplan-Meier equation, there was no difference in surgical reintervention between the two groups in time and number of surgical reoperations. Actuarial freedom from surgery at two, four and seven years was 87%, 87% and 77% for overall patient population, 89%, 83% and 78% for group 1, and 87%, 87% and 75% for group 2 ($p>0.05$ (Figure 6).

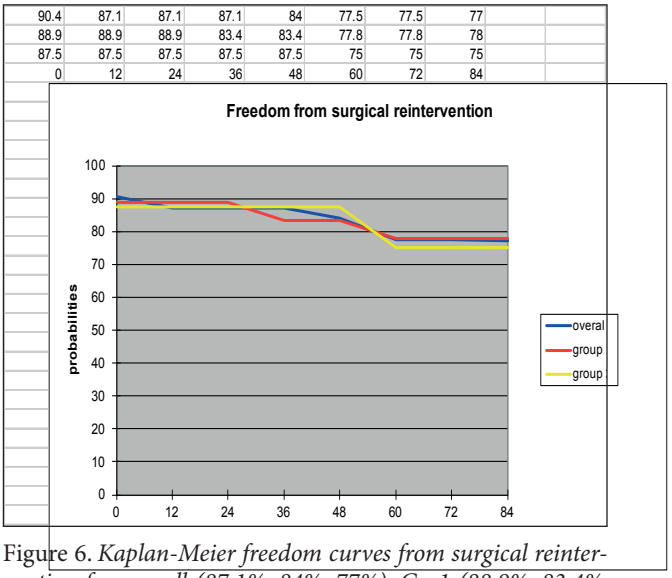

Figure 6. *Kaplan-Meier freedom curves from surgical reintervention for overall (87.1%, 84%, 77%), Gr. 1 (88.9%, 83.4%, 78%) and Gr.2 (87%, 87%, 75%) for 2, 4 ad 7 years*

DISCUSSION

Congenital aortic stenosis occurs at an incidence of 5%-6%, more common in male than in female children (13). The pathophysiological background is most commonly found in the bicuspid aortic valve (BAV), although there is a monocuspid but also an amorphous aortic valve. It is a progressive disease, especially in bicuspid aortic valve, which in adulthood is also called BAV syndrome due to usually progressive accompanying changes in the entire left ventricular outflow tract (mitral and aortic valve, ascending aorta, aortic arch, aortic isthmus). AoVP has become the method of choice in the treatment of aortic stenosis from neonatal period to adulthood, and has recently entered fetal age. Since the first dilatation of aortic stenosis in 1983 (1) and first dilatation of critical aortic stenosis in 1986 (7), both in neonatal and later age, pediatric cardiology has been of increasing interest (2-5). It has been established, in fact, as a palliative method for the treatment of aortic stenosis with the aim of postponing cardiac surgery until later childhood or already adult age. The success reports of the method refer to early (immediate) results (2-4), intermediate-term follow-up results (3,4), and then to the results of long-term follow-up

monitoring of AoVP (5, 6). Although there is still debate about justification, especially in some conditions (9), the method has nevertheless proved to be very successful, both in critical and non-critical aortic stenosis (8, 9), not only according to the degree of success but also for a relatively small number of complications (8, 11,12). The balloon dilatation technique in pediatric cardiology emerged as early as 1950 with Rubio and Limon who dilated tricuspid and pulmonary valves with a modified catheter (14). A balloon catheter with a double lumen was constructed by Gruntzig *et al.* (15). It took almost 30 years from the first heart balloon intervention to AoVP in children (1,11, 14-18). We are using the current criteria for the diagnosis of aortic stenosis, critical aortic stenosis, heart failure, ventricular function, and cardiovascular status according to the NYHA criteria listed in the Materials and Methods section (4,12,19-21).

In our study, a statistically significant difference between the two groups was clearly evident in the clinical symptoms of severe heart failure (NYHA IV), echocardiographic assessment of severe LV insufficiency, and the development of severe aortic valve insufficiency after AoVP, all to the detriment of group 1 *versus* group 2 ($p<0.01$). All procedures in our study were performed *via* the percutaneous femoral artery approach except for one patient where balloon catheter was introduced antegradely *via* mitral valve. Retrograde femoral arterial approach is most common (12, 22-25), and other approaches are rarely described and are used when the usual approach is not available for some reason, primarily in neonatal period, *via* carotid artery (26), transumbilical (27) and anterograde *via* mitral valve (28, 29). We measured immediate effects of dilatation and further development of the disease over a 7 years (mediate-term follow up at 2, 4 and 7 years). Direct pressure drop in the whole group and subgroups is shown in Table 2 and Figures 2 and 3. Gradients were measured by direct passage of the catheter through the valve. We found more severe aortic valve insufficiency in three (16.7%) patients from group 1 or 8.8% of all patients, without severe aortic insufficiency in group 2 ($p<0.01$). Data on a steeper gradient drop and higher number of aortic insufficiency in group 1 as compared to group 2 may be important in discussing improving indications for AoVP. Immediate result and mid-term follow-up are possible, as reported in different publications (2, 3, 25). The first series reported by Lababidi in 1984 shows the peak-to-peak systolic gradient across the aortic valve to have decreased form 113±48 mm Hg to 32±15 mm Hg ($p<0.001$) after AoVP. Very mild aortic insufficiency was noted in 10 (43%) patients after balloon dilatation and two patients required surgery because of high-grade aortic insufficiency. Acute results following balloon AoVP reported during a decade (1983-1992) following their description have been tabulated elsewhere (25). Similar results were reported in our study for the whole group (70.62±20.78, max 120, min 45 at 20; 0.03±13.7, max 65, min 0 mm Hg; $p<0.05$) and similar for group 1 and group 2. Rao showed early AoPV results in 10 centers in which newborns were mostly excluded, in others almost entirely similar to our group 2 with a residual gradient immediately after dilatation of 22.46 mm Hg, and in our group 2 the mean gradient was 24.81 mm Hg. In our study, Table 2 shows a statistical difference in the post-dilatation gradient between group 1 and group 2, which was due to a steeper pressure drop in LV in group 1 (group 1 *vs.* group 2, $p<0.05$). However, there was no statistically significant difference between the two groups in the gradient rising at mid-term follow up (2, 4, and 7 years). A steep gradient drop in group 1 was a possible reason for the development of post-dilatation aortic insufficiency. According to the presented results, a gradual increase of the gradient (up to a maximum of 50 mm Hg) is visible, and severe aortic insufficiency developed in three patients (see graphic presentation of events in Figure 3). In the literature, we found exact descriptions of intermediate-term follow-up by Galal (12) and in the aforementioned article by Rao, with a review of a large number of papers (25). With careful examination of the results, restenosis defined as a peak-to-peak gradient of ≥50 mm Hg was found in 23% of children, some requiring surgical valvotomy or repeat balloon valvuloplasty. The degree of aortic insufficiency remained stable during intermediate-term follow-up. There are the similar results in other publications (12, 25, 29-32).

The Kaplan-Maier estimate was used to calculate actuarial probabilities, followed by a log-rank test. All results of the actuarial freedom from any re-intervention, re-dilatation and surgery for overall patient population, group 1 and group 2 at 2, 4, and 7 years in our study are shown in Table 3 and Figures 5 and 6. Figure 1 shows that more than 60% of children after 7 years were exempted from additional interventions on the aortic valve. Group 1 children were least spared from re-interventions (about 56%), and group 2 children were spared significantly better (about 75%) ($p<0.05$). Figure 5 shows that after 7 years, about 83% of all children were spared from re-dilatation. Group 1 children were spared from re-dilatation in about 78%, and group 2 children in as much as 95% of cases ($p<0.05$). According to the presentation in Figure 6, there was no difference in the need of surgical intervention between the two patient groups in all periods of mid-term follow-up (2, 4 and 7 years). After 7 years, between 75% and 78% of children did not need surgical reintervention (NS). In Croatia, the method was introduced in 1999, and the first publication was published in 2005 (33).

The question of predictors of two important problems arises, namely, progressive post-dilatation aortic valve stenosis in long-term follow-up and immediate or rapidly progressive post-dilatation aortic insufficiency, especially in group 1. Predictors of post-dilatation progressive stenosis were studied by Galal *et al.* and Rao *et al.* (12, 31). Based on multivariate logistic regression analysis of the two groups of patients (pre-dilatation gradient >50 mm Hg and <50 mm Hg), they concluded that the main predictor of progressive post-dilatation stenosis was the residual peak-to peak gradient >30 mm Hg after first dilatation. Sholler *et al.* studied the possible influence of the dilatation technique itself on the occurrence of post-dilatation stenosis, but found no additional predictor significance in the derivation technique (34). Subsequent studies, including their own, particularly bicuspid aortic valve studies, have shown that an important factor in restenosis is conditioned by the morphology of the aortic valve itself (35).

Aortic insufficiency appears to be the most severe complication of AoVP, which is not the case in primary cardiac surgical commissurotomy (12). Among our patients, severe aortic insufficiency was developed by three patients from group 1 (16.6%). The first had AO insufficiency after re-dilation, 5 days after initial dilatation. Due to LV dysfunction, K-D-S procedure was also performed 8 days later, but the patient died and histologically fibroelastosis of the left ventricle was later demonstrated. It is not impossible that the patient was prejudiced for dilatation of the aortic valve because he had the criteria for 'borderline' hypoplastic left heart syndrome (HLHS). Two children in group 1 had re-dilatation, but one developed progressive grade III aortic insufficiency and was operated on according to Ross at 27 weeks of age. He also had the criteria for partial Shone syndrome. The third patient also underwent re-dilatation after initial dilatation, but was operated on according to Ross due to persistent insufficiency with stenosis. Galal refers to as many as seven out of 26 (28%) patients, of whom three (8%) received Ross surgery due to grade III aortic valve insufficiency. Other studies have also shown patients with progressive grade I aortic insufficiency, but the true cause has not yet been determined. The possible reasons include too high difference in post-dilatation gradient (35), mono-commissure valve with post-dilatation prolapse (36), poor or amorphous morphology of bicuspid aortic valve (12), too high ratio of balloon/aortic annulus (36, 35) excessive gradient reduction, and possible unrealistic selection of some patients for balloon dilatation (37, 38). The investigators believe that the relatively high degree of aortic insufficiency immediately after initial dilatation becomes a predictor for later development of severe aortic insufficiency. It appears, however, that children with monocuspid or markedly poor aortic valve morphology should be suggested primarily for cardiac surgery correction and not primarily for balloon dilatation. In some patients, dilatation of the aortic valve is forced in order to get rid of the left ventricle, and according to the strictest criteria, the child belongs to the HLHS, so the situation should be considered prudently.

Numerous complications have been described in the literature, such as transient bradycardia with premature extrasystole during balloon inflation, and therefore inflation in more than 5 seconds is not recommended. Blood loss with the need of transfusion, femoral thrombosis due to which anticoagulation therapy (heparin, streptokinase, thrombectomy), perforation of the myocardium and mitral valve damage, coronary artery occlusion, cerebrovascular incidents, 2 transient ischemia and 35 transient myocardial infarction are also mentioned (2, 24, 25, 35, 40). Complications in our group 1 appeared individually in 6 patients and included ventricular perforation without tamponade, severe cardiac impairment during catheterization (the patient died later), severe arrhythmias during the procedure and successful drug treatment, aortic insufficiency grade IV requiring immediate surgery, and progressive insufficiency, early Ross. In group 2, we had only two complications, in one patient arrhythmias with early post-dilatation surgery (commissurotomy and drug treatment of arrhythmia), and in the other serious arrhythmia that required drug treatment.

Although we did not show long-term follow-up for more than 7 years, we included in discussion some important research results that speak of dilatation of the aortic valve from the experience of studies that lasted a long time. Fratz *et al.* in single center long-term follow-up study (Deutsches Herzzentrum München) of AoVP reviewed 17.5 years of follow-up data on all 188 patients, dividing them into those aged <1 month and >1 months. The main attention was to find out whether AoVP efficacy at long term could be a good guideline to prevent and postpone aortic valve surgery in different groups of patients. The study showed that long-term results of AoVP of congenital aortic stenosis in pediatric patients and their efficacy in preventing or postponing aortic valve surgery was a therapeutic procedure of choice for the treatment of congenital aortic valve stenosis in pediatric patients (40). Similar results have been published from mid-term and long-term follow-up studies (2, 4, 8, 12, 22, 36). Fratz *et al.* conclude that the results of a long-term follow-up study of AoPV congenital aortic valve stenosis in pediatric patients are very good and effective in postponing aortic valve surgery. About one-third of these patients are exempted from the need of surgical approach for ten years. The results of our studies are similar. In a multicenter retrospective survey of more than 1000 patients, which included patients from Olgahos-

pital (Stuttgart), as did out study, Ewert *et al.* (41) show a high survival rate. The results of mid-term follow-up and long-term follow-up are similar to the results of other long-term follow-up studies (40, 42, 43, 44). According to these studies, special attention should be paid to the criteria set in early neonatal age, which relate to neonates with critical aortic stenosis selected for biventricular repair (44). In their long-term follow-up study conducted over a ten-year period, McCrindle *et al.* found patient survival to be 72.9% (44). In neonates, the prognosis appears more severe, clearly related to 'borderline' LV and the challenge of recognizing which is suitable or not for a biventricular management strategy (45). Recently, in some patients with severe aortic stenosis in fetal age, which may be borderline to the HLHS, intrauterine dilatation of the aortic valve begins. Some of these patients have a further successful course in postpartum palliative non-surgical methods (additional dilatation), similar to the findings from the period of immediate-term results. It seems that one should be very careful not only in the criteria for intrauterine dilatation but also in the expectations for further treatment on two ventricles. Early complications also appear to occur with dilatation as a consequence of an excessive desire to conceive to avoid early cardiac surgery. Regardless of the topic, the method of balloon dilatation of the aortic valve has a firm place in the treatment of children with aortic stenosis and postponing of cardiac surgery (46, 47).

CONCLUSION

Percutaneous balloon valvotomy provides an effective palliative interventional method in the treatment of infants and children with aortic valve stenosis. The majority of problems in the early and late period after dilatation appear in the group of patients with critical aortic stenosis. Ealy and late onset and development of high-grade aortic insufficiency after aortic valve dilatation should probably be reduced by avoiding to attain the greatest difference possible, but moderately reducing the gradient. The development of aortic insufficiency is not caused by the corresponding choice of the balloon size, but probably by immaturity of the aortic valve tissue in critical aortic stenosis. We hypothesize, based on our own study and literature review, that the number of early complications would be reduced if a proportion of patients with monocuspid aortic valve and amorphous aortic valve anatomy were primarily referred for cardiac surgery. We are also of the opinion that in most patients with 'borderline' HLHS, the decision to correct two ventricles should not be overestimated, but rather that some children should be referred for the first palliative HLHS operation.

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S A Ž E T A K

PERKUTANA AORTNA VALVULOPLASTIKA BALONOM U DJECE

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Procijenili smo učinkovitost aortne valvuloplastike (AoVP) kod 34 djece koja su podvrgnuta dilataciji aortne valvule balonom tijekom 7 godina (od veljače 1997. do veljače 2014.) u dvije ustanove (Stuttgart i Zagreb). Prevladavala su muška djeca (28/6; p<0,01), srednja dob (±SD) kod dilatacije bila je 35,55±55,59 mjeseci (minimum 1 dan, maksimum 14,2 godine), a tjelesna težina 13,1±15,9 kg (minimum 2640 g, maksimum 57 kg). Pacijenti su podijeljeni u dvije skupine. Skupinu 1 činila su novorođenčad i dojenčad mlađa od 2 mjeseca kod prve dilatacije s kriterijima za kritičnu aortnu stenozu (N=18), a skupinu 2 djeca starija od 2 mjeseca kod dilatacije (N=16). Šesnaest od 34 (47 %) pacijenata nije imalo kliničkih simptoma, 12 (35,3 %) je imalo dispneju pri mirovanju i pojačano se znojilo tijekom hranjenja (NYHA III), a 6 (17,6 %) pacijenata je imalo simptome teškog srčanog zatajenja (NYHA IV). Svi pacijenti s NYHA IV bili u skupini 1 (n=6) prema 0 u skupini 2 (p<0,05). Prema ehokardiografskom nalazu funkcija lijevog ventrikla (LV) bila je normalna u 16 (47 %), umjereno oslabljena u 12 (35,3 %) i teško oslabljena u 6 (17,6 %) pacijenata. Svi pacijenti s teško oštećenom funkcijom LV pripadali su skupini 1. (n=6) prema 0 u skupini 2. (p<0,05]. AoVP balonom izvedena je retrogradno nakon perkutane punkcije femoralne arterije kod svih osim jednog pacijenta kod kojega je balonski kateter uveden anterogradno putem mitralne valvule (MV). Kvocijent anulusa aortnog zalistka/u odnosu na površinu tijela (BSA) (mm/m^2) bio je 30,97±10,02 (maksimum 47,5, minimum 12,02) i 37,60±5,99 u odnosu na 23,03±7,86 u skupini 1 prema skupini 2 (p<0,05). Omjer Ao/Ba (mm/mm) za cijelu skupinu bio je 0,85±0,09 te 0,81±0,11) u skupini 1 i 0,89±0,05) u skupini 2 (skupina 1 prema skupini 2, p<0,01). Odmah nakon dilatacije srednja sistolička vrijednost gradijenta tlaka na aortnoj valvuli smanjuje se sa 70,62±20,78 (maksimum 120, minimum 45 mm Hg) na 20,03±13,7 (maksimum 65, minimum 0 mm Hg.) u cijeloj skupini (p<0,05) te sa 73,23±21,57 (maksimum 120, minimum 50 mm Hg) na 15,25±11,09 (maksimum 40, minimum 0 mm Hg) u skupini 1 (p<0,05) i sa 67,78±20,21 (maksimum 111, minimum 45 mm Hg) na 24,81±14,71 (maksimum 65, minimum 10 mm Hg) u skupini 2 (skupina 1 prema skupini 2, p<0,05) (mjerenje kateterom). Rezultati termina praćenja proučavani su kod 31 (91 %) pacijenta 3,5-84 (20,91±22,19) mjeseca. Nakon AoVP nađen je kontinuirano rastući rezidualni gradijent aortne valvule (31,35±12,01; maksimum 50, minimum 15 mm Hg), ali je i dalje bio značajno niži (p<0,001) nego prije valvuloplastike. Ukupna stopa preživljavanja nakon 7 godina iznosila je 91 %. Oslobađanje od tri moguće skupine re-intervencija (bilo koja re-intervencija, kirurška re-intervencija; ponovna dilatacija) bila je 77, 74, 61; 87, 84, 77; 90, 90, 83 u 2, 4 i 7 godina za cijelu skupinu te u skupini 1 prema skupini 72, 67, 56 prema 87, 87, 75 (p<0,05); 89, 83, 78 prema 87, 87, 75 (NS); 83, 83, 78 prema 100, 100, 94 (p<0,05) u 2, 4 i 7 godina. Stupanj aortne insuficijencije neposredno nakon kateterizacije nije se značajno povećao; samo je 1 pacijent razvio aortnu insuficijenciju koja je liječena kirurškom rekonstrukcijom zalistaka na dan intervencije. Nakon praćenja aortna insuficijencija povećala se na stupanj III. u 3 (10 %) i na stupanj II. u 7 (23,3 %) pacijenata. Sve troje bolesnika s visokim stupnjem aortne insuficijencije pripadali su skupini 1 (prema 0 u skupini 2; p<0,05). Osam (26 %) pacijenata zahtijevalo je ponovnu intervenciju, a samo 4 (13 %) ponovnu AoVP plus operaciju. Od 8 pacijenata kojima je bila potrebna operacija 4 (13 %) su operirana tijekom razdoblja od 27 do 78 mjeseci, a 4 u roku od mjesec dana nakon dilatacije. Jedan pacijent umro je tjedan dana nakon dilatacije, ponovne dilatacije i kirurškog zahvata zbog fibroelastoze (potvrda histološka). *Zaključak:* Perkutana balonska valvuloplastika je učinkovita palijativna intervencijska metoda u liječenju novorođenčadi i djece sa stenozom aortne valvule. Većina problema u ranom i kasnom razdoblju nakon dilatacije pojavljuje se u skupini bolesnika s kritičnom aortnom stenozom u novorođenačkoj i ranoj dojenačkoj dobi.

Ključne riječi: aortna stenoza, interventna kateterizacija, balonska aortna valvuloplastika, novorođenčad, djeca, neposredan i srednjoročan ishod liječenja

CLINICAL AND PATHOLOGICAL PRESENTATIONS OF PATIENTS WITH HPV POSITIVE OROPHARYNGEAL CARCINOMA – A SOUTH CROATIAN STUDY

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Objective: The objective of this study was to analyze the influence of human papilloma virus (HPV) in patients with oropharyngeal squamous cell carcinoma (OPSCC) from southern Croatia on survival, clinical outcomes, and pathological features. *Methods:* We analyzed HPV DNA presence and p16 immunohistochemistry staining in 68 formalin-fixed paraffin-embedded samples from patients diagnosed with OPSCC at the Split University Hospital Center between 2013 and 2017. Histologic features were analyzed using a light microscope. Clinical data were retrospectively collected from patient records and analyzed for HPV status. *Results:* In this study, 10.29% of patients were HPV positive (HPV+). Lymphocyte invasion was more prominent in p16 positive OPSCC. Overall survival (OS) was better in HPV+ and p16+ patients. HPV status is a significant prognostic variable for patients from south Croatia with OPSCC. *Conclusion:* HPV seems to have a minor influence on OPSCC in south Croatia in comparison to other Western European countries and the USA. Although the influence of HPV on survival was significant, traditional risk factors were more important in the carcinogenesis of OPSCC in our population.

Key words: Croatia, head and neck neoplasms, histology, human papilloma virus 16, oropharyngeal neoplasms, squamous cell carcinoma

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INTRODUCTION

Head and neck carcinoma is the sixth leading cancer by incidence worldwide, with 550,000 new cases and 300,000 new deaths emerging every year. In 90% of cases, it is classified as squamous cell carcinoma (HNSCC). Usually, this cancer affects men far more than it affects women (1,2). Traditionally, these cancers have been strongly associated with risk factors such as tobacco and alcohol exposure (3). Because of the harmful effect smoking has on carcinogenesis, many states have enforced actions against smoking. Because of this, there has been a steady decline in smoking incidence around the world. However, even though smoking rates are declining, the incidence of oropharyngeal cancers in the USA is rising (4-6). This is due to a shift in the etiology of oropharyngeal carcinoma, i.e., human papillomavirus (HPV). Recently, more cases are being attributed to certain HPV infections, with types HPV-16 and HPV-18 emphasized as the most commonly isolated (3,4). These high-risk oncogenic types encode oncoproteins E6 and E7 which dysregulate cell cycle control and induce cellular transformation of primary squamous epithelial cells by binding to tumor suppressor proteins p53 and pRB (7).

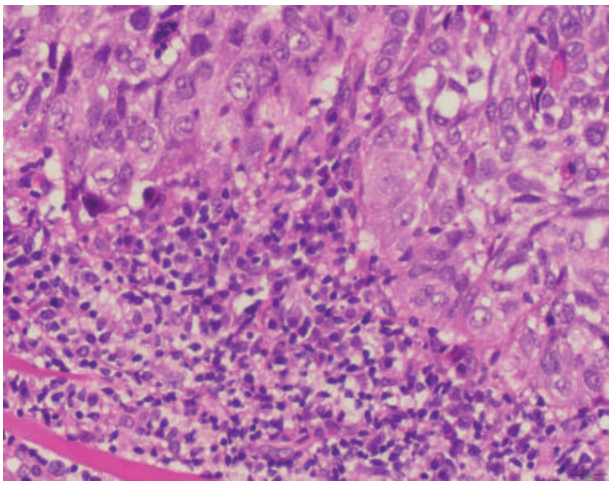

Figure 1. *Typical histologic presentation of HPV+ OPSCC. Syncytial clusters of atypical poorly differentiated squamous epithelial cells are surrounded by dense mononuclear infiltration.*

Clinical differences have been recorded among patients in regard to HPV status. HPV positive (HPV+) patients are known to have a more favorable survival rate than HPV negative (HPV-) patients, which is due to their better therapy response (8,9). These patients are typically characterized as younger white men of a higher socioeconomic status, without traditional risk factors such as smoking and alcohol exposure (10). Squamous cell carcinomas of the oropharynx, especially the palatine and lingual tonsils, make up most of the HPV+ head and neck carcinomas (11).

Not only are there differences in the clinical presentation of HPV+ oropharyngeal squamous cell carcinomas (OPSCC), but histologic differences have been clearly described. HPV+ OPSCC displays a basaloid appearance, lacking prominent keratinization. The tumor infiltrates surrounding tissue as expansive lobules. These lobules are usually surrounded by infiltrating lymphocytes (Figure 1). Usually, there is minimal to no desmoplastic reaction (12,13).

The geographic distribution of HPV+ OPSCC differs fairly among countries. Viral etiology outnumbers traditional risk factors in North America and northern Europe (4,14,15), while HPV- OPSCC still indicates the prevalent role of traditional risk factors in southern Europe (16). In Croatia, head and neck carcinomas make up 7% of all cases in men and 1% in women, with the trend for oropharyngeal carcinomas staying relatively consistent from 2010 to 2014 with about 100 new cases emerging *per* year (17). Recently, a study performed by Božinović *et al*. analyzed HPV status in OPSCC in northern Croatia. In this study, HPV RNA was found in 29.3% of cases (18).

In order to get a complete view on how HPV impacts patients with OPSCC in Croatia, we evaluated HPV status in patients from southern Croatia, analyzed the clinical and histologic features, and determined survival rates.

MATERIALS AND METHODS

Data collection

We collected 68 formalin-fixed paraffin-embedded (FFPE) tissue samples diagnosed with oropharyngeal carcinoma at the Split University Hospital Center between 2013 and 2017. Epidemiological data were collected from the computer database of the Department of Pathology, and narrow localization of tumors from the history of diseases at the Department of Ear, Nose and Throat Diseases with Head and Neck Surgery, Split University Hospital Center. The inclusion criterion was sufficient material in FFPE sample for real-time polymerase chain reaction (qPCR) analysis and hematoxylin-eosin (H&E) staining. Subjects who did not have enough tissue in the paraffin block for PCR analysis were excluded from the study. The study was approved by the Bioethical Board of the Split University Hospital Center (00-03/19-01/78).

Microscopy and qPCR

Every paraffin block was cut to a thickness of 5 mm for histologic analysis and to a thickness of 10 mm for molecular analysis. After fixation, histologic samples were dewaxed in xylene (3x5 minutes) and rehydrated in a gradient of ethanol (100% 1x5 minutes and 96% 1x5 min) to water. The samples were immersed in the hematoxylin solution for 5 minutes and stained by rinsing in running water. The samples were then immersed in eosin solution for 3 minutes, washed and dehydrated in an alcohol gradient (75% 1x5 min and 100% 1x5 min), clarified by brief immersion in xylene, and covered with mounting medium and a microscopic cover slip. The slides were analyzed by an Olympus BX51 (Olympus Corporation, Shinjuku, Tokyo, Japan) light microscope. The degree of cancer differentiation, inflammatory stroma reaction, keratinization, desmoplasia, and growth pattern were analyzed by two pathologists.

The tumor-nodes-metastases (TNM) classification was done according to the 8th edition American Joint Committee on Cancer and the Union for International Cancer Control (UICC) TNM classification of malignant tumors (19).

DNA isolation was performed by affinity chromatography according to Sigma-Aldrich protocol with GenElute™ FFPE DNA Purification Kit. The method begins with the process of dewaxing the paraffin sample in a series of washings with xylene and 96% ethanol. The sample was then dissolved with proteinase K, RNase and dissolution buffer A, after which the samples were incubated for one hour at 55 °C and for one hour at 90 °C. RL buffer and 96% ethanol were added to the obtained lysate and the solution was placed in spin columns. Nucleic acids bind to the spin column by an ionic gradient, and the contaminant passes smoothly through the column or is retained at the top of the column. To further remove impurities, bound DNA was washed with elution solution A. DNA was then eluted with elution buffer B. After DNA isolation, qPCR was performed with selected primers, GP5+/GP6+ primers. SYBR Green dye was used. The ready-made SYBR Green Master Mix (containing buffers, dNTPs and SYBR Green dye), the above primers, DNA/RNase-free water and an isolated DNA sample were mixed. The Applied Biosystems™ 7500 Real-Time PCR Systems were used to read the samples.

Fluorescence data on all 68 samples in 40 PCR cycles were extracted and modelled logistically and exponentially due to the biochemical course of the reaction. Since DNA is amplified 2^n times (n=number of cycles) during qPCR, the primer is integrated into the positive DNA into the newly formed strands and emits fluorescence that can be measured spectrophotometrically in each cycle. As the amount of primers and dNTPs is limited, logistic or exponential growth in the samples is expected. Positive samples were considered to be those for which these two models were more likely than the linear model in both examples, i.e., the one that best described the negative control (20). The probability of the model was calculated based on the Akaike information criterion (AIC).

Immunohistochemistry

Tissue microarrays were assembled from the 68 samples. Each slide consisted of eight samples. The p16 was done using anti-p16ink (CINtec p16, Ventana, Roche Holding AG, Oro Valley, Arizona, United States) antibodies on Ventana Ultrabenchmark (Ventana, Roche Holding AG, Oro Valley, Arizona, United States) using Ventana Ultraview staining kit (Ventana, Roche Holding AG, Oro Valley, Arizona, United States). The p16 expression was assessed by two pathologists. Samples were considered positive if the overexpression of p16 was more than 70% of tumor cells (Figure 2).

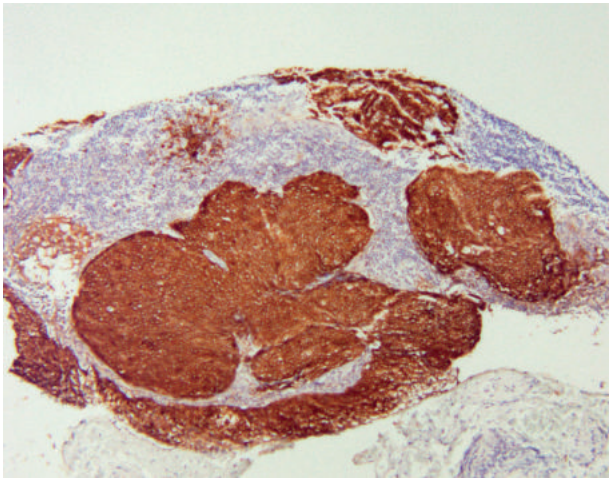

Figure 2. *p16 immunostaining on HPV+ OPSCC tissue.*

Statistics

Patient and sample data were entered into Microsoft Excel and analyzed with the GraphPad Prism 8.1 statistical program (GraphPad software, La Jolla, CA USA) and SPSS (version 26, IBM Corp., Armonk, New York, SAD). Differences between categorical variables were tested by the χ^2-test and Fisher exact test. Differences between continuous variables were tested with t-test for unpaired samples. The Shapiro-Wilk test was used to check the normality of the distribution. Missing data were categorized as unknown and removed from analysis. Samples with histologic features that could not be assessed were excluded from contingency calculations. Kaplan-Meier analysis was used to calculate survival data based on p16 status, HPV status, clinical stage, and therapy. Individual variable impact on survival was calculated *via* univariate Cox-proportional hazard regression models based on age, sex, HPV DNA presence, p16 status, T classification, N classification, distant metastasis presence, clinical stage, and type of therapy. A multivariate model was made considering the aforementioned variables, calculating AIC and removing insignificant variables. The level of statistical significance was set at $p<0.05$.

RESULTS

Out of the 68 samples collected that were diagnosed with OPSCC, 10.29% were HPV DNA positive, and 19.12% were p16 positive. Male sex was predominant in both HPV+ and HPV- groups, making up 71.43% and 81.97% of cases, respectively. There was no significant age difference between HPV positive and negative patients (p=0.6296, t-test), nor was there a difference regarding p16 status (p=0.1832, t-test). Anatomically, the most common location of HPV+ tu-

Table 1. *Patient clinical characteristics with respect to HPV and p16 status*

Variables	HPV + n (%)	HPV – (%)	p16 + (%)	p16 – (%)
Sex				
Men	5 (7.35)	50 (73.53)	11 (16.17)	44 (64.71)
Women	2 (2.94)	11 (16.17)	2 (2.94)	11 (16.17)
Tumour location				
Tonsils	5 (71.43)	17 (25)	5 (7.35)	17 (25)
Base of tongue	1 (1.47)	27 (39.71)	4 (5.88)	24 (35.29)
Soft palate	0	11 (16.17)	3 (4.41)	8 (11.76)
Palatine arch	0	3 (4.41)	1 (1.47)	3 (4.41)
Location unknown	1 (1.47)	3 (4.41)	0	3 (4.41)
Average age	65,14	63,12	59,85	64,15
Median age	64	62	62	62
Clinical stage				
Stage 1	1 (1.47)	13 (19.11)	2 (2.94)	12 (17.65)
Stage 2	1 (1.47)	0	1 (1.47)	0
Stage 3	1 (1.47)	15 (22.06)	3 (4.41)	13 (19.11)
Stage 4	3 (4.41)	10 (14.71)	5 (7.35)	8 (11.77)
T status				
T1	0	5 (7.35)	0	5 (7.35)
T2	1 (1.47)	9 (13.24)	2 (2.94)	8 (11.76)
T3	2 (2.94)	5 (7.35)	2 (2.94)	5 (7.35)
T4	1 (1.47)	17 (25)	6 (8.82)	12 (17.65)
N status				
N0	0	6 (8.82)	1 (1.47)	5 (7.35)
N1	3 (4.41)	11 (16.17)	5 (7.35)	9 (13.24)
N2	0	16 (23.53)	3 (4.41)	13 (19.11)
N3	2 (2.94)	5 (7.35)	1 (1.47)	6 (8.82)
M status				
M0	3 (4.41)	30 (44.12)	6 (8.82)	27 (39.71)
M1	3 (4.41)	9 (13.24)	5 (7.35)	7 (10.29)

mors were the tonsils (p=0.0157, Fisher exact test) (Table 1). Both patient groups presented more commonly in later clinical stages. Smoking data were collected on 13 patients only, out of which 92.37% were current or ex-smokers. Therapy data were found for 77.94% of patients. The majority of patients were treated with combined surgery and chemoradiotherapy (54.72%), while others underwent primary chemoradiotherapy (Table 2).

Patients with HPV+ OPSCC had a better survival rate than patients with HPV- OPSCC, although not statistically significant (p=0.7769) (Table 3, Figure 3). On the other hand, patients diagnosed with lower clinical stages had a significantly better outcome (p=0.0009). Patients that underwent combined surgical and chemoradiotherapy had a better outcome than those treated with primary chemoradiotherapy (p=0.0036).

Cox-proportional hazard regression models were made for univariate models and a multivariate model was formed. Both the univariate and multivariate models were calculated for 40 patients for which all of the clinical data were collected in order to reduce the risk of bias. Combined therapy confirmed to be beneficial in both the univariate model (p=0.002) and multivariate model (p=0.008) (Table 4). Presence of distant metastases worsened survival (p=0.021), given the univariate model (Table 5). The observed variables in our multivariate model were HPV status, sex, T status, N status, and therapy. In our multivariate model, several variables proved to be statistically significant. HPV+

Table 2. *Overall survival of patients with OPSCC based on HPV status, p16 status, clinical stage, and therapy*

Variables	Median survival	p	Hazard ratio	95% confidence interval	
				Lower	Upper
HPV status					
HPV +	1440	0.7769	1.156	0.4248	3.144
HPV -	930				
p16 status					
p16 +	1440	0.6277	1.218	0.5496	2.697
p16 -	930				
Clinical stage					
Stage 1	Undefined*	0.0009			
Stage 3	1740				
Stage 4	450				
Therapy					
Nonsurgical	450	0.0036	3.123	1.45	6.728
Combined therapy	1890				

Table 3. *Multivariate proportional hazard regression model for clinical and histologic features*

Variables	p	hazard ratio	95% confidence interval	
			Lower	Upper
HPV status				
Negative		1		
Positive	0.019	0.081	0.01	0.665
Sex				
Male		1		
Female	0.018	0.076	0.009	0.639
T status				
T1	0.407			
T2	0.339	4.450	0.209	94.858
T3	0.236	4.720	0.363	61.447
T4	0.352	0.524	0.135	2.041
N status				
N0	0.050			
N1	0.026	0.023	0.001	0.637
N2	0.006	0.037	0.004	0.389
N3	0.041	0.164	0.029	0.927
Therapy				
Nonsurgical		1		
Combined	0.008	0.123	0.026	0.572

patients, women and patients treated with combined surgery and chemoradiotherapy all had a more favorable outcome. The presence of metastases in lymph nodes had a better outcome, but it decreased for higher N stages.

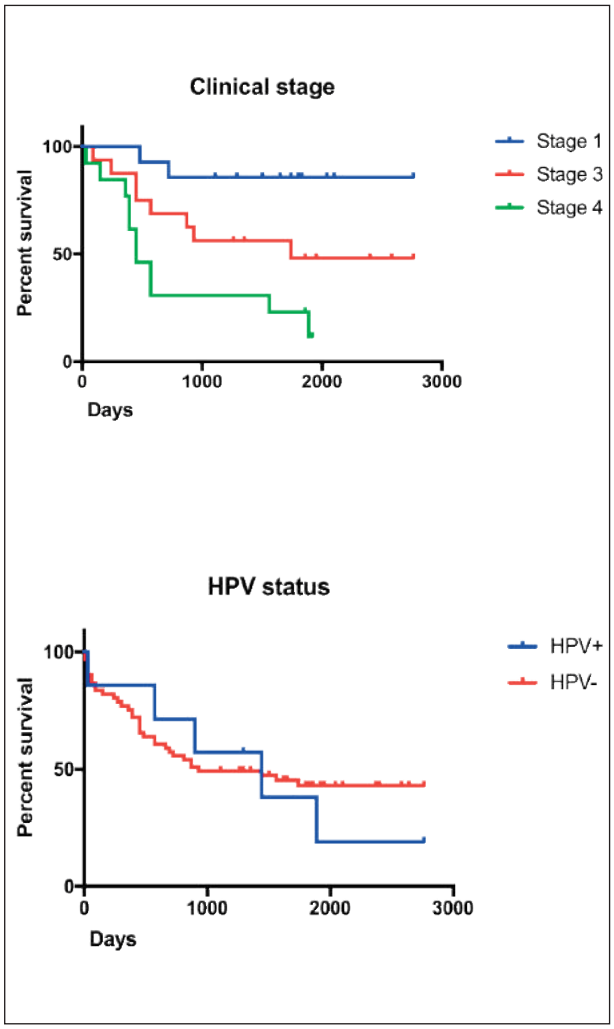

Figure 3a. *Overall survival of patients with OPSCC based on clinical stage and HPV status.*

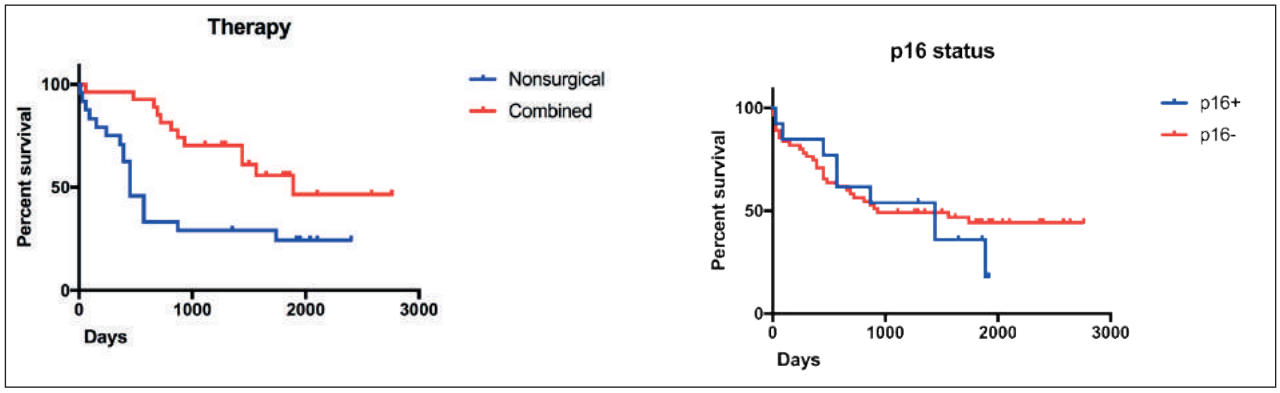

Figure 3b. *Overall survival of patients with OPSCC based on p16 status, and applied therapy presented in Kaplan-Meier curves.*

Table 4. *Univariate proportional hazard regression of patient survival for clinical and histologic features*

Variables	p	Hazard ratio	95.0% confidence interval		Overall significance
			Lower	Upper	
HPV status					0.230
Negative		1			
Positive	0.240	0.475	0.137	1.645	
p16 status					0.548
Negative		1			
Positive	0.550	0.746	0.286	1.948	
Sex					0.319
Male		1			
Female	0.329	2,070	0,480	8,926	
Age	0.103	1.035	0.993	1.079	0.100
T stage					0.038
T1		1			
T2	0.100	0.180	0.023	1.392	
T3	0.027	0.184	0.041	0.824	
T4	0.378	0.623	0.217	1.783	
N stage					0.039
N0		1			
N1	0.043	0.103	0.012	0.927	
N2	0.035	0.242	0.065	0.907	
N3	0.318	0.550	0.170	1.777	
M stage					
M0		1			0.016
M1	0.021	2.882	1.170	7.143	
Clinical stage					0.006
Stage 1		1			
Stage 2	0.003	0.096	0.021	0.447	
Stage 3	0.747	0.710	0.089	5.685	
Stage 4	0.148	0.493	0.189	1.285	
Therapy					0.001
Nonsurgical		1			
Combined	0.002	0.195	0.07	0.541	

Table 5. *Tumour histology characteristics*

Variables	HPV +	HPV -	p	p16 +	p16 -	p
Dysplasia of surface epithelium			>0.9999			0.6867
Present	7 (10.29)	49 (72.06)		10 (14.71)	46 (67.65)	
Not present	0	12 (17.65)		3 (4.41)	9 (13.24)	
Tumour architecture			0.4066			>0.9999
Irregular cords and nests	1 (1.47)	22 (32.35)		4 (5.88)	19 (27.94)	
Expanding lobules	6 (8.82)	37 (54.41)		9 (13.24)	34 (50)	
Cannot be determined	0	2 (2.94)		0	2 (2.94)	
Desmoplasia			0.7029			0.7527
Prominent	3 (4.41)	21 (30.88)		4 (5.88)	20 (30.88)	
Often absent	4 (5.88)	37 (54.41)		9 (13.24)	32 (47.06)	
Cannot be determined	0	3 (4.41)		0	3 (4.41)	
Keratinization			0.4093			0.2045
Prominent	4 (5.88)	21 (30.88)		7 (10.29)	18 (26.47)	
Minimal or absent	3 (4.41)	40 (58.82)		6 (8.82)	37 (54.41)	
Differentiation			0.0861			>0.9999
Moderately differentiated	7 (10.29)	38 (55.88)		4 (5.88)	36 (52.94)	
Basaloid/poorly differentiated	0	23 (33.82)		9 (13.24)	19 (27.94)	
Inflammation reaction			0.1972			0.0257
Weak	2 (2.94)	39 (57.35)		4 (5.88)	37 (54.41)	
Intermediate	2 (2.94)	10 (14.71)		5 (7.35)	7 (10.29)	
Strong	3 (4.41)	12 (17.65)		4 (5.88)	11 (16.18)	

Out of the histologic features, inflammation was the only variable that showed a statistically significant difference regarding p16 status. p16+ samples had a stronger inflammatory reaction than p16- tumors (p=0.0257, Fisher exact test).

DISCUSSION

The aim of this study was to assess the impact of HPV on OPSCC in southern Croatia, and to describe clinical and histologic features of OPSCC. In this study, 10.29% of patients were HPV positive, which is less than in North America and West Europe (3,14,15). Although western countries seem to have a higher burden of HPV, South European countries seem to report lower prevalence (4,16,21-26). In a recent northern Croatian study, Božinović *et al.* report on 29.3% of HPV positive cancer cases, showcasing a difference in distribution within Croatia (18). Northern Croatia seems to follow a trend that befits western countries, while southern Croatia would be categorized with other South European regions.

Human papillomavirus proved to be an important prognostic factor in the survival of our patients. Our multivariate Cox regression-hazard model showed a more favorable outcome in patients with HPV+ OPSCC. It is widely described that HPV is a favorable prognostic factor, and that patients with HPV+ OPSCC have a better therapy response than those with HPV- OPSCC (27), and therefore better survival (28-30). The reason why HPV is a positive prognostic factor is unclear. This might be due to the lack of p53 mutation in patients with HPV+ OPSCC (31), making them more radiosensitive and more sensitive to chemotherapy, resulting in better outcome (32,33).

Patients with HPV+ OPSCC are usually described as white men of a higher socioeconomic status and of a younger age than patients with HPV- OPSCC (10). Although our patients were predominantly men, we did not find any age differences between the two patient groups. Similar findings have been reported in studies in Europe (18,22,23,34). This indicates the impact that classical risk factors such as cigarette smoking and alcohol consumption have in our population.

In Croatia, smoking is an important factor in carcinogenesis because more than 25% of adults smoke tobacco products every day (35). In 2011, Bergman Marković *et al.* announced that a higher proportion of smokers are in coastal cities than in inland Croatia

(36). Alcohol consumption is another important factor in carcinogenesis. In 2009, Bencević-Striehl *et al*. reported that coastal Croatia was second in the prevalence of alcohol consumption in both sexes, immediately after Slavonia in men and northern Croatia in women (37). It is possible, therefore, that the lower percentage of HPV+ OPSCCs in the sample of our patients is inversely related to the higher rate of smokers and alcohol consumption in southern Croatia, which contributes to the development of HPV- OPSCC.

Generally, HPV+ OPSCC is histologically described as poorly differentiated squamous epithelial cells that do not keratinize, without desmoplastic stroma and surrounding epithelial dysplasia (12). In this study, we did not find a statistically significant association of these parameters of a typical histologic picture with HPV tumor status. Both studies from Slovenia and northern Croatia did not manage to find significant difference in regards to histologic grade in HPV+ and HPV- OPSCC (18,21). In 2018, Liu *et al*. concluded that the structure of HPV+ OPSCC in patients who were smokers was histologically more similar to HPV- OPSCC (38). This confirms that traditional risk factors were prevalent in both groups.

As expected, we found that HPV+ OPSCC was most commonly found on the palatine tonsils. It is believed that HPV+ OPSCC originates from the epithelium of tonsillar crypts, while HPV- OPSCC emerges from the surface epithelium (39). Similar to cervical transformation zones, the tonsils are characterized by deep invaginations of mucosal surface called crypts. These tonsillar crypts are lined by monolayered epithelium, showing similarities to mucosal basal keratinocytes (40). Deep tonsillar crypts facilitate the transfer of external antigens to lymphoid tissue and the presentation to antigen-presenting cells, which stimulates lymphocytic infiltration (41,42). Stronger lymphocyte infiltration we found in HPV+ OPSCC, which most often occurs in the tonsils, can be partly explained by the reaction of lymphatic cells to viral antigens.

CONCLUSION

In conclusion, traditional risk factors are the cause of OPSCC, as well as of many other cancers in our environment. Prophylactic vaccination against HPV is still not mandatory in Croatia, so in the future we can expect a natural increase in the number of HPV+ OPSCC in today's generation of young people, who are slowly adopting lifestyle behaviors as seen in western countries. Our study implies that histologic features of HPV+ OPSCC change towards that of HPV- OPSCC in populations where traditional risk factors are still the main cause of OPSCC. Although there are no significant differences between most of the histologic features, HPV+ status still has a more favorable outcome.

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S A Ž E T A K

KLINIČKI I PATOFIZIOLOŠKI PRIKAZ BOLESNIKA S OROFARINGEALNIM RAKOM PLOČASTIH STANICA POZITIVNIH NA HUMANI PAPILOMAVIRUS – ISTRAŽIVANJE U JUŽNOJ HRVATSKOJ

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Cilj: Svrha ovog istraživanja bila e analizirati utjecaj humanog papilomavirusa (HPV) na preživljavanje, kliničke pokazatelje i patohistološke značajke u ispitanika oboljelih od orofaringealnog raka pločastih stanica (OPSCC) u južnoj Hrvatskoj. ***Metode:*** Istražili smo prisutnost HPV DNK i imunohistokemijsko bojanje na p16 u 68 u parafinske blokove uklopljenih uzoraka tkiva ispitanika oboljelih od OPSCC-a i liječenih u Kliničkom bolničkom centru Split u razdoblju od 2013. do 2017. godine. Svjetlosnim mikroskopom utvrđene su histološke značajke tkiva. Retrospektivno smo prikupili kliničke podatke ispitanika i proučili ih s obzirom na HPV status. ***Rezultati:*** U ovom je istraživanju 10,29% pacijenata pozitivno na HPV (HPV+). Invazija limfocita značajnija je u ispitanika s p16 pozitivnim (p16+) OPSCC-om. Ukupno preživljavanje (OS) bolje je u HPV+ i p16+ ispitanika. HPV je značajan prognostički čimbenik u ispitanika koji boluju od OPSCC-a iz južne Hrvatske. ***Zaključak:*** Čini se da je HPV manje utjecajan uzročni čimbenik nastanka OPSCC-a u južnoj Hrvatskoj u usporedbi sa zapadnoeuropskim zemljama i SAD-om. Iako je HPV značajan čimbenik preživljavanja, tradicionalni čimbenici rizika pokazali su se važnijim karcinogenima za nastanak OPSCC-a u našoj populaciji.

Ključne riječi: Hrvatska, neoplazme glave i vrata, histologija, humani papilomavirus 16, orofaringealne neoplazme, planocelularni karcinom

CUMULATIVE PREGNANCY RATES AFTER FRESH AND FIRST SUBSEQUENT TRANSFER OF THAWED EMBRYOS: IS IT TIME TO CHANGE PRACTICE?

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The aim: was to clarify parameters that contribute to successful pregnancy outcomes from one oocyte retrieval cycle with the least procedure steps. ***Methods:*** This retrospective study included 42 stimulated IVF cycles with fresh embryo transfers (fresh ET) and the subsequent 42 frozen embryo transfer cycles (FET) performed between January 2012 and December 2015. ***Results:*** The observed clinical pregnancy rate of 21.4% in stimulated cycles with fresh embryo transfers was significantly lower compared with the pregnancy rate of 52.4% in cycles with thawed embryo transfers (p=0.015) indicating impaired endometrium quality in stimulated IVF cycles. Most of the patients (78.6%) failed to achieve pregnancy after fresh ET, but more than half of them (57.6%) succeeded to achieve pregnancy after FET. The cumulative pregnancy rate after fresh ET and the first subsequent FET was 73.8% per initiated cycle. ***Conclusion:*** The results suggest that not only the presence of supernumerary good-quality blastocysts but also a receptive endometrium is needed for a successful IVF outcome. Our findings suggest that ovarian stimulation protocol had an impact on the pregnancy rate in the fresh cycle and that a better chance of conceiving was after FET. Thus, IVF outcomes can be improved with a better embryo transfer strategy.

Key words: freeze-all cycle, fresh embryo transfer, frozen-thawed embryo transfer

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INTRODUCTION

Over the last decade, frozen embryo transfer (FET) has become an essential part of IVF/ICSI treatment enabling elective single embryo transfer thereby reducing the risk of multiple pregnancies while increasing the cumulative pregnancy rate and also minimizing overall treatment costs (1-3).

The introduction of vitrification as a method of cryopreservation improve embryo cryopreservation techniques resulting in a greater embryo survival rate and high pregnancy rate after FET (4) and even higher pregnancy rate compared with pregnancy rate after fresh embryo transfer (fresh ET) in stimulated cycles (5-8). Furthermore, recent studies showed reduced pregnancy complications, birth defects, and perinatal outcomes after FET compared with fresh-ET (ie: ectopic pregnancies, perinatal mortality, small for gestational age, preterm birth, low birth weight, antepartum haemorrhage) (9-11).

Observed lower pregnancy rate after fresh ET has been associated with the negative impact of exogenous ovarian stimulation on the endometrium. Both embryo quality and endometrial receptivity are critical for successful implantation. It is important to synchronize embryo-endometrium interactions by improving the uterine microenvironment. The supraphysiologic levels of estradiol and progesterone during and after supra-ovulation can affect and alter gene expression in the endometrium (12), endometrial morphology (13),

the window of implantation and cause asynchronicity between the embryo and endometrium, especially in high-responders or younger women (5, 14). Premature elevation of progesterone on the day of hCG administration is associated with a reduced pregnancy rate after fresh embryo transfer (15). Studies have shown that ovarian stimulation and elevated estradiol levels can also cause adverse effects on early placentation and therefore affect fetal growth and development (16,17).

Implications of supra-ovulation on endometrial quality support the strategy of cancelling fresh embryo transfer and cryopreservation of all viable embryos ("freeze-all" strategy). Embryo transfer should be performed in cycles with proper endometrial development whether natural or prepared by hormone replacement therapy (HRT). The "freeze-all" strategy can further improve IVF/ICSI outcomes, providing a higher pregnancy rate and greater safety for both the mother and baby. Despite that, fresh embryo transfer is still the predominant approach in most clinics, while the "freeze-all" strategy is applied primarily in patients with a risk of OHSS development.

The aim of this study was to observe and compare clinical pregnancy rates between transfers of day 5 blastocysts in the supra-ovulation cycle and vitrified-warmed cycle from the same oocyte retrieval in order to suggest an adequate embryo transfer strategy.

PATIENTS AND METHODS

Patients

A retrospective study of 42 couples undergoing controlled ovarian stimulation for IVF procedure at the Clinical Hospital "Sveti Duh" between January 2012 and December 2015 was performed.

Inclusion criteria were: women younger than 42 years old; patients participating in both, fresh and frozen day 5 blastocyst ET cycle from the same oocyte retrieval procedure; basal FSH< 10 IU/l, TSH< 2 mIU/l.

Infertility was caused by both partners in most cases (20 couples). There were 15 couples with female infertility (10 with PCO, 5 with tubal factor), and in 7 cases caused male factor of infertility.

The results of routine haematological, serological, microbiological, and molecular tests (differential blood count, sedimentation, routine serological assays for blood donors, presence of anaerobic and aerobic bacteria, Human Papillomaviruses, *Chlamydia trachomatis* as well as *Ureaplasma and Mycoplasma* in cervical swabs) did not reveal acute or chronic infections in our patients. Patients with endometriosis and pelvic inflammatory disease were excluded.

Ovarian stimulation protocols

All patients were examined with transvaginal ultrasound using Siemens Sonoline G40 (Siemens Medical Solutions USA) transvaginal probe EC9-4. On day 1 or 2 of the menstrual cycle, if the endometrial thickness was < 5 mm and/or if there were no ovarian cysts >15 mm in diameter present the cycle was started and the patient was instructed to start hormonal stimulation, and was advised to attend for ultrasound scan following 5 days of injections. Supraovulation was achieved with daily injections of rFSH (Puregon, MSD, USA or Gonal F, Merck Serono, London) using the step-down protocol. Administration of rFSH started on day 2 and continued daily until r-hCG (Ovitrelle, Merck Serono, London) was given. The starting dose was calculated according to age, AMH level, and antral follicle count. Starting dose of rFHS was: 150 IU for patients under 30; 200 IU rFSH for patients between 31 and 35; 250 IU for patients between 36 and 38 and 300 IU rFSH for patients older than 38 years. GnRH antagonists (Cetrotide; Merck Serono London or Orgalutran MSD, USA) were introduced from day 7 and continued daily until r-hCG was given. Criteria for hCG administration were at least 2 follicles of > 18 mm in diameter and endometrial thickness > 7 mm.

Oocyte retrievals were performed transvaginally with a single-lumen needle 34/35 hours after r-hCG injection. Sedation options were peroral sedatives and analgetics, intravenous pethidine or general anaesthesia.

In vitro fertilization, vitrification, and embryo transfer procedure

Semen samples were collected by masturbation on the day of follicle aspiration. After checking semen quality, semen samples were washed and centrifuged at 300xg in the sperm washing medium (Sydney IVF Sperm Medium, K-SISM-20, William A. Cook Australia Pty. Ltd., Brisbane, Australia) and subsequently processed by the swim-up method.

A day in advance, Petri dishes were prepared with fertilization medium drops (Sydney IVF Fertilization Medium, K-SIFM-20, William A. Cook Australia Pty. Ltd., Brisbane, Australia) and covered with oil (Liquid Paraffin oil, Medicult, Origio, Måløv, Denmark).

After follicular aspiration, oocytes were washed free from follicular fluid. Oocyte maturity was assessed

after mechanical dissection of cumulus oophorus till the corona radiata. Oocyts were preincubated for 4h at 37°C in 6% CO_2 in humidified air.

For the IVF procedure, oocytes were inseminated with $40x10^3$ to $80x10^3$ of motile sperm, depending on semen morphology and motility.

For the ICSI procedure, oocytes were prepared with enzyme (Hyaluronidase; SAGE In-vitro Fertilization, Inc., Trumbull, CT 06611 USA) mechanically denuded and washed in a fertilization medium.

Microinjection was performed using: a Petri dish with fertilization drops and polyvinylpyrrolidone solution (PVP 7%; SAGE In-vitro Fertilization, Inc., Trumbull, CT 06611 USA) covered with oil (prepared two hours in advance) and micropipettes (Holding and ICSI Pipette; Cook Ireland Ltd, National Technological Park Limerick, Ireland).

Fertilization was checked between 18-20 h after insemination. If two pronuclei did not appear, fertilization was rechecked once more after another 24 h. Zygotes were transferred using the 140 μm pipette (Cook Ireland Ltd, National Technological Park Limerick, Ireland) to the Petri dish with cleavage medium (Sydney IVF Cleavage Medium K-SICM-20, William A. Cook Australia Pty.Ltd., Brisbane, Australia) the prepared day before.

On day 3, embryos were transferred using the 170 μm pipette (Cook Ireland Ltd, National Technological Park Limerick, Ireland) into blastocyst medium (Sydney IVF Blastocyst Medium K-SIBM-20, William A. Cook Australia Pty.Ltd., Brisbane, Australia) prepared the day before.

On day 5, the quality of blastocysts was evaluated according to Gardner's score criteria (18, 19).

Embryo transfer was performed after an ultrasound evaluation of the uterus and ovaries. Pending the patient's age and previous infertility history, one or two high-quality (5AA, 4AA, 4AB) blastocysts were transferred per patient.

All embryo transfers were done using a catheter set (Embryo Transfer Catheter Set, Labotect GmbH, Labor-Technik, Gottingen, Germany) under ultrasound guidance.

Freezing protocol – vitrification method

Blastocyst vitrification was performed using vitrification solutions (Vitrification Media, Kitazato BioPharma Co. Ltd 81, Nakajima, Fuji, Shizuoka, Japan) stabilized at room temperature and open system carrier (Cryotop, Kitazato BioPharma Co. Ltd 81, Nakajima, Fuji, Shizuoka, Japan). Blastocysts were incubated in an equilibration solution for 15 minutes, then placed in a vitrification solution and washed a few times for 60 seconds, and put on the carrier. Carrier was plunged into liquid nitrogen and capped.

On the day of embryo transfer, blastocysts were thawed using a thawing kit stabilized at 37°C (Thawing Media, Kitazato BioPharma Co. Ltd 81, Nakajima, Fuji, Shizuoka, Japan). Cryotop with the blastocysts was placed directly from liquid nitrogen into a thawing solution. After a minute blastocysts were transferred into the diluent solution for 3 minutes, then transferred in the washing solution for 5 minutes and washed additionally for 1 minute in another washing solution. Following thawing, blastocysts were put in blastocyst medium and incubated for 2 hours until embryo transfer.

All transferred blastocysts were graded prior to ET.

Luteal phase support

Luteal phase support has been accomplished with the micronized progesterone (Utrogestan; Laboratories Piette International S.A., Brussels, Belgium) 600 mg/day starting from the day after oocyte retrieval.

Endometrial preparation for frozen-thawed embryo transfer

The FET procedure included freeze-all patients, patients after unsuccessful fresh cycle procedures (no pregnancy or spontaneous miscarriage), as well as patients with successful fresh procedures and live birth delivery.

Transvaginal ultrasonographic estimation of endometrial thickness was performed for scheduling the frozen embryo transfer. The HRT protocol started on day 2 of the cycle with daily administration of 6 mg estradiol (Estrofem, Novo Nordisk A/S, Bagsvaerd, Danmark). An ultrasound scan was performed between days 10 and 12 of the cycle and if the endometrial thickness was > 8 mm vaginal micronized progesterone (Utrogestan, 600 mg was introduced daily. On the 5th day of progesterone intake, a frozen/thawed blastocyst transfer was performed.

Pregnancy definition

Clinical pregnancy was defined as ultrasound visualization of the gestational sac and positive heart action of the embryo.

Statistical methods

Comparisons of continuous variables were performed by Student's t-test and Mann-Whitney U test. For paired groups comparison Wilcoxon and McNemar's tests were used. All statistical analyses were performed using SPSS software. P<0.05 was considered significant for all measures.

Table 1. *IVF procedure data*

	Fresh cycle (n=42)
Patient age (years)	33.36 ± 4.2 (25-40)
Retrieved oocytes (n)	10.57 ± 3.9 (4-20)
Cultivated oocytes (n)	8.57 ± 2.5 (4-12)
Fertilized oocytes (n)	6.40 ± 1.6 (3-10)
Fertilization rate (%)	77.05 ± 14.4 (50-100)
Good quality embryos (n)	3.19 ± 0.7 (2-5)
Transferred embryos (n)	1.26 ± 0.4 (1-2)
Cryopreserved embryos (n)	1.93 ± 0.7 (1-3)

Values listed as mean ± standard deviation (min-max value)

RESULTS

The study included 42 patients in stimulated cycles with fresh blastocyst transfer and subsequent frozen blastocyst transfer using sibling embryos from the same retrieval. The female patient age and IVF procedure data are presented in Table 1.

The study included a population of patients with a good response to ovarian stimulation with an average of 10 retrieved oocytes and with two and more good quality day-5 blastocysts per cycle.

Of a total of 134 good-quality blastocysts, 39% of them were transferred in the initiated stimulated cycle and 48% were cryopreserved, thawed, and transferred in the FET cycle.

There was no statistically significant difference in patients' age, the number of retrieved, cultivated, and fertilized oocytes, as well as the number of transferred embryos between the groups, was distinguished by the type of cycle (fresh and FET) and cycle outcome (pregnant and non-pregnant) (Table 2).

There was no statistically significant difference (p= 0.054) between the number of transferred embryos in fresh cycles (1.26±0.4) and in FET cycles (1.52±0.5). Comparison between pregnancy rate after FET cycle and fresh cycle ET has shown a statistically significant higher pregnancy rate after FET (p=0,015).

A description of undergone procedures with the number of patients according to cycle outcome was presented in Figure 1.

Table 2. *Fresh and FET cycles: characteristics of pregnant and not pregnant patients*

Type of cycle	Fresh cycle			FET cycle		
Cycle outcome	Pregnant (21.4%)	Not pregnant (78.6%)	P-value	Pregnant (52.4%)	Not pregnant (47.6%)	P-value
Age (years)	34.67±3.8 (28-39)	33.00±4.3 (25-40)	0.300a	33.64±4.1 (26-40)	33.85±4.4 (25-40)	0.871a
Retrieved oocytes (n)	8.56±3.0 (5-12)	11.12±4.0 (4-20)	0.075b	11.27±3.6 (4-20)	9.80±4.2 (5-20)	0.066b
Cultivated oocytes (n)	7.33±2.2 (4-10)	8.91±2.5 (4-12)	0.127b	9.00±2.6 (4-12)	8.10±2.4 (4-12)	0.212b
Fertilized oocytes (n)	5.67±1.2 (3-7)	6.61±1.7 (4-10)	0.171b	6.68±1.7 (4-10)	6.10±1.6 (3-10)	0.268a
Fertilization rate (%)	79.97±16.6 (60-100)	76.26±14.0 (50-100)	0.503a	77.20±15.9 (50-100)	76.88±13.2 (58-100)	0.943a
Transferred embryos (n)	1.44±0.5 (1-2)	1.21±0.4 (1-2)	0.303b	1.59±0.5 (1-2)	1.45±0.5 (1-2)	0.367b

Values listed as mean ± standard deviation and (min-max) value.
[a] Student's t-test
[b] Mann-Whitney U test

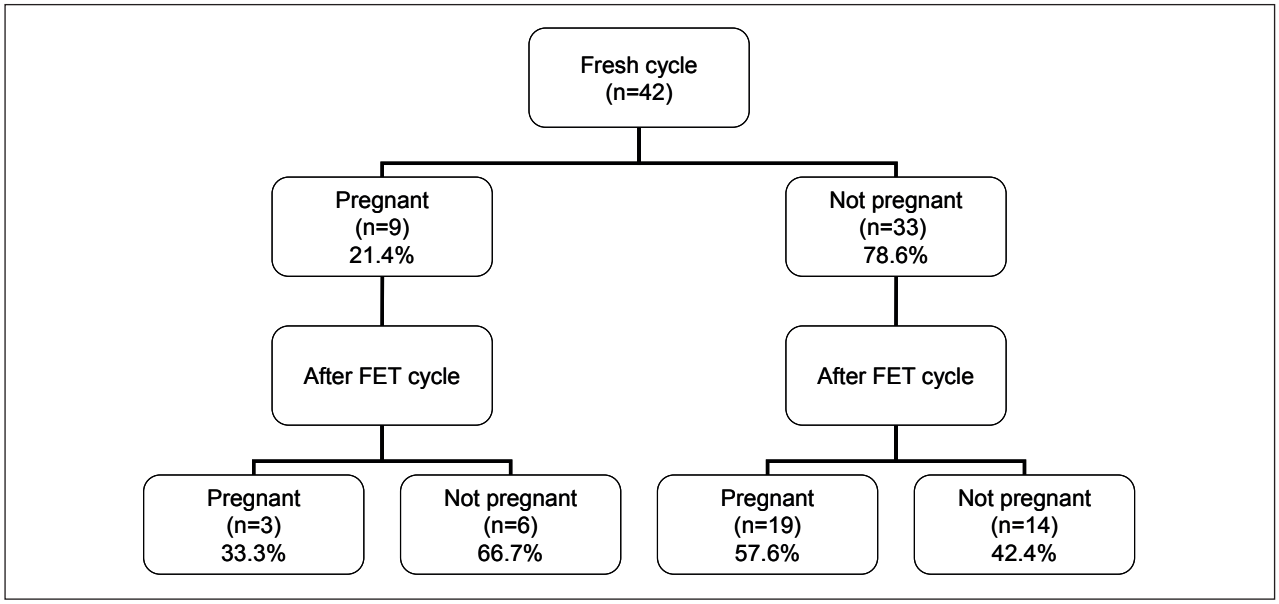

Figure 1. *Outcome anagram*

The clinical pregnancy rate per embryo transfer was 21.4% after fresh ET and 52.4% after the FET cycle. The cumulative pregnancy rate after fresh ET and the first subsequent FET was 73.8% per initiated cycle.

DISCUSSION

From our study, the main finding was a significantly (p=0.015) lower pregnancy rate per transfer (21.4%) after fresh blastocyst ET compared with the pregnancy rate per transfer after blastocyst FET (52.4%), with no influence on the patient's age, the number of retrieved, cultivated and fertilized oocytes. All our patients have been stimulated with a short antagonist protocol and the cumulative pregnancy rate including the first subsequent FET with HRT was 73%.

Toftager *et al* (20) observed a higher cumulative live birth rate with antagonist protocol after fresh ET and all subsequent FET compared to agonist protocol. This group of authors also recommended antagonist protocol for obese women; the protocol had lower OHSS risk and should be the first choice of treatment for ART. Nevertheless, older patients may still benefit from the agonist protocol.

There is growing evidence of impaired endometrial receptivity after ovarian stimulation, which might be related to a lower pregnancy rate when fresh ET is performed (5). Poorer obstetric and perinatal outcome in ART pregnancies was observed in fresh ET compared to FET and to spontaneous conception (9,21-24). Ovarian stimulation with consequent supraphysiologic levels of estradiol (16,17,25) and premature progesterone elevation (12, 15, 26) affects the expression of more than 200 genes related to implantation (12, 27). In the endometrium, these changes cause morphological modifications (13) and impaired maturation, receptivity, and embryo-endometrium asynchrony (28,29). Endometrial impairment can occur both under GnRH agonist and GnRH antagonist stimulation protocols (30).

Nowadays, cryopreservation techniques are effective procedures with a high embryo survival rate and with similar potential for implantation as fresh embryos (4, 31). Cryopreservation techniques give us the opportunity to delay embryo transfer and perform it in a more suitable endometrium.

Shapiro *et al.* (6,7), suggested "freeze all" cycles and subsequent FET cycles for normal and high responders and patients with prior fresh blastocyst implantation failure. The freeze-all strategy is a procedure in which all viable embryos are cryopreserved in the fresh cycle and transferred in subsequent cycles (32, 33, 34). It may not be necessary for all patients, but it should be considered for patients with a high risk of OHSS (35), high estradiol, and/or early elevated progesterone.

CONCLUSION

Our findings suggest that ovarian stimulation protocol had a strong impact on the pregnancy rate in the fresh cycle and that a better chance of conceiving is after FET with HRT. For further improving the success

rate of fresh stimulated cycles and IVF procedures in general, while making decisions between fresh ET and "freeze-all" it is crucial to consider the type of stimulation protocol and trigger applied, estradiol and progesterone levels, and ultrasound endometrial quality.

The limitation of our study includes not a uniform number of blastocysts for transfer but the aim of the study was to compare pregnancy rates in fresh and frozen cycles between patients themselves. Patients undergoing different treatment options showed undoubtedly better results using thawed embryos. Another limitation is not testing progesterone on hCG day as during that period we do not routinely test progesterone. Still, our conclusion is on the path that endometrial receptivity is impaired in the fresh cycle and according to previously mentioned studies high progesterone could be one of the potential reasons for impaired receptivity (5).

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SAŽETAK

KUMULATIVNA STOPA TRUDNOĆA OSTVARENA NAKON PRIJENOSA ZAMETAKA U SVJEŽEM CIKLUSU I PRVOM NAREDNOM CIKLUSU S ODMRZNUTIM ZAMETCIMA: JE LI VRIJEME ZA PROMJENU PRAKSE?

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Cilj rada je razjasniti parametre koji pridonose uspješnom ostvarivanju trudnoće iz jednog započetog postupka prikupljanja jajnih stanica uz najmanji broj postupaka koji slijede. ***Metode:*** U retrospektivnu studiju uključeno je 42 stimulirana IVF ciklusa s prijenosom svježih zametaka ("svježi ET") i 42 ciklusa prijenosa kriopohranjenih zametaka ("FET") učinjenih između siječnja 2012. i prosinca 2015. ***Rezultati:*** Zabilježena je značajno niža stopa kliničkih trudnoća (p=0,015) nakon prijenosa svježih zametaka u stimuliranim ciklusima (21,4 %) u usporedbi sa stopom trudnoća nakon prijenosa odmrznutih zametaka (52,4 %) što ukazuje na smanjenu kvalitetu/receptivnost endometrija u stimuliranim IVF ciklusima. Ukupna, kumulativna stopa trudnoća nakon "svježeg ET" i FET postupka iznosila je 73,8 % po započetom ciklusu. ***Zaključak:*** Rezultati ukazuju da je osim kvalitetnih blastocisti za uspješnost IVF postupka nužan i receptivni endometrij. Uočen je utjecaj protokola stimulacije jajnika u svježem ciklusu na stopu trudnoća i veća uspješnost začeća nakon FET postupka. Stoga bi se uspješnost IVF postupka mogla poboljšati boljom strategijom prijenosa zametaka.

Ključne riječi: "freeze-all" ciklus, prijenos svježih zametaka, prijenos kriopohranjenih zametaka

PREVALENCE, RISK FACTORS AND PREGNANCY OUTCOMES OF LABOR INDUCTION IN CROATIA – A NATIONAL ONE-YEAR STUDY

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Objective: The aim of this study was to determine the prevalence of labor induction in Croatia, as well as the main risk factors and adverse pregnancy outcomes associated with labor induction. *Materials and methods:* A cross-sectional study was performed using data from medical birth certificates collected in 2019 in Croatia. *Results:* Among 36,603 deliveries in 2019, the prevalence of labor induction was 14.1%. Women whose labor was induced were older, had a higher body mass index (BMI), and more frequent gestational weight gain above recommendations compared to women with spontaneous onset of labor (p<0.001). Induced labors were more frequent in pregnancies with gestational diabetes, gestational hypertension, preeclampsia, and fetal growth restriction (p<0.001 all). Women with induced labor had a higher incidence of cesarean section, vacuum extraction, postpartum hemorrhage, shoulder dystocia, and more frequently delivered infants above 4000 g (p<0.05 all). Logistic regression showed that maternal age, pre-pregnancy BMI, gestational diabetes, gestational hypertension, preeclampsia, fetal growth restriction, and gestational age at delivery were significant predictors of labor induction (p<0.001 all). *Conclusions.* The prevalence of labor induction in Croatia is 14.1%. Labor induction is associated with important risk factors and adverse perinatal outcomes, which can partially be attributed to the mode of labor onset. All of these should be taken into account when performing this obstetric procedure.

Key words: labor induction, prevalence, risk factors, cesarean section, logistic regression analysis

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INTRODUCTION

Induction of labor refers to the initiation of labor before its spontaneous onset and, when performed for appropriate indications, can have significant benefits for both the mother and the child (1). This obstetric procedure is performed in circumstances when the risk of waiting for spontaneous onset of labor is considered to be greater than the risk of inducing labor, i.e., in prolonged pregnancies, pregnancies burdened with prelabor rupture of membranes, fetal compromise, or maternal complications such as hypertensive disorders of pregnancy, diabetes mellitus or gestational diabetes, cholestasis, fetal growth restriction (FGR), and others (2,3).

According to the World Health Organization (WHO) data, labor induction is of increasing prevalence worldwide and present in up to one in four deliveries in developed countries, whereas its prevalence is lower in developing countries (4). Namely, up to 32.6% of labors in England are induced as compared with 25.7% in the USA and 22% in France, while official national data for Croatia are lacking (5-7).

Studies have recognized certain risk factors and obstetric complications associated with labor induction. Older pregnant women (above 35 years of age), those with a higher pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) above recommendations are more likely to have their labor induced

(8-10). Maternal complications associated with labor induction include chorioamnionitis and postpartum hemorrhage from uterine atony (11,12), whereas studies regarding the mode of delivery following labor induction report conflicting results. Some observational studies indicate that induced labors have an increased risk of cesarean section (CS) (13,14), while others relate this association to various prepartum and intrapartum factors not directly linked with labor induction (15). These include certain pregnancy complications and nulliparity. Conversely, results of a 2015 literature review and 2014 meta-analysis suggest that the term labor induction may, in fact, be associated with a decreased risk of CS (16,17), while a study by Grobman *et al*. (18) suggests that induction of labor at 39 weeks of gestation in low-risk nulliparous women results in a significantly lower frequency of CS compared to expectant management.

The aim of this study was to determine the prevalence of labor induction in Croatia and to detect the main risk factors and adverse pregnancy outcomes associated with labor induction in our population.

MATERIALS AND METHODS

This was a cross-sectional study performed using data from medical birth certificates (MBC) collected in 2019 in Croatia as part of perinatal statistics data reporting to the Croatian Institute of Public Health (CIPH). Completing MBCs is mandatory for all doctors attending labor at all labor units in the country. The study included pregnant women who gave birth in 2019 in Croatia. Inclusion criteria were spontaneous or induced onset of labor, while women who delivered by elective CS were excluded from the study.

Registered and approved methods of labor induction in Croatia in 2019 included mechanical methods, i.e. amniotomy and transcervical balloon catheter; and pharmacological methods , i.e., oxytocin infusion and prostaglandin E2 (2,3). Prostaglandin E1 was neither registered nor approved in 2019 for labor induction in Croatia.

The parameters assessed in this study were pre-pregnancy BMI and GWG. Based on the WHO classification system, women were assigned to 4 groups according to pre-pregnancy BMI, as follows: underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obese (≥30.0kg/m^2) (19). The optimal GWG for each pre-pregnancy BMI group was defined *per* Institute of Medicine (IOM) recommendations, as follows: 13-18 kg for underweight women, 11-16 kg for normal BMI women, 7-11 kg for overweight women, and 5-9 kg for obese women (20). Other parameters analyzed were the frequency of pregnancy complications (incidence of gestational diabetes, gestational hypertension, preeclampsia and FGR), labor complications (postpartum hemorrhage, retained placenta, shoulder dystocia and episiotomy rate), as well as neonatal complications (birth weight below 2500 g and above 4000 g, Apgar score <7 after 5 minutes, and NICU admission).

Statistical analyses were performed using SPSS ver. 21.0. Homogeneity of variance was tested using Levene's test. Continuous variables were presented as mean ± standard deviation (SD), while categorical data were presented as frequency and percentage. Differences between groups of independent continuous variables were analyzed using the t-test, while differences in the occurrence of individual conditions were compared using the χ^2-test.

Logistic regression was performed to ascertain the effects of maternal age, pre-pregnancy BMI, gestational age at delivery, and pregnancy complications such as gestational diabetes, gestational hypertension, preeclampsia and FGR on labor induction. In addition, logistic regression analysis was performed to ascertain the effects of maternal age, pre-pregnancy BMI, newborn weight, and induction of labor on three selected outcomes, i.e., likelihood of non-vaginal delivery, shoulder dystocia, and 5-min Apgar score <7. Statistical significance was defined as $p<0.05$. Ethical approval for the study was obtained from the CIPH Ethics Committee for Public Health Research, grant number 381-08-83-20-2.

RESULTS

A total of 36,632 deliveries in 2019 in Croatia were reported to CIPH using MBCs. Data regarding the onset of labor were missing in 29 (0.08%) MBCs and they were excluded from the study, leaving 36,603 deliveries for further analysis. Elective CS was performed in 4,576 cases and 253 labors were involved in perinatal death, excluding these cases from the analysis. Labor was induced in 5,153 (14.1%) deliveries, while the remaining 26,621 deliveries had a spontaneous onset.

General characteristics of women with spontaneous and induced onset of labor are presented in Table 1. Compared to women with spontaneous onset of labor, women with an induced onset were older, more frequently overweight before pregnancy, and were more frequent in the IOM GWG category above recommended. Differences in all these characteristics were statistically significant ($p<0.001$).

Table 1. *Characteristics of pregnant women with spontaneous and induced onset of labor in Croatia in 2019*

	Spontaneous onset	Induced onset	p-value
Age (years) (mean ± SD)	30.6±5.4	31.0±5.4	<0.001
<18	0.7%	0.3%	<0.001
18-35	82.3%	82.0%	
>35	17.0%	19.2%	
Pre-pregnancy BMI (kg/m2) (mean ± SD)	23.6±4.2	24.5±4.7	<0.001
<18.5	5.4%	3.6%	<0.001
18.5-24.9	66.9%	60.2%	
25.0-29.9	19.8%	24.1%	
≥30.0	7.9%	12.2%	
GWG; IOM category+ (kg) (mean ± SD)	13.8±5.4	14.1±5.5	<0.001
Below	21.3%	17.1%	<0.001
In range	35.5%	33.9%	
Above	43.2%	49.1%	
GA at delivery (weeks)++ (mean ± SD)	39.1±1.8	39.6±1.6	<0.001
<37	12.0%	8.10%	<0.001
37+0-38+6	13.3%	13.4%	
39+0-40+6	58.1%	46.5%	
41+0-41+6	13.8%	25.8%	
≥42	2.9%	6.2%	

SD = standard deviation; GWG = gestational weight gain; IOM = Institute of Medicine; GA = gestational age

+According to the Institute of Medicine weight gain recommendations. Available at: https://www.acog.org/Clinical-Guidance-and-Publications/Committee-Opinions/Committee-on-Obstetric-Practice/Weight-Gain-During-Pregnancy

++Classification of deliveries from 37 weeks into early term, full term, late-term and post-term according to recommendations from the Defining "Term" Pregnancy Workgroup (22)

Table 2. *Pregnancy outcomes of deliveries with spontaneous and induced onset in Croatia in 2019*

Pregnancy outcome	Spontaneous onset n (%)	Induced onset n (%)	p-value
Mode of delivery			<0.001
Vaginal	22304 (83.8)	3957 (76.7)	
Vacuum extraction	398 (1.5)	106 (2.1)	
Cesarean section	3918 (14.7)	1090 (21.2)	
Pregnancy complications			
Gestational diabetes	1311 (5.0)	534 (10.4)	<0.001
Gestational hypertension	308 (1.2)	190 (3.7)	<0.001
Preeclampsia	59 (0.2)	48 (0.9)	<0.001
FGR	253 (1.0)	199 (3.9)	<0.001
Labor complications			
Retained placenta	362 (1.4)	90 (1.7)	0.033
Postpartum hemorrhage	46 (0.2)	17 (0.3)	0.021
Shoulder dystocia	44 (0.2)	17 (0.3)	0.014
Episiotomy	7255 (27.3)	1240 (24.1)	<0.001
Neonatal complications			
BW <2500g	1134 (4.3)	200 (3.9)	0.215
BW >4000g	2903 (10.9)	731 (14.2)	<0.001
5-min Apgar <7	158 (0.6)	24 (0.5)	0.225
NICU admission	1234 (4.6)	214 (4.2)	0.118

FGR = fetal growth restriction; BW = birth weight; NICU = neonatal intensive care unit

Labor was induced more frequently in pregnancies with complications and this group had a higher incidence of CS and instrumental delivery (i.e., vacuum extraction). The induced labor group also had a higher incidence of all labor complications analyzed in this study. Women whose labor had been induced gave birth more frequently to infants with birth weight above 4000 g. All pregnancy complications and outcomes analyzed are presented in Table 2.

A logistic regression model with labor induction as outcome revealed that maternal age, maternal pre-pregnancy BMI, gestational age at delivery and pregnancy complications such as gestational diabetes, gestational hypertension, preeclampsia and FGR were significant predictors of induction of labor. This is presented as Model I in Table 3, R square was 6.3%.

Other logistic regression models analyzed the effects of maternal age, pre-pregnancy BMI, newborn weight and induction of labor on the likelihood of non-vaginal delivery, shoulder dystocia and 5-min Apgar score <7, and are presented in Table 3.

Although each of the variables in Model II (outcome of non-vaginal delivery) had a statistically significant effect on outcome (p<0.001), outcome prediction was highly limited due to the low R square (3.2%). Model III (outcome of shoulder dystocia) suggested that newborn weight was a significant predictor of shoulder dystocia [OR 1.002 (95% CI 1.001-1.002)], i.e., the odds of shoulder dystocia increase with birth weight increase. However, as in Model II, the R square in Model III was low (6.6%). Model IV (outcome of 5-min Apgar <7) suggested that newborn weight was a significant predictor of low 5-min Apgar score [OR 0.998 (95% CI 0.0997-0.998)], i.e., the odds of low 5-min Apgar decrease as birth weight increases. R square in Model IV was 25.6%. The models revealed that labor induction was not a significant predictor of either shoulder dystocia or low Apgar score.

Table 3. *Logistic regression models for Model I-IV, i.e., induction of labor, not delivering vaginally, shoulder dystocia, and 5-min Apgar <7 as outcomes*

Variable	Odds ratio	95% CI	p-value
Model I – outcome of induction of labor (R square 6.3%)			
Maternal age	1.020	1.013-1.026	<0.001
Maternal BMI	1.034	1.026-1.041	<0.001
Gestational diabetes	2.045	1.820-2.298	<0.001
Gestational hypertension	2.751	2.244-3.373	<0.001
Preeclampsia	4.022	2.617-6.181	<0.001
FGR	5.529	4.520- 6.763	<0.001
GA at delivery	1.269	1.238-1.301	<0.001
Model II – outcome of not delivering vaginally (R square 3.2%, $\chi 2$=505.629, p<0.001)			
Maternal age	0.983	0.977-0.989	<0.001
Maternal BMI	0.953	0.946-0.960	<0.001
Newborn weight	1.000	1.000-1.001	<0.001
Induction of labor	1.484	1.369-1.609	<0.001
Model III – outcome of shoulder dystocia (R square 6.6%, $\chi 2$=44.503, p<0.001)			
Maternal age	1.040	0.983-1.101	0.169
Maternal BMI	1.033	0.973-1.096	0.285
Newborn weight	1.002	1.001-1.002	<0.001
Induction of labor	0.686	0.353-1.333	0.266
Model IV – outcome of 5-min Apgar <7 (R square 25.6%, $\chi 2$=451.388, p<0.001)			
Maternal age	0.991	0.962-1.021	0.547
Maternal BMI	1.014	0.976-1.053	0.473
Newborn weight	0.998	0.997-0.998	<0.001
Induction of labor	1.070	0.657-1.741	0.787

BMI = body mass index; FGR = fetal growth restriction; CI = confidence interval

DISCUSSION

The prevalence of labor induction in Croatia of 14.1% is lower than in several other Western European countries where it is reported to be above 20% (5, 7), but similar to the prevalence of 13.8% in one clinical center in Croatia (21). Even though accurate comparison is difficult due to the lack of data on the modes of labor onset from the majority of European countries, it seems that the labor induction rate in Croatia is still below the European average. Several ideas might explain this. Obstetric practice possibly differs among countries where some national guidelines are more prone to labor induction compared to others. Also, there might be a difference in data collection; labor induction can be performed on a favorable and unfavorable cervix. It is possible that some analyses include both groups, whereas others only focus on labor induction with unfavorable cervices, without accentuating it in the Methods section.

Maternal overweight and high GWG, advanced maternal age, and prolonged pregnancy are factors previously found to be positively associated with labor induction (8-10). These observations were confirmed in this study accentuating the necessity of good preconceptional and antenatal care. Special emphasis should be given to educating women regarding weight management before and during pregnancy in order to keep the rates of labor induction within reasonable limits. A higher mean gestational age at delivery in the induced labor group was expected as the main indication for labor induction is prolonged pregnancy with the aim of avoiding post-term pregnancy risks (12).

Labor induction was performed more frequently in pregnancies with complications, and some of these complications were indications for labor induction. Our study also showed that pregnant women with gestational diabetes or gestational hypertension had a twofold greater risk of undergoing labor induction compared to pregnant women without these complications. These risks increase up to fourfold and fivefold for pregnancies burdened with preeclampsia or FGR. Therefore, in order to reduce the incidence of labor induction, additional preventive measures for reducing pregnancy complications should be implemented.

The association of labor induction with a higher incidence of CS found in this study is in concordance with some previously published studies (13,14) and contradictory to results from other studies (16,18). The higher incidence of CS in the induced labor group found in this study must be observed with caution as it might not be directly associated with labor induction but with various other antenatal factors. These include pregnancy complications, which necessitated labor induction in the first place, prolonged pregnancy, higher maternal age or BMI, neonatal weight above 4000 g, or others. Further studies are necessary to better evaluate these associations, possibly by comparing induction of labor with expectant management rather than spontaneous onset of labor. Regression analysis performed also suggests that the outcome of 'non-vaginal delivery' can be predicted with labor induction. However, due to the low explanatory power of the model used and a multitude of other risk factors unrelated to investigated variables, the results of this model should be interpreted with caution.

Other complications associated with labor induction identified in this study, such as instrumental delivery (i.e., vacuum extraction), shoulder dystocia, retained placenta and postpartum hemorrhage, are also im-

portant. These are partly linked to labor induction but also may arise from other antenatal and intrapartum factors. Nonetheless, all listed complications are significantly associated with the induced onset of labor, making such deliveries to fall in the high-risk category. Hence, the management of these labors requires additional attention, as well as careful and critical planning of labor induction. Increased neonatal weight represents a well-known risk factor for shoulder dystocia (23) but the association of neonatal weight with 5-min Apgar score values remains more complicated. Results of some studies disprove the negative effect of macrosomia on 5-min Apgar scores, while other studies corroborate it (24,25). The practice of labor induction has been shown by randomized controlled trials to benefit neonatal outcomes, reducing the incidence of fetal distress, stillbirth, and other postnatal complications (26,27). Further work and additional studies are necessary to better understand these associations.

Limitations of the study include the absence of data regarding the indications and methods of labor induction and the cervix status (favorable or unfavorable) prior to induction. Studies have concluded that these parameters significantly impact outcomes of labor induction and might influence the results. Also, it would have been interesting to have included data on the indications for CS in order to differentiate those directly related to labor induction from those that were not. The strength of this study is that it was a national-based study performed on the whole population of pregnant women and deliveries in Croatia in 2019. This large sample with no inclusion bias represents a valuable addition to understanding the risk factors and pregnancy outcomes associated with labor induction.

CONCLUSION

The prevalence of labor induction in Croatia in 2019 was 14.1%. Women whose labor was induced were older, had higher BMI, GWG above recommendations, and more frequent pregnancy complications. Induced labor was more often burdened with labor complications, which could partially be attributed to the mode of labor onset.

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SAŽETAK

UČESTALOST, RIZIČNI ČIMBENICI I ISHODI TRUDNOĆA KOD INDUKCIJE PORODAJA U HRVATSKOJ – NACIONALNO JEDNOGODIŠNJE ISTRAŽIVANJE

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Cilj: Indukcija porođaja važan je opstetrički zahvat koji se provodi u sve većem broju porođaja diljem svijeta s ciljem smanjenja perinatalnog pobola i smrtnosti. Cilj ovog istraživanja bio je utvrditi učestalost indukcije porođaja u Hrvatskoj te glavne rizične čimbenike i nepoželjne ishode povezane s indukcijom porođaja. ***Materijali i metode:*** Provedeno je presječno istraživanje pomoću podataka sakupljenih pri prijavi porođaja u Hrvatskom zavodu za javno zdravstvo u 2019. godini u Republici Hrvatskoj. ***Rezultati:*** Na ukupno 36.603 porođaja u 2019. godini u Republici Hrvatskoj učestalost indukcije porođaja bila je 14,1 %. Trudnice kod kojih je porođaj bio induciran bile su starije, imale su veći indeks tjelesne mase i češće prirast tjelesne težine u trudnoći iznad preporučenog u usporedbi s trudnicama koje su imale spontani početak porođaja (p<0,001 sve). Porođaj je češće induciran kod trudnoća s komplikacijama poput gestacijskog dijabetesa, gestacijske hipertenzije, preeklampsije i intrauterinog zastoja u rastu ploda (p<0,001 sve). Trudnice kojima je porođaj induciran češće su rodile carskim rezom, vakuum-ekstrakcijom, češće su imale postpartalno krvarenje, distociju fetalnih ramena te su češće rađale novorođenčad iznad 4000 g u usporedbi s trudnicama u kojih je porođaj započeo spontano (p<0,05). Logistička regresija pokazala je kako su dob trudnice i indeks tjelesne mase prije trudnoće, gestacijski dijabetes, gestacijska hipertenzija, preeklampsija, intrauterini zastoj u rastu ploda i gestacijska dob kod porođaja značajni prediktori indukcije porođaja (p<0,001 sve). ***Zaključak:*** Učestalost indukcije porođaja u Hrvatskoj je 14,1%. Indukcija porođaja povezana je s važnim rizičnim čimbenicima i nepoželjnim perinatalnim ishodima koji se djelomice mogu pripisati načinu početka porođaja. O svemu navedenom treba voditi računa kada se savjetuje i planira navedeni opstetrički zahvat.

Ključne riječi: indukcija porođaja, učestalost, rizični čimbenici, carski rez, logistička regresija

EPIDEMIOLOGICAL DATA ON RENAL BIOPSIES IN SOUTHERN CROATIA – A SINGLE CENTER REPORT OF 22-YEAR EXPERIENCE AT SPLIT UNIVERSITY HOSPITAL CENTER

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The Croatian Registry of Native Renal Biopsy (CRNRB) was established in 2019. Thus, in this study, we present retrospective data on kidney biopsies in adult patients performed at the Split University Hospital Center from 1994 to 2019 before the CRNRB establishment. The aim of the study was to show epidemiological data on glomerular diseases in southern Croatia in order to compare them with others and provide data for the establishment of the CRNRB. During the study period, 110 patients (mean age 46.6±15.4, age range 17-76 years), 68 men and 42 women, underwent renal biopsy at the Department of Internal Medicine, Split University Hospital Center in Split. Data on age, sex, serum creatinine, urinalysis, daily proteinuria, and complications after biopsy were collected and related to indication for biopsy and pathological diagnosis. Light and immunofluorescence analysis was supplemented by electron microscopy in 63.5% of cases. Indications for biopsy were nephrotic syndrome (64.5%), asymptomatic urinary tract abnormalities (12.7%), and acute renal failure of unknown cause (9.1%). The most common diagnosis was IgA nephropathy (IgAN) (20.9%), the prevalence of which decreased during the study period. IgAN was followed by focal segmental glomerulosclerosis (FSGS) (19.1%), membranous nephropathy (13.6%), lupus nephritis and minimal change disease (8.2%), crescentic glomerulonephritis (5.4%), membranoproliferative glomerulonephritis (4.5%), mesangial proliferative glomerulonephritis (3.6%), amyloidosis (3.6%), Henoch-Schönlein nephritis (3.6%), and Alport syndrome (2.7%). Other forms of glomerular diseases were rarely found. IgAN was most frequently found in men (26.5%) and FSGS in women (21.4%). These data can be included in the historical epidemiological observation of glomerular diseases in Southeastern Europe. The guidelines for performing biopsies need to be constantly updated to improve preventive and therapeutic strategies.

Key words: biopsy-proven renal disease, epidemiology, glomerulonephritis, renal biopsy, renal pathology, registry

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INTRODUCTION

There are more than one million end-stage renal disease (ESRD) patients worldwide (1-3), and the prevalence and incidence of chronic kidney disease and ESRD show a rising trend. The number of patients requiring chronic replacement therapy is expected to increase by 60% by 2030 (4). Renal biopsy remains the gold standard for diagnosis (5), therapeutic management, and outcome prediction in patients with renal parenchymal disease. Unfortunately, there is currently little consensus on the proper indications and clinical utility of this procedure (6), and the decision to perform renal biopsy is usually based on personal opinion and/or individual center policy. Glomerular disease is on the rise and currently ranges from 6.5 to 27 persons/million person/year (p.m.p) (1, 2, 4). In the last report of the ERA-EDTA registry (7), the average

prevalence and incidence of dialysis patients without specific renal diagnosis was 15% to 16%, ranging from 1.5% in Croatia to 38% in Romania. The highest biopsy rates to date have been reported in the Australian database (215 p.m.p./year) (8) and in the experience of individual centers at Helsinki University Hospital, Finland (176 p.m.p./year) (9) and Olmsted County, USA (up to 175 p.m.p./year) (10). In contrast, very low rates have been reported from a regional database in Romania (11.3 p.m.p./year) (11). The lack of clear guidelines for the indication of renal biopsy may hinder the epidemiological classification of kidney disease, as well as the future validation of biomarkers (2).

Therefore, it is necessary to use uniform guidelines for renal biopsies, which will improve regional renal biopsy registries that are an important source for epidemiological studies. These registries would be a useful tool for planning a preventive and therapeutic approach to reduce the burden of patients with chronic kidney disease.

Data are available on national registries of biopsy-proven kidney disease established in Italy (12, 13), Denmark (14), Brazil (15), Spain (16, 17), Czech Republic (18, 19) and Saudi Arabia (20). In addition, there are a number of macroregional reports from individual centers and limited national registries for renal biopsies in several other countries (2). The Croatian national registry of native kidney biopsies was established through the efforts of colleagues from all Croatian centers and represents a promising basis for epidemiological data (21).

The aim of this study was to collect epidemiological data on kidney disease in the Dalmatian area of Croatia before establishment of this registry. These data would provide a source of useful information on the prevalent underlying disease and the opportunity to improve protocols for preventive medicine and therapeutic approach. In addition, combining data with renal replacement therapy registries would be a useful strategy for evaluating long-term outcomes of patients with kidney disease in our country, as well as in neighboring parts of Europe.

MATERIALS AND METHODS

The study was performed with the approval of the Ethics Committee of the School of Medicine, University of Split, in accordance with the Declaration of Helsinki. Data were obtained retrospectively from hospital records and pathological data from the Department of Pathology on all biopsies performed at the Department of Nephrology and Dialysis, Split University Hospital Center from 1996 to 2019. During this period, 110 biopsies were performed, 68 in men and 42 in women.

For each case, the following data were collected: age, sex, clinical and histopathologic diagnosis, levels of nitrogen waste products, urinalysis, 24-hour proteinuria, glomerular filtration rate (measured creatinine clearance using a laboratory referral protocol), and relevant clinical data. Renal tissue cylinders were processed according to standardized procedures. Light microscopy (LM) and immunofluorescence (IF) were analyzed at the Department of Pathology, Split University Hospital Center, and electron microscopic analysis (EM) was performed at the Department of Pathology, School of Medicine, University of Zagreb. Glomerular diseases were classified according to the relevant pathological literature (22). The following clinical data were collected: indication for renal biopsy, arterial pressure, presence of nephrotic (NS) or nephritic syndrome (NeS), and complications of renal biopsy.

Biopsies were indicated by a nephrologist and performed under local anesthesia after routine examination had been performed previously. Complete medical history was also obtained and physical examination, including blood pressure measurement, was performed. A series of necessary laboratory tests were performed, such as complete blood count and coagulation parameters. All biopsies were performed using a percutaneous, automated, spring-loaded biopsy instrument (Biopty TM) under real-time ultrasound guidance and a 14-G biopsy needle.

The indications for biopsy were as follows: nephrotic proteinuria (≥3.5 g/dU) (64.5%), asymptomatic urinary abnormalities (AUA) defined with proteinuria of non-nephrotic range with or without hematuria (12.7%), unexplained acute renal failure (ARF) (clinically 'silent' reduction of glomerular filtration rate which lasts for days and weeks, without arterial hypertension and with various urinalysis changes) (9.1%), kidney disorders in patients with diverse clinical presentations of systemic vasculitis (4.5%), nephritic syndrome (NeS) (dysmorphic erythrocyturia, various ranges of proteinuria, arterial hypertension and glomerular filtration rate reduction) (2.7%), kidney biopsy in patients with systemic lupus erythematosus (SLE) in order to adjust therapeutic protocol (2.7%), and isolated hematuria (IH) (0.9%). We took blood pressure ≥140/90 mm Hg as arterial hypertension.

Ethical statement

Split University Hospital Center Ethics Committee, File No. 2181-147-01/06/M.S.-19-2.

Statistical analysis

All analyses were performed with SPSS statistical software package (version 23.0). Continuous variables were expressed as median with interquartile range and categorical variables as frequency and percent.

RESULTS

During the study period, 110 adult Caucasian patients (68 men and 42 women) underwent percutaneous renal biopsy. Their mean age was 46.6±15.4 (range 17-78) years. Mean creatinine at the time of the procedure was 150.5 μmol/L, and mean daily proteinuria 6.58 g/d (Table 1). The most common indication for biopsy was NS in 71 (64.5%) patients. AUA was the indication for biopsy in 14 (12.7%) and ARF in 10 (9.1%) patients. Clinical presentation of systemic vasculitis (4.5%) and acute NeS (2.7%) were the least common reasons for biopsy. Pathological urine findings with or without renal function worsening were the reason for biopsy in 3% of patients with SLE (Figure 1).

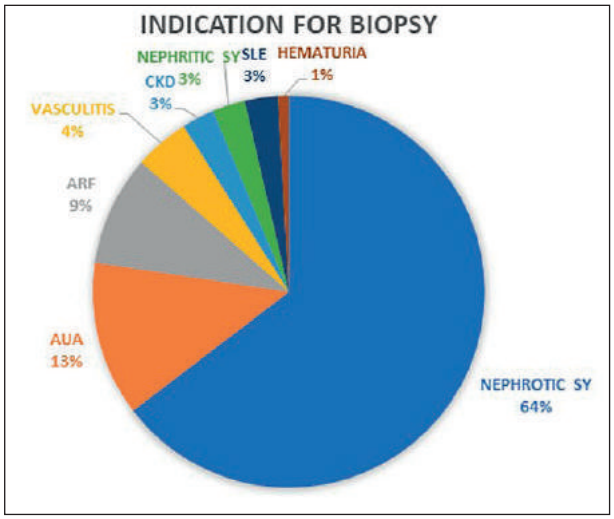

FIGURE 1. *Indication for renal biopsy. The most frequent indication for biopsy was NS, found in 71 patients (64.5%). AUA was the indication for a biopsy in 14 (12.7%) and ARF in 10 patients (9.1%).*

Legend: AUA: Asymptomatic urinary abnormalities; ARF: Acute renal failure; SLE: Systemic lupus erythematosus; SY: syndrome; CKD: chronic kidney disease

Complete pathological analysis was performed on all 110 specimens, with the exception of electron microscopy, which was performed in only 73 (64%) cases. Figure 2 shows relative distribution of renal disease diagnosed on biopsy. The most common renal disease detected by biopsy was IgA nephropathy (IgAN) in 23 (20.9%) cases, followed by focal segmental glomerulosclerosis (FSGS) in 21 (19.1%), membranous nephropathy (MGN) in 15 (13.6%), lupus nephritis (LN) and minimal change disease (MCD) in 9 (8.2%), and membranoproliferative glomerulonephritis (MPGN) in 5 (4.5%) patients. Four patients had mesangial proliferative glomerulonephritis (MePGN) (3.6%) and amyloidosis (AM) (3.6%) in their histopathologic diagnosis. Four (3.6%) patients with clinical signs of systemic vasculitis were diagnosed with Henoch-Schönlein purpura (HSP). Crescentic glomerulonephritis (CGN) (ANCA-related and anti-GBM) accounted for 6 (5.4%) cases. Hereditary nephritis including Alport syndrome (AS) and thin membrane disease (TMD) was detected in 4 (3.6%) cases. Other renal diseases were rarely diagnosed, e.g., IgM glomerulonephritis (IgMGN), endoproliferative glomerulonephritis (EPGN), C1q nephropathy, tubulointerstitial nephritis (TIN), and myeloma kidney (MK), each of which was diagnosed in 1 (0.9%) patient.

Regarding sex, IgAN (78.3% *vs.* 21.7%) and FSGS (57.1% *vs.* 42.9%) were more common in men, in contrast to LN, which was more common in women (88.9%). FSGS was the most common finding in women overall (21.4%) and in men with IgAN (26.5%).

Table 1. *Clinical characteristics of most common glomerulonephritis*

GN	Mean age (years)	Proteinuria (g/dU)	Creatinine clearance (mL/s)	NS (%)	Hypertension (%)	ARF (%)	Male (%)
IgA GN	42.5	5.36	1.01	52.1	90	4.3	78.3
FSGS	51.1	6.48	1.24	85.7	68.4	4.8	57.1
LN	49.3	5.67	1.3	55.5	55	-	11.1
MGN	50.4	9.62	1.49	73.3	57.1	6.7	66.7
MPGN	57.8	10.44	0.79	100	100	-	60
MCD	42.2	11.47	1.37	100	12.5	-	60

IgA GN: IgA glomerulonephritis; FSGS: focal segmental glomerulosclerosis; LN: lupus nephritis; MGN: membranous glomerulonephritis; MPGN: membranoproliferative glomerulonephritis; MCD: minimal change disease; ARF: acute renal failure

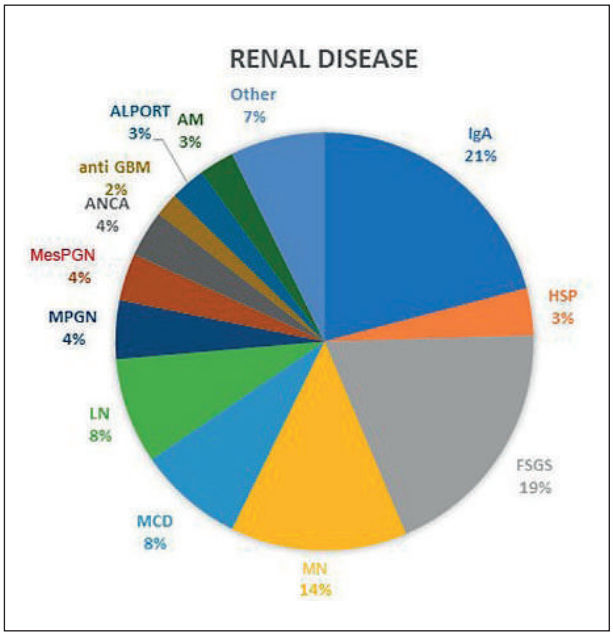

FIGURE 2. *The frequency of certain diagnoses made by kidney biopsy. The most frequently biopsy-proven renal disease was IgAN, found in 23 specimens (20.9%), followed with FSGS in 21 patients (19.1%), MGN in 15 (13.6%), LN and MCD in 9 patients (8.2%), and MPGN in 5 patients (4.5%).*

Legend: IgA: IgA nephropathy; FSGS: focal segmental glomerulosclerosis; MGN: membranous glomerulonephritis; LN: lupus nephritis; MCD: minimal change disease; MPGN: membranoproliferative glomerulonephritis; MePGN mesangial proliferative glomerulonephritis; ANCA: neutrophil cytoplasmic antibody-associated glomerulonephritis; anti GBM: anti-glomerular basement membrane disease.

In patients with IgAN, the mean age was 42.5 (range 19-66) years, mean proteinuria 5.36 (range 0.92-24.5) g/dU, and mean creatinine clearance 1.01 (range 0.13-2.35) mL/s. The most common clinical presentation was NS (52.1%), followed by AUA (21.7%), chronic renal failure (CRF) (8.7%), ARF, NeS, and isolated hematuria (4.3% each). Arterial hypertension was present in 90% of patients.

Among patients with the clinical picture of nephrotic syndrome (NS), the most common pathohistological finding was FSGS in 18 (25.4%), IgAN in 12 (16.9%), MGN in 11 (15.5%), MCD in 9 (12.7%), MPGN in 5 samples (7%), followed in decreasing order by LN and AM in 4 cases each (5.6%) (Figure 3).

Asymptomatic urine abnormalities were found in 5 (35.7%) patients with IgAN and 3 (21.4%) patients with MGN; FSGS and AS were each found in 2 (14.3%) samples (Figure 4).

The most common histopathologic findings in patients with equivocal ARF, involving 20% each, were anti-GBM and ANCA-related CGN.

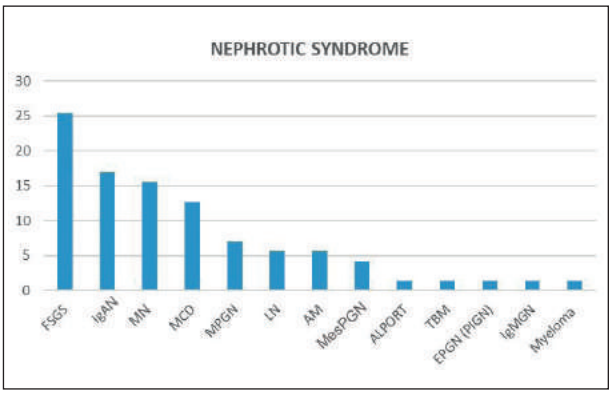

FIGURE 3. *Frequency of nephrotic syndrome in certain entities. Among patients with clinical presentation of nephrotic syndrome, the most often pathohistological finding was FSGS in 18 (25.4%), IgAN in 12 (16.9%), MGN in 11 (15.5%), MCD in 9 (12.7%), MPGN in 5 specimens (7%), followed in decreasing order with LN and AM, each in 4 cases (5.6%).*

Legend: FSGS: Focal segmental glomerulosclerosis; IgA: IgA nephropathy; FSGS: focal segmental glomerulosclerosis; LN: lupus nephritis; MCD: minimal change disease; MesPGN: mesangial proliferative glomerulonephritis; ANCA: neutrophil cytoplasmic antibody-associated glomerulonephritis; anti GBM: anti-glomerular basement membrane disease; IgMGN: IgM glomerulonephritis; Anti-Tubular Basement-Membrane (TBM) nephritis; EPGN (PIGN): proliferative endocapillary glomerulonephritis; MGN: Membranous glomerulonephritis; MCD: Minimal change disease; MPGN: Membranoproliferative glomerulonephritis

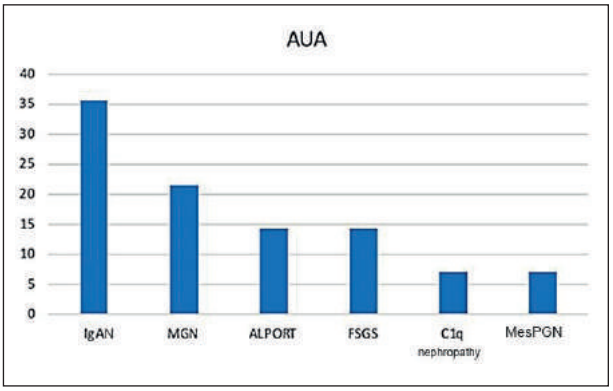

FIGURE 4. *Frequency of certain entities in patients with Asymptomatic urinary abnormalities. AUA were presented in 5 patients with IgAN (35.7%), 3 patients with MGN (21.4%); FSGS and AS were each found in 2 specimens (14.3%).*

Legend: AUA: Asymptomatic urinary abnormalities; IgAN: IgA glomerulonephritis; MGN: Membranous glomerulonephritis; FSGS: Focal segmental glomerulosclerosis; Alport : Alport syndrome; MesPGN: mesangial proliferative glomerulonephritis

Only one serious clinical complication requiring intervention was recorded, i.e., retroperitoneal hemorrhage, which was successfully resolved by an interventional radiological procedure.

DISCUSSION

Numerous reports have been published describing the incidence, correlation of clinical epidemiological data, and histopathologic findings from various renal biopsy databases throughout the world (2, 8-29). Unfortunately, the results are not always easy to compare for several reasons such as inconsistencies in biopsy indications, different time frames for different epidemiological studies, lack of renal biopsy registration due to voluntary data collection, and inconsistencies in the definition of a clinical syndrome and histopathologic classification (2). Some reports included pediatric and adult populations (12, 13, 15-19), whereas others included adults exclusively (11, 20, 21). In addition, some reports collected data exclusively on glomerular biopsy-proven kidney disease (2, 21). In the present paper, we present the results of a 22-year retrospective study of biopsy-proven glomerular and non-glomerular kidney disease in adults older than 18 years because colleagues from the Department of Pediatrics, Split University Hospital Center had previously presented their own data (23, 29). One of the main difficulties in comparing epidemiological data is different approach resulting from different practice of renal biopsy in different countries, as well as in different centers of the same country, partly due to socioeconomic status and availability of biopsy. A wide range of indications for biopsy, ranging from a relatively liberal approach to performing biopsy only when therapy adjustment is required based on histopathologic findings, results in a wide range of biopsy rates, ranging from 215 p.m.p./year, as reported in an Australian database (8) to 176 p.m.p./year at Helsinki University Hospital, Finland (9). Disagreement about the need of biopsy is particularly important in the case of AUA syndrome. In addition, there are different definitions of AUA syndrome, as well as of other clinical renal syndromes used in different epidemiologic reports, which may pose another difficulty for appropriate comparison. In our report, as well as in some other reports (11, 24), the term AUA is used for non-nephrotic proteinuria, isolated or in association with hematuria; in other reports, macroscopic hematuria is considered a separate syndrome (13, 15, 16, 19). AUA syndrome is the most common reason for biopsy reported in two national registries (12, 18, 19), two macroregional reports (9, 15), and in a single-center database (24). In our study, AUA was the second most common reason for biopsy (12.7%). The most common underlying histopathologic finding in the AUA group was IgAN (35.7%), followed by MGN (21.4%), FSGS, and AS (14.3%). IgAN was also diagnosed in single cases of IH (1%). This is consistent with the results of histopathologic findings in two large national registries in the Czech Republic and Italy (12, 18, 19), where IgAN was the most common finding in patients with AUA. IgAN as isolated renal disease or as systemic vasculitis with renal involvement (HSP) was the most common histopathologic finding (24%) in our study. These findings are consistent with 6 of 8 reports from national registries (Italy, Spain, Denmark, Scotland, Czech Republic, and Japan) (12-14, 16-19, 28), 3 macroregional ones (western France, Finland, Victoria-Australia) (8, 9, 27), and in more single-center databases (2). In contrast, FSGS has been reported as the most important histopathologic pattern in African American and Hispanic populations, with an increasing trend in adult Caucasians in the United States (10, 25, 30, 31), and in Brazil and Uruguay (15, 32). Different results have been reported from other European countries; MGN was the predominant histopathologic finding in Macedonia (33), and MPGN in Romania (11). As it is well known, IgAN occurs predominantly in AUA, and in countries with strict biopsy policies, the incidence and prevalence depend largely on biopsy practice. Even in Croatia, different centers have different approaches to the need of renal biopsy, with the two largest centers in northern Croatia taking a more liberal stance. In our center, we have so far accepted the expectation attitude and perform biopsies only in cases where diagnosis is not possible based on clinical data and other relevant tests, e.g., unexplained renal failure, worsening of glomerular filtration rate, and/or worsening of daily proteinuria despite ongoing treatment. This might be a reason for the underestimation of IgAN in our study. Of note, the most common clinical presentation in the group of patients with an underlying pattern of IgAN in our study was NS (52.1%), which is somewhat unexpected and in contrast to most other studies in which AUA was the most common clinical presentation (12, 13, 15-17, 21). This could be due to the fact that performing biopsy earlier in the disease course could change the clinical presentation.

Nephrotic syndrome was the most common indication for performing biopsy in 64.5% of cases. We accept proteinuria above 3.5 g/dU as a sufficient criterion, with or without other clinical manifestations. Our results are consistent with most registry and database reports (2, 34), the results of three national registries (15, 17, 32), three macroregional studies (11, 21, 35), and additional reports from individual centers (2). The most common histopathologic diagnosis underlying nephrotic proteinuria was FSGS (25.4%), followed by IgAN (16.9%), MGN (15.5%), and MCD (12.7%). In the United States, a high prevalence with increasing trend of biopsy-proven primary FSGS has been reported in the African American population (10, 23, 25), and recent data show an increasing trend of incidence in the Caucasian population (10, 25). FSGS, followed by MGN, is the most common histologic pattern reported in Croatia (35). Our findings are in contradiction with most other study reports (MGN as the most common underlying histopathologic pattern of NS (12-14, 26).

In our study, MGN was the third most common histopathologic finding, accounting for 14% of cases. In the literature, reports on MGN are generally inconsistent. Although MGN is the most common GN in the elderly population, and considering the increase in life expectancy and aging of the population, there are reports on a lower proportion of MGN in primary GN, for unclear reasons (30). In contrast, MGN was the second most common primary GN in the Italian national registry (13), and an increase in the proportion of MGN among primary GN has been reported in the Spanish national registry (17).

Uniformity in the use of IF and EM in confirming the histopathologic diagnosis is another difficulty in comparing the results of particular epidemiological studies. These techniques, especially EM, are limited, mainly because of the low economic resources in some countries (2). It is known that IF is important for confirming the histopathologic diagnosis and some clinical entities, such as IgAN and pauci-immune glomerulonephritis. It could be speculated that an increase in the incidence of IgAN may be due to an increase in the IF technique. On the other hand, EM is an important technique to confirm the correct diagnosis of MCD and inherited diseases and to distinguish primary from secondary FSGS. As previously reported, ultrastructural analysis using EM was required in 21% of cases to confirm the correct diagnosis, in addition to LM and IF. Thus, the availability of a particular technique could influence the histopathologic pattern. Reports on the use of IF are incomplete in most studies, with few exceptions, i.e., Spain 90% (17), Denmark 78% (14), and a regional report in Croatia 100% (35). According to the available data, EM is used even less frequently, e.g., in Italy 38% (13), Brazil 9% (15), Spain 23% (17) and Croatia 100% (35). We routinely performed IF in all samples, in contrast to EM, which was performed in 66% of cases, although EM could not be performed in our center so far. The missing data from EM were mainly due to technical problems. The most common histopathologic findings in patients presenting with ARF were CGN, ANCA, and anti-GBM (5.4%). Of note, unusual histopathologic findings such as FSGS and MGN were found in a certain percentage of patients. The other primary GN were found less frequently in our study. We found combined histopathologic patterns in seven patients; FSGS was combined with TMD in three cases, and we found renal vasculitis and TMD in one sample; HSP, MCD, and MGN were combined with acute TIN in one case each. LN was the most common secondary GN, found in 8% of cases, predominantly in women (88.9%). Similar results have been reported in larger national and macroregional registries and in single-center registries (8, 11, 13, 16, 18, 25). In Czech Republic, Japan, and Scotland, diabetic nephropathy has been reported as the predominant secondary form of biopsy-proven glomerular disease (2). Only one clinically serious complication of biopsy was recorded (0.9%); the frequency is consistent with reports (5).

We found that the pattern of distribution in our study was largely similar to that reported from other European countries (2, 9, 16, 17, 19, 33). We might even suspect more differences due to our overly restrictive biopsy practice and low biopsy rate, with the possibility that some histopathologic patterns are underestimated. Therefore, it was necessary to expand the spectrum of indications for diagnosis in order to establish a Croatian national registry of biopsy-proven kidney disease comparable to others in Europe and worldwide. Different data observed in different studies could be due to geographic heterogeneity, changes in environmental and lifestyle factors, and biopsy practice.

CONCLUSION

In conclusion, longitudinal follow-up, observing changes in the clinicopathologic pattern of kidney disease in separate centers/national registries, and combining these data with registries of renal replacement therapies would be a particularly useful tool to assess long-term outcomes in our patients and potential outcomes after renal transplantation. Our data can contribute to the guidelines for performing kidney biopsies because these inputs can help improving preventive and therapeutic strategies, as well as testing appropriate preventive and therapeutic measures to reduce the burden of chronic kidney disease.

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SAŽETAK

EPIDEMIOLOŠKI PODATCI BUBREŽNIH BIOPSIJA U JUŽNOJ HRVATSKOJ – IZVJEŠTAJ O 22-GODIŠNJEM ISKUSTVU KBC-A SPLIT

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Hrvatski registar nativne bubrežne biopsije (CRNRB) uspostavljen je 2019. godine. Stoga u ovom istraživanju prikazujemo retrospektivne podatke biopsija bubrega odraslih bolesnika KBC-a Split obavljenih od 1994. do 2019. godine prije uspostave CRNRB-a. *Cilj rada* bio je prikazati epidemiološke podatke o glomerularnim bolestima u južnoj Hrvatskoj radi usporedbe s drugima i dobivanja podataka za uspostavu CRNRB-a. U promatranom razdoblju 110 bolesnika (raspon dobi 17-76 godina, srednja dob 46,6±15,4 godina), 68 muškaraca i 42 žene, bilo je podvrgnuto biopsiji bubrega na Klinici za unutarnje bolesti KBC-a Split. Podatci o dobi, spolu, kreatininu u serumu, analizi mokraće, dnevnoj proteinuriji i komplikacijama nakon biopsije prikupljeni su i povezani s indikacijom za biopsiju i patološkom dijagnozom. Analiza svjetlosnom mikroskopijom i imunofluorescencijom dopunjena je elektronskom mikroskopijom u 63,5 % slučajeva. Indikacije za biopsiju bile su nefrotski sindrom (64,5 %), asimptomatske abnormalnosti mokraćnog sustava (12,7 %) i akutno zatajenje bubrega nepoznatog uzroka (9,1 %). Najčešća dijagnoza bila je IgA nefropatija (IgAN) (20,9 %), učestalost koje se smanjila tijekom promatranog razdoblja. Nakon IgAN-a slijede žarišna segmentna glomeruloskleroza (FSGS) (19,1 %), membranska nefropatija (13,6 %), lupusni nefritis i bolest minimalnih promjena (8,2 %), polumjesečasti glomerulonefritis (5,4 %), membranoproliferativni glomerulonefritis (4,5 %), mezangijski proliferativni glomerulonefritis (3,6 %), amiloidoza (3,6 %), Henoch-Schönleinov nefritis (3,6 %) i Alportov sindrom (2,7 %). Drugi oblici glomerularnih bolesti rijetko su nađeni. IgAN je najčešće nađen u muškaraca (26,5 %), a FSGS u žena (21,4 %). Ti se podatci mogu uključiti u povijesno epidemiološko promatranje glomerularnih bolesti u jugoistočnoj Europi. Smjernice za izvođenje biopsije bubrega potrebno je stalno ažurirati kako bi se poboljšale preventivne i terapijske strategije.

Ključne riječi: biopsijom dokazana bubrežna bolest, epidemiologija, glomerulonefritis, biopsija bubrega, patologija bubrega, registar

ONEČIŠĆENJE ZRAKA I ASTMA

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Prema podatcima Svjetske zdravstvene organizacije većina populacije živi u područjima nezadovoljavajuće kvalitete zraka, što utječe na ljudsko zdravlje, posebno na dišni sustav. Astma je kronična bolest dišnog sustava karakterizirana hiperaktivnošću bronha koja je posredovana upalom. Kratkoročna izloženost onečišćenju zraka povećava pojavu simptoma i egzacerbacija u osoba oboljelih od astme, povećava broj posjeta hitnim službama i hospitalizacija te smrtnost od bolesti, a neke komponente smanjuju plućnu funkciju. Novijim je istraživanjima pokazano da onečišćenje zraka djeluje i na povećanje prevalencije astme u djece. Vjeruje se da su glavni mehanizmi kojima je posredovano nepovoljno djelovanje onečišćenja zraka povećanje oksidativnog stresa u dišnom sustavu pojedinca, promjena odgovora imunološkog sustava, aktivacija upale, povećanje osjetljivosti na utjecaj alergena te remodeliranje dišnih puteva. Onečišćenje zraka neće jednako djelovati u svih zdravih pojedinaca i osoba oboljelih od astme. Ovi će učinci biti izraženiji u genetski predisponiranih pojedinaca, a smatra se da važnu ulogu u posredovanju ovih učinaka imaju i epigenetski mehanizmi. Intervencijama na globalnoj i lokalnoj razini mogli bismo utjecati na poboljšanje kvalitete života i ishoda milijuna ljudi oboljelih od astme, ali i zdravih pojedinaca. Intervencije na globalnoj razini uključuju smanjenje emisija štetnih plinova i čestica putem prometa, industrije i poljoprivrede, okretanje obnovljivim i čišćim izvorima energije, a na lokalnoj razini javna upozorenja o onečišćenju zraka, edukacija bolesnika, razvoj prometne mreže biciklističkih staza i pješačkih zona.

Ključne riječi: astma, onečišćenje zraka, egzacerbacije astme, upalni putovi, genetski i epigenetski čimbenici

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GLAVNE ZNAČAJKE ASTME

Astma je kronična bolest dišnog sustava koja je karakterizirana hiperreaktivnošću bronha koja je posredovana upalom. Očituje se simptomima dišnog sustava – kašljem, zviždanjem, otežanim disanjem, stezanjem u prsima. U dječjoj dobi češća je u muškog spola, dok je astma koja se javlja u odrasloj dobi učestalija u žena. Najviše prevalencije ove bolesti bilježe se u razvijenim zemljama svijeta (Švedska, Ujedinjeno Kraljevstvo, Australija, Sjedinjene Američke Države) (1-4). S druge strane, smrtnost od posljedica astme najviša je u slabije razvijenim zemljama (5). Hrvatska je prema ukupnom broju oboljelih od astme unutar svjetskog prosjeka, s prevalecijom bolesti od 5 % (6).

Riječ je o heterogenoj bolesti koju čini više fenotipova, a sukladno tome, različiti patofiziološki mehanizmi utječu na njen razvoj. Nastaje međudjelovanjem genetskih i okolišnih čimbenika, ali još uvijek nije sasvim jasno u kojoj mjeri i koji sve čimbenici imaju utjecaj. Neki od poznatih etioloških čimbenika koji povećavaju rizik za razvoj astme su prematuritet, mala porođajna težina, starija dob majke pri porođaju, zagađenje zraka, izloženost duhanskom dimu, pretilost, alergijska atopija, učestale virusne infekcije (7).

Kronična upala u podlozi astme nastaje poremećenjem ravnoteže proupalnih i protuupalnih čimbenika. Mnoge upalne stanice sudjeluju u ovom procesu (mastociti, neutrofili, eozinofili, limfociti), ali glavnu ulogu imaju T pomoćnički limfociti (Th1 i Th2 stanice). Aktivacija tih stanica rezultira otpuštanjem citokina kao što su interleukin (IL) 2, IL-4, IL-5, IL-9 i IL-13 te interferona alfa (α). Ovi upalni medijatori reguliraju kaskadu događaja koja dovodi do nakupljanja upalnih stanica u području mukoze i submukoze bronha, otpuštanja drugih medijatora upale (histamin, leukotrieni, prostaglandini), hipertrofije glatkog mišićja bronha te povećane produkcije sluzi uz smanjenu učinkovitost njenog odstranjivanja (poremećen mukocilijarni transport). Posljedično nastaje hiperreaktivnost bronha koja varira u vremenu i intenzitetu. Dugoročno, ona posredovanjem procesa remodulacije, može dovesti do trajnog, ireverzibilnog suženja bronha. Čimbenici koji provociraju pojačavanje upale i suženje bronha, uzrokujući egzacerbaciju astme su virusne infekcije, alergeni, onečišćenje zraka, tjelesni napor, emocionalni stres, a u nekih bolesnika i acetilsalicilna kiselina (7). Dugo je u znanstvenim krugovima bila prihvaćena hipoteza higijene, prema kojoj rano izlaganje mikrobiološkim čimbenicima preusmjerava odgovor imunološkog sustava s Th2 prema Th1 stanicama, čime se sprječava alergijski odgovor i smanjuje vjerojatnost nastanka astme. Hipotezu su podržavali podatci o većoj prevalenciji astme u razvijenim zemljama, gdje su uvjeti života doveli do boljih higijenskih uvjeta i manje izloženosti djece infektivnim agensima, no danas se zna da je patofiziologija astme mnogo kompleksniji proces nego što je to ova hipoteza dala naslutiti (8).

VRSTE I IZVORI ONEČIŠĆENJA ZRAKA

Onečišćenje zraka odnosi se na pojavu čestica i plinova u zraku koji mogu nepovoljno djelovati na ljudsko zdravlje. Posredovano je prirodnim procesima (šumski požari, vulkani, pelud biljaka) te antropogenim djelovanjem (poljoprivreda, promet, industrija), što je postalo posebno značajno nakon industrijske revolucije u 19.stoljeću. Glavne sastavnice onečišćenja u atmosferi mogu se podijeliti na lebdeće čestice (engl. *particulate matter,* PM) i plinove. Najznačajniji plinoviti onečišćivači su ozon (O_3), sumporni dioksid (SO_2), dušikov dioksid (NO_2), ugljikov monoksid (CO) i dioksid (CO_2) te hlapljivi organski spojevi. Oni se najvećim dijelom otpuštaju u atmosferu izgaranjem fosilnih goriva, ali mogu i nastati u zraku reakcijama iz prekursora. Tako na primjer, ozon nastaje u atmosferi djelovanjem UV zračenja na dušikov monoksid, hlapljive organske spojeve, druge plinovite prekursore. Lebdeće su čestice mješavina sitnih kapljica ili krutih tvari, koje uključuju prašinu, čađu, fragmente bakterija, dim, metale i drugo. Radi metoda mjerenja kvalitete zraka podijeljene su u nekoliko kategorija prema promjeru čestica. Grube čestice (PM_{10}) promjera su 2,5 do 10 μm, fine čestice ($PM_{2.5}$) između 2,5 i 0,1 μm, a ultra fine čestice ($PM_{0.1}$) su manje od 0,1 μm. Ova podjela ima i kliničko značenje budući da o veličini čestica ovisi dubina njihovog prodiranja u dišni sustav.

Prema podatcima Svjetske zdravstvene organizacije, 2019. godine 99 % svjetske populacije živjelo je u područjima gdje kvaliteta zraka nije bila zadovoljavajuća, a najlošiju kvalitetu zraka imaju slabije razvijene zemlje (Bangladeš, Indija, Pakistan, Afganistan).

Dozvoljene količine pojedinog onečišćivača u atmosferi propisane su zakonski, a Hrvatska je, kao država članica, podložna i direktivama Europske unije. Prema podatcima više od 70 mjernih postaja za praćenje kvalitete zraka u Hrvatskoj iz 2021. godine, onečišćenje zraka najprisutnije je u urbanim područjima kontinentalnog dijela zemlje (Slavonski Brod, Sisak, Kutina, Zagreb). Najveći su problem u Hrvatskoj lebdeće čestice u zraku, a s obzirom na nepovoljan geografski položaj veliki dio tog zagađenja potječe iz susjednih država. Najviše se koncentracije onečišćivača u atmosferi bilježe u hladnijem dijelu godine s iznimkom ozona koji ovisi o Sunčevu zračenju te je stoga najviši u ljetnim mjesecima i to u području Istre i Dalmacije.

Da smo daleko od zadovoljavajuće kontrole kvalitete zraka ukazuju sljedeći podatci. Za PM_{10} lebdeće čestice prema Zakonu o zaštiti zraka dozvoljena koncentracija s obzirom na zaštitu zdravlja ljudi iznosi do 50 μg/m^3 na dnevnoj bazi, a unutar godine dana ne bi smjela biti prekoračena u više od 35 puta. Ipak, u 2021. godini dozvoljena je koncentracija u Osijeku prekoračena ukupno 91 dan, u Koprivnici tijekom 39 dana, u Kutini 48 te u Sisku tijekom 50 dana. U navedenim su područjima dozvoljene koncentracije prekoračene u svim godinama mjerenja, od 2013. do 2021. godine. U području Istre i Dalmacije dominantan je problem ozon, čije su ciljne vrijednosti koncentracija s obzirom na zaštitu zdravlja ljudi prekoračene u razdoblju od 2019. do 2021. godine (9-13).

Osim onečišćenja zraka u vanjskom okolišu, ljudsko je zdravlje ugroženo onečišćenim zrakom u unutarnjim prostorima. Prosječni suvremeni čovjek provede većinu svog života u zatvorenom prostoru. S obzirom na smanjene mogućnosti ventilacije, u unutarnjem prostoru zagađenje može biti i nekoliko puta veće nego u vanjskom. Neki izvori onečišćenja u unutrašnjem prostoru su duhanski dim, kućna ložišta, dimnjaci, plinske peći, boje i lakovi, sredstva za čišćenje te plijesni (13).

UČINCI ONEČIŠĆENJA ZRAKA NA POGORŠANJE I RAZVOJ ASTME

Dosad su provedena mnoga istraživanja s ciljem utvrđivanja utjecaja kratkoročnog izlaganja onečišćenom zraku na oboljele od astme. Budući da onečišćenje zraka nastaje djelovanjem više plinovitih komponenti i čestica, nije uvijek moguće jasno odrediti koji se učinak može pripisati kojoj tvari. Zato se u istraživanjima često promatra utjecaj ukupnog onečišćenja koje dolazi iz istog izvora, primjerice onečišćenje zraka uzrokovano prometom. Onečišćenje zraka povećava pojavu simptoma i egzacerbacija u osoba oboljelih od astme, povećava broj posjeta hitnim službama i hospitalizacija, a neke komponente smanjuju plućnu funkciju u takvih bolesnika (tablica 1) (9,14-18). U izloženih je bolesnika povećana potrošnja lijekova, sama je bolest lošije kontrolirana, a bilježi se i smanjena kvaliteta života i povećana smrtnost od posljedica astme (19,20). Osim lošeg utjecaja u oboljelih od astme, brojnim novijim istraživanjima utvrđena je poveznica onečišćenja zraka i povećane prevalencije astme. Djeca izložena onečišćenju zraka uzrokovanog prometom te duhanskom dimu imat će veći rizik razvoja bolesti u odnosu na neizloženu djecu (21-23). Iako postoje podatci koji ukazuju na utjecaj i na razvoj astme u odraslih, potrebna su daljnja, pažljivije strukturirana istraživanja kako bi se ta poveznica potvrdila (24-26).

MOGUĆI MEHANIZMI NEPOVOLJNOG DJELOVANJA ONEČIŠĆENJA ZRAKA U BOLESNIKA S ASTMOM

Relativno su nam poznati učinci onečišćenja zraka u bolesnika s astmom, no mehanizmi kojima je ovo djelovanje posredovano još nam uvijek nisu dovoljno poznati. Oni se intenzivno istražuju u nadi da će nam bolje razumijevanje omogućiti smanjenje njihovog utjecaja i razvoj metoda zaštite bolesnika i zdravih pojedinaca. Vjeruje se da su glavni mogući mehanizmi povećanje oksidativnog stresa u dišnom sustavu pojedinca, promjena odgovora imunološkog sustava, poticanje nastanka upale, povećanje osjetljivosti na utjecaj alergena te remodeliranje dišnih puteva (slika 1). Neki agensi imaju izravno oksidativno djelovanje povećavajući koncentracije slobodnih radikala u dišnom sustavu. Drugi agensi mogu djelovati neizravno putem mehanizma aktivacije upale. Naime, u području upale

Tablica 1. *Učinci pojedinih sastavnica onečišćenja zraka u oboljelih od astme*

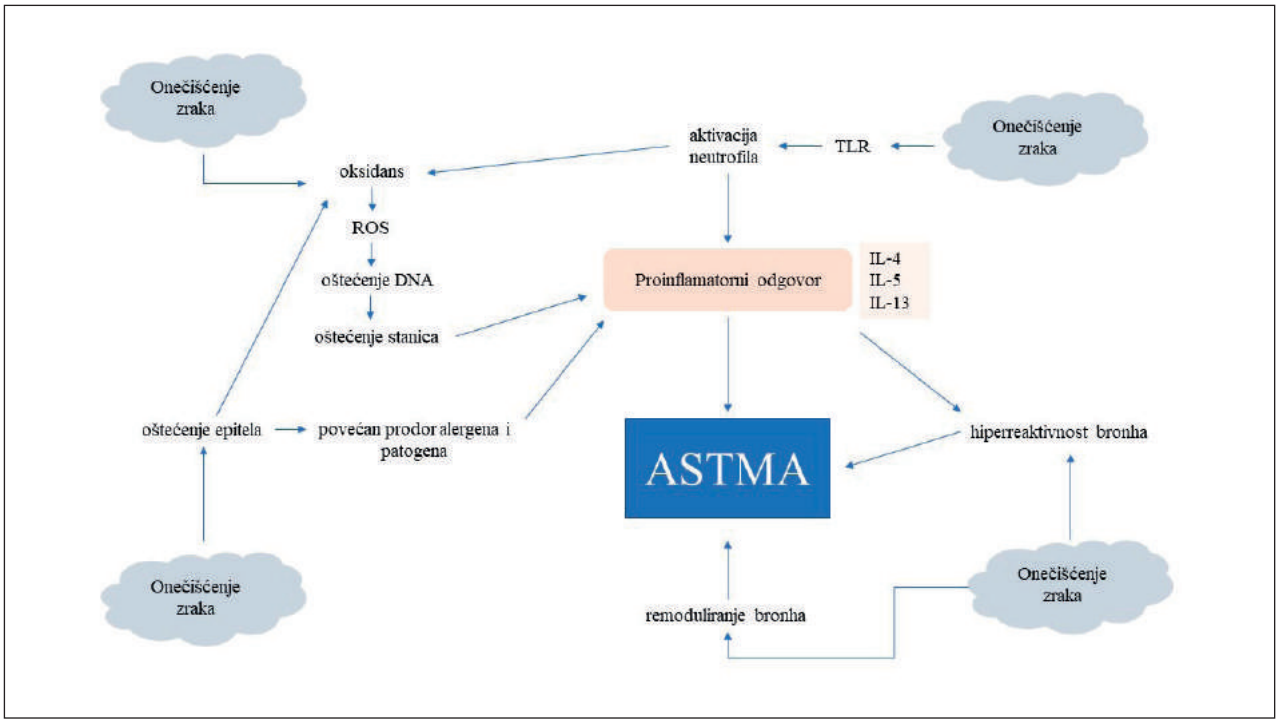
Tablica prikazuje kako najznačajnije komponente onečišćenja zraka (ozon, sumporni dioksid, ugljikov monoksid, dušikov dioksid te čestice) djeluju na simptome, egzacerbacije, broj hospitalizacija, plućnu funkciju i kvalitetu života u oboljelih od astme.

(Prilagođeno iz izvora: Tiotiu AI, Novakova P, Nedeva D i sur. Impact of Air Pollution on Asthma Outcomes. Int J Environ Res Public Health 2020;17(17):6212.)

Zagađivač	Simptomi astme	Egzacerbacije astme	Hospitalizacije zbog astme	Plućna funkcija	Kvaliteta života
PM_{10}	↑	↑	↑	↓	↓
$PM_{2.5}$	↑	↑	↑	↓	↓
Ozon	-	↑	↑	↓	↓
NO_2	↑	↑	↑	↓	↓
SO_2	↑	↑	↑	↓	↓
CO	-	↑	-	-	↓

dolazi do povećanja broja neutrofila, koji mogu i sami povećati stvaranje slobodnih radikala. Sustavni antioksidansi suprotstavljaju se djelovanju viška slobodnih radikala, no samo do određene razine mogu sprječavati stanično oštećenje. Kada ipak do njega dođe, pokreću se proupalni mehanizmi, koji će posredovati daljnje oštećenje tkiva. Stanično i međustanično oštećenje učinit će tkivo podložnijim prodoru i djelovanju različitih patogena (u prvom redu virusa) i alergena, koji su najčešći provocirajući čimbenici za nastanak egzacerbacija bolesti. Neke komponente onečišćenja zraka aktiviraju određene unutarstanične signalne puteve (na primjer, djelovanjem na „Toll-like" receptore – TLR). Dolazi do lučenja proupalnih citokina (primjerice, IL-4, IL-5. IL-13) te aktivacije Th17 i Th2 odgovora, koji imaju važnu ulogu u nastanku astme. Poveća se i proizvodnja imunoglobulin E (IgE) protutijela, koja dovodi do neprimjerenog odgovora imunološkog sustava na alergene (27-29). Mehanizmi kojima je posredovano djelovanje onečišćenja zraka u pogoršanju i nastanku astme mnogobrojni su i kompleksni, a svaka njegova sastavnica ima svoje specifičnosti. Primjerice, ugljikov dioksid produljuje sezonu polinacije, povećava količinu i alergenost stvorene peludi, djelujući neizravno na povećanje učestalosti egzacerbacija astme (30). Sumporov dioksid je jaki iritans dišnog sustava. Kao plinoviti agens dospijeva u male dišne puteve te djeluje na osjetne receptore provocirajući bronhokonstrikciju i povećanje produkcije sluzi čime djeluje na smanjenje plućne funkcije, pogoršanje simptoma astme i potiče nastanak egzacerbacija (31). Lebdeće čestice važna su sastavnica onečišćenja zraka uzrokovanog prometom. One, osim djelovanja već navedenim mehanizmima, mogu na sebe vezati teške metale, komponente bakterija, virusa, alergena, unoseći ih duboko u dišni sustav te na taj način provociraju oksidativni stres i upalu (32).

Važno je razumjeti da onečišćenje zraka neće jednako djelovati u svih zdravih pojedinaca i osoba oboljelih od astme. Ovi će učinci biti izraženiji u genetski predisponiranih pojedinaca. Na nprimjer, osobe s određenim polimorfizmima gena koji kodiraju za enzim glutation-S-transferazu, važan antioksidans, bit će podložniji djelovanju prizemnog ozona u razvoju astme,od osoba s drugim polimorfizmima (33). Polimorfizmi gena koji kodiraju za „Toll-like" receptore TLR2 i TLR4 prepoznati su kao važni u patogenezi djelovanja onečišćenja zraka na razvoj astme u dječjoj dobi (34). Osim genetskih, čini se da važnu ulogu u posredovanju ovih učinaka imaju i epigenetski mehanizmi (metilacija deoksiribonukleinske kiseline - DNA, modifikacija histona i interferencija ribonukleinskom kiselinom - RNA) (35). Primijećeno je da je prenatalna izloženost duhanskom dimu povećala rizik nastanka astme u djece (36). Također, djeca čije su bake bile izložene duhanskom dimu



Sl. 1. *Neki od važnijih mehanizama posrednika u djelovanju onečišćenja zraka na nastanak i pogoršanje astme. Vjeruje se da su glavni mogući mehanizmi nepovoljnog djelovanja onečišćenja zraka u oboljelih od astme povećanje oksidativnog stresa u dišnom sustavu pojedinca, promjena odgovora imunološkog sustava, promocija upale, povećanje podložnosti organizma utjecaju alergena te remodeliranje dišnih puteva.*

imala su veću vjerojatnost razvoja astme, neovisno o izloženosti majke istome agensu (37). Vjeruje se da su u ovim slučajevima okolišni čimbenici utjecali na promjenu epigenetskih svojstava, koja su prenošena na sljedeće generacije (36). U jednom istraživanju osobe koje su bile izložene djelovanju onečišćenja zraka imale su izraženu hipermetilaciju lokusa Foxp3, što je dovelo supresije regulatornih T limfocita i pomicanje ravnoteže imunološkog odgovora u smjeru Th2 staničnog odgovora, čija je važnost u patogenezi astme prethodno već istaknuta (38). Prema istraživanju iz 2012. godine, djeca koja su imala visoke razine metilacije beta-2 adrenergičnog receptora (ADRB2 5'-UTR) imala su veći rizik razvoja teške astme kada su bila izložena djelovanju dušikovog dioksida, dok u djece s niskim vrijednostima metilacije nije bilo značajne poveznice onečišćenja zraka i težine astme (39). Mnoga su dosadašnja saznanja nastala istraživanjima na životinjskim i *in vitro* modelima. Budući da je ovo područje kompleksno i zahtjevno za istraživanje na humanim modelima, bit će potrebno dulje vrijeme i brojna dodatna istraživanja da steknemo veće razumijevanje mehanizama na koje onečišćenje zraka uzrokuje učinke prepoznate u opservacijskim studijama (35).

RASPRAVA I ZAKLJUČAK

Nastojanje da razumijemo načine na koje onečišćenje zraka utječe na morbiditet astme omogućava nam da to znanje primijenimo u zaštiti od njegovog štetnog djelovanja. Na globalnoj razini treba težiti smanjenju emisija štetnih plinova i čestica putem prometa, industrije i poljoprivrede okrećući se obnovljivim i čišćim izvorima energije. Djelovanje na tako širokoj razini zahtijeva angažman cjelokupne međunarodne zajednice, ali i određeno vrijeme prilagodbe novim uvjetima. Međutim, oboljelima od astme, kao i zdravom dijelu populacije, rješenja trebaju sada. Na regionalnoj razini može se djelovati razvojem prometne infrastrukture koja će omogućiti učinkovitiji javni prijevoz i sigurno putovanje za bicikliste i pješake, kako bi se smanjila upotreba motornih vozila. Bolesnicima s astmom preporuča se živjeti na barem 300 metara udaljenosti od velikih prometnica, budući da će se koncentracija čestica i plinova u zraku djelomično smanjiti na toj udaljenosti. Također, u vrijeme kada su povećane koncentracije onečišćenja u atmosferi, trebali bi izbjegavati izlazak iz domova i boravak u vanjskim prostorima. Od velike pomoći mogli bi biti i javni regionalni alarmi koji bi određenim danima upozoravali na potencijalno štetne koncentracije onečišćenja u zraku. Tijekom vožnje u automobilima preporuča se ne otvarati prozore te koristiti unutarnje kruženje zraka (14,40). Prema nekim istraživanjima prehrana bogata voćem i povrćem ima zaštitni učinak na razvoj alergijskih bolesti u djece izložene onečišćenju zraka. Pretpostavlja da bi za takav učinak moglo biti zaslužno njihovo antioksidativno djelovanje (41). Iako još nema definitivnih preporuka, istražuje se korist uzimanja suplemenata vitamina C, D i E na zaštitu od djelovanja onečišćenja zraka na razvoj i loše ishode astme (42).

U istraživanju objavljenom 2019. godine analizirani su podatci 18 europskih država o koncentracijama određenih komponenti u onečišćenom zraku te podatci o broju novih slučajeva astme u djece. Prema procjeni temeljenoj na tim podatcima, 15-33 % slučajeva astme u djece moglo bi se spriječiti značajnim smanjivanjem koncentracija NO_2, $PM_{2.5}$ i ugljika u zraku (43). Dobro provedenim intervencijama mogli bismo spriječiti razvoj milijuna slučajeva astme u svijetu, kao i mnoge komplikacije bolesti. Zabranom pušenja u zatvorenim javnim prostorima već smo uspjeli postići korak unaprijed za zdravlje populacije. Podizanjem svijesti o ovom globalnom problemu sigurno možemo postići i mnogo više.

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SUMMARY

AIR POLLUTION AND ASTHMA

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According to the World Health Organization data, the majority of the population live in areas with poor air quality, which has an impact on human health, particularly respiratory system. Asthma is a chronic respiratory disease induced by inflammation due to an imbalance of proinflammatory and anti-inflammatory factors. Short-term exposure to air pollution causes an increase in asthmatic symptoms, number of exacerbations, visits to emergency services, hospitalizations, an increase in mortality, and some air pollutants may also decrease lung function. Furthermore, according to the latest research, air pollution can also increase the occurrence of asthma in children. It is considered that the main mechanisms by which air pollution mediates the development and exacerbation of asthma are an increase in oxidative stress in the individual's respiratory system, a change in the immune system response that promotes inflammation, an increase in the body's susceptibility to the influence of allergens, and airway remodeling. The effect of air pollution will be more pronounced among genetically predisposed individuals. In addition to genetic pathways, epigenetic mechanisms appear to have a significant part in mediating these effects. Interventions at the global and local scale might contribute to the improvement of the quality of life and outcomes in millions of asthmatic patients, but also in healthy individuals. Global interventions include reduction of harmful gas and particle emissions from transportation, manufacturing, and agriculture, and transition to renewable and cleaner energy sources is indispensable. Some interventions at the local scale may be public air pollution warnings, patient education, development of better infrastructure for cyclists and pedestrians, etc.

Key words: asthma, air pollution, asthma exacerbations, inflammation pathways, genetic and epigenetic factors

POSEBNOST DIJAGNOSTIKE LAJMSKE NEUROBORELIOZE

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Lajmska neuroborelioza (LNB) nastaje hematogenim rasapom borelija u središnji živčani sustav (SŽS), a opisan je i prodor putem perifernog živca. Razvija se serozni meningitis sa ili bez pareze kranijalnog živca što je prevladavajuća klinička slika. Najčešće je zahvaćen *n. facialis.* Za razliku od Sjeverne Amerike, u Europi mogu biti zahvaćeni i drugi kranijalni živci, što se povezuje s prevaliranjem različitih vrsta borelija - u Europi najčešće *B. garinii, B. bavariensis* i *B. afzelii,* a u Sjevernoj Americi samo *B. burgdorferi* sensu stricto. Meningoradikulitis ili Bannwarthov sindrom tipična je slika LNB samo u Europi. Simptomi LNB većinom nisu tipični i mogu sličiti različitim neurološkim bolestima, pa dijagnozu često nije jednostavno definirati. Dijagnostika LNB mora uključivati analizu likvora u kojem je značajan nalaz pleocitoze, što upućuje na serozni meningitis kojem je potrebno dokazati povezanost s borelijama. Mikrobiološka dijagnostika LNB obuhvaća kultivaciju, zahtjevnu i dugotrajnu metodu (9-12 tjedana) koja se radi isključivo u referentnim centrima, te molekularnu (PCR) i serološku dijagnostiku. Zbog malog broja borelija u likvoru kao i relativno male količine likvora koja se šalje za analizu, molekularna je dijagnostika često lažno negativna. Stoga je serološka dijagnostika ključna za dokazivanje LNB. Serologija se radi iz istovremeno uzetih uzoraka seruma i likvora u kojima se određuju specifična protutijela IgM i IgG te ukupni imunoglobulini i/ili albumini. Serum i likvor moraju se analizirati istom metodom u istim uvjetima kako bi se mogla odrediti intratekalna sinteza specifičnih protutijela, tj. izračunati indeks protutijela (*antibody index,* AI). Specifična protutijela u lajmskoj boreliozi nastaju relativno sporo, a s duljinom trajanja infekcije njihova količina se povećava. U ranoj LNB protutijela u likvoru ne mogu se uvijek otkriti, iako je prisutna pleocitoza. Kasnu LNB u pravilu prati jaki imunosni odgovor i uz pleocitozu često se nalazi pozitivan AI. Nakon infekcije, vremenom se likvor normalizira i pleocitoza se više ne nalazi, iako specifična protutijela u likvoru mogu ostati dugo prisutna, čak i uz pozitivan AI. Stoga je potrebno pratiti imunosni odgovor u krvi i likvoru od dana kada se bolesnik javi zbog simptoma i zatim nakon jednog, tri, šest i dvanaest mjeseci, radi procjene korelacije nalaza i bolesti. Dijagnoza LNB mora biti u skladu s kliničkim, epidemiološkim i anamnestičkim podatcima te laboratorijskim nalazima, posebno u likvoru. LNB je 1) potvrđena ako uz kliničku sliku postoji pleocitoza i intratekalna sinteza specifičnih protutijela; 2) vjerojatna ako intratekalna sinteza nije potvrđena, a specifična protutijela su prisutna u krvi bolesnika; 3) LNB nije vjerojatna ako nema pleocitoze ili nema analize likvora, iako su prisutna specifična protutijela u krvi bolesnika, a klinička slika i epidemiološka anamneza nisu karakteristični. CXCL13 je biljeg koji može biti koristan kao dodatni test premda nije specifičan za LNB - u likvoru je povišen i prati akutnu upalu. Ako postoji mogućnost, LNB bi trebalo potvrditi kultivacijom i molekularnom dijagnostikom. Interpretacija laboratorijskih i kliničkih nalaza zahtijeva znanje i iskustvo. Informacije se trebaju sagledati u skladu s okolnostima i specifičnosti bolesnika, tako da je svaki bolesnik poseban dijagnostički izazov.

Ključne riječi: lajmska neuroborelioza, *Borrelia burgdorferi,* mikrobiološka dijagnostika, serološka dijagnostika, likvor, likvorski indeks protutijela

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UVOD

Borelije i lajmska borelioza

Borelije su tanke, duge i vrlo pokretne spirohete. Vrste koje nalazimo u ljudi razvrstane su prema kliničkim entitetima koje uzrokuju u dvije skupine: borelije kompleksa *Borrelia burgdorferi* sensu lato (BBSL) koje uzrokuju lajmsku boreliozu (LB) i borelije koje se povezuju s povratnom groznicom. Kompleks BBSL obuhvaća više od dvadeset vrsta borelija od kojih su neke uobičajeni humani patogeni, kao što *Borrelia (B.) afzelii*, *B. garinii*, *B. bavariensis*, *B. burgdorferi* sensu stricto i *B. spielmanii*, a za neke, kao što su *B. valaisiana*, *B. lusitaniae* i *B. bissettiae*, patogenost nije sigurno dokazana. Borelije kompleksa BBSL prenose tvrdi štitasti krpelji, a borelije povratnih groznica (*B. recurrentis*, *B. duttonii*) prenose meki krpelji i tjelesne uši. Među borelije povratnih groznica filogenetski spada i *B. miyamotoi* koju prenose iste vrste tvrdih krpelja koji prenose BBSL, a dokazana je u nekoliko imunokompromitiranih bolesnika s infekcijom središnjeg živčanog sustava (SŽS) (1-4).

Borelije kompleksa BBSL prirodno se nalaze u različitim divljim, ali i domaćim životinjama. Posebno važni domaćini su glodavci i ptice. S jednog na drugog domaćina BBSL prenose krpelji vrste *Ixodes* spp. U razvojnom ciklusu iksodesi za prijelaz iz jednog stadija u drugi, od jajašca preko ličinke do nimfe i odrasle jedinke trebaju krvni obrok. Žrtvu ne traže aktivno već čekaju na vlatima trave i niskom raslinju kako bi se nožicama zakvačili za potencijalnog domaćina u prolazu, nakon čega hodaju po njemu tržeći idealno mjesto za prihvaćanje kliještima i ubadanje rilca sa zubićima. Ubod krpelja ne boli ni ne svrbi jer se u slini nalazi specifičan protein koji djeluje kao anestetik i omogućava pričvršćivanje nakon čega započinje sisanje krvi. Ako je krpelj zaražen, borelije iz intestinalnog trakta slinom prelaze u novog domaćina. Jako je važno što prije krpelja otkriti i skinuti s kože polaganim povlačenjem pincetom kojom ga se uhvati ispod tijela kako bi se otpustile nožice i izvuklo rilce, jer vrijeme pričvršćivanja krpelja odnosno vrijeme do infekcije traje od nekoliko sati do nekoliko dana. Nakon izvlačenja ubodno mjesto treba dezinficirati. Rano uklanjanje krpelja najbolji je način prevencije lajmske borelioze. Pretpostavlja se da je za prijenos borelija iz krpelja potrebno najmanje 10 do 24 sata. Čovjek je slučajni domaćin u životnom ciklusu krpelja u kojem mu zaraženi krpelj može prenijeti borelije, ali istovremeno i prekinuti prirodno održavanje borelija, jer nije vjerojatno da će se novi krpelj borelijama inficirati na čovjeku (1).

Rasprostranjenost borelija i bolesti koje mogu uzrokovati povezani su s geografskom rasprostranjenosti vektora. Iksodesi, a time i LB, rašireni su samo u područjima sjeverne polutke (5). Prevalencija LB značajno se razlikuje između pojedinih europskih područja, a dodatno velike razlike postoje u odnosu na Sjevernu Ameriku. U Europi su u ljudi izolirane *B. afzelii*, *B. garinii*, *B. bavariensis*, *B. burgdorferi* sensu stricto i *B. spielmanii*. Za razliku od Europe gdje su značajne sve vrste BBSL, u Sjevernoj Americi dominantno je za čovjeka patogena *B. burgdorferi* sensu stricto. *B. mayonii* pripada među BBSL, jako rijetko je dokazana kao humani patogen u Sjevernoj Americi, ali ne i u Europi (3). Raširenost različitih vrsta borelija povezana je uz učestalost i različitost kliničkih slika. Literaturni podatci o LB trebaju se stoga oprezno primjenjivati ovisno o regionalnim specifičnostima (6).

Općenito, u kliničkoj slici LB dominantna je kožna promjena *erythema migrans* (EM) (1). Primjerice, u Sloveniji koja je endemska za LB, više od 95 % borelijskih infekcija prođe kao EM. EM kao i druge kožne promjene [multipli EM, borelijski limfocitom, *acrodermatitis chronica atrophicans* (ACA)] u Europi najčešće uzrokuje *B. afzelii* (>90 %), za razliku od Sjeverne Amerike gdje se kožne promjene uglavnom prezentiraju kao pojedinačni ili multipli EM koje uzrokuje isključivo *B. burgdorferi* sensu stricto. EM najčešće prolazi bez posljedica spontano ili uz antibiotsku terapiju. Ako dođe do diseminacije borelija iz kože, najčešće će se razviti lajmska neuroborelioza (LNB) s kliničkom slikom seroznog meningitisa koji često prati pareza jednog od kranijalnih živaca, najčešće facijalnog. Najčešći uzročnik LNB u Europi je *B. garinii* (oko 65 % slučajeva), a u Sjevernoj Americi *B. burgdorferi* sensu stricto (1,5).

Širenje borelija iz kože može biti usmjereno na velike zglobove (npr. koljeno) i uzrokovati artritis. Lajmski artritisi u Europi su rijetki (< 4 %) dok se često opisuju u Sjevernoj Americi (oko 40 % slučajeva) što se povezuje s organotropizmom dominantne *B. burgdorferi* sensu stricto. Iz zglobova borelije su u Europi izolirane vrlo rijetko, a prema vrsti identificirane su različite vrste europskih borelija (1,5).

Osim navedenih najčešćih kliničkih manifestacija, borelijsku infekciju može pratiti niz netipičnih simptoma kao što su glavobolja, mialgije, artralgije, opća slabost, umor, smetnje koncentracije i drugo. Budući da se slične tegobe mogu javljati i u zdravoj populaciji te u drugim bolestima, postavljanje dijagnoze LB nije jednostavno posebno u endemskim područjima (1,5).

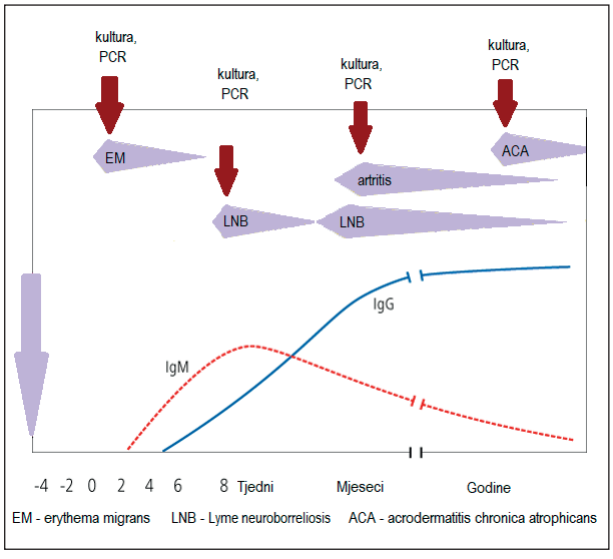
Lajmska neuroborelioza (LNB)

Kod sumnje na LNB dijagnostički postupak treba započeti prikupljanjem kliničkih i epidemioloških podataka uz procjenu vjerojatnosti borelijske infekcije. Važni su podatci o ugrizu krpelja i boravku u priro-

di posebno u endemskim područjima tijekom sezone krpelja od travnja do rujna kao i podatak o ranijoj tipičnoj kožnoj promjeni EM. Borelije mogu ući u SŽS hematogeno, a opisano je putovanje borelija do SŽS i uzduž perifernog živca. LNB se razvije u oko 10-15 % borelijskih infekcija (1,5,7).

Unutar šest mjeseci od borelijske infekcije kao odgovor na prodor borelija u SŽS može se razviti serozni meningitis s parezom kranijalnog živca ili bez pareze, što se opisuje kao rana LNB. Kliničke slike i učestalost rane LNB razlikuju se u Europi i Sjevernoj Americi. U Europi se u sklopu borelijskog meningitisa mogu razviti pareze različitih kranijalnih živaca - najčešće je zahvaćen *n. facialis* (u 80 %), zatim *n. abducens, n. vestibularis, n. opticus, n. oculomotorius* i drugi, dok u Americi serozni meningitis prati isključivo pareza vezana za *n. facialis* (5,8-10). Jednostrana ili obostrana pareza kranijalnog živca razvije se u manje od polovine europskih bolesnika sa seroznim meningitisom, a češće se razvije tzv. Bannwarthov sindrom. Garin-Bujadoux-Bannwarthov sindrom ili meningoradikulitis je tipična klinička slika za LNB. Opisuje se samo u Europi, a povezuje se s infekcijom i organotropizmom vrste *B. garinii*. Bannwarthov sindrom nije poznat u Sjevernoj Americi (5,7,9). Rijetko se u sklopu LNB opisuje kranijalni vaskulitis koji se najčešće manifestira kao akutni ishemički inzult (8-10).

Kronična periferna polineuropatija obično je povezana s kroničnom kožnom promjenom ACA i u Europi se opisuje kao kasna manifestacija LB koju najčešće uzrokuje vrsta *B. afzelii* (11). Kasna periferna polineuropatija iznimno rijetko je opisana u Sjevernoj Americi, gdje se povezuje s kroničnim lajmskim artritisom i vrstom *B. burgdorferi* sensu stricto (5,9-11).



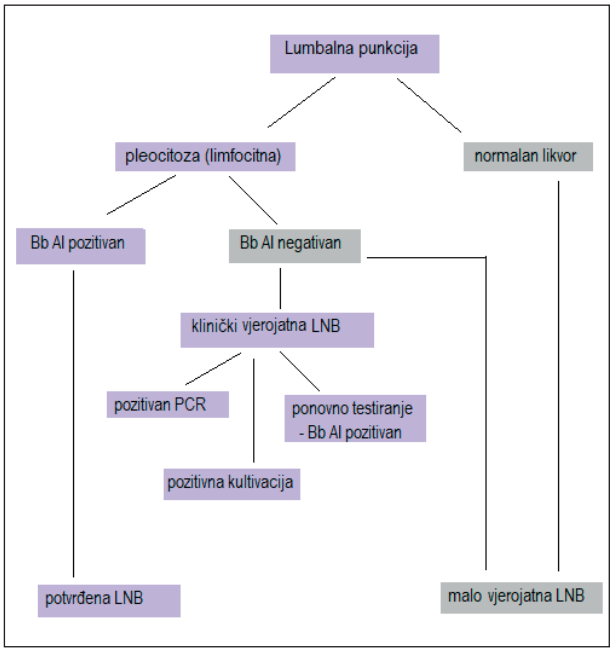
Slika 1. *Razvoj kliničkih slika od ugriza krpelja do pojave simptoma i moguće mikrobiološke metode za dijagnostiku lajmske borelioze.*

Nespecifični simptomi koji se pojavljuju u raznim neurološkim bolestima kao što su npr. multipla skleroza, miastenija gravis, kognitivno propadanje, sindrom Guillian-Barre, spastična pareza, optički neuritis, pareza facijalnog živca nepoznate etiologije, a koje se pokušava povezati s borelijskom infekcijom, dodatno su opterećenje u intepretaciji značenja dijagnostičkih testova za LNB (1,8-10). Kod navedenih bolesti potrebno je isključiti LNB što ponekad nije jednostavno, jer osobe koje su ranije imale LB dugo pa i doživotno zadržavaju rezidualna protutijela koja se ne mogu jednoznačno povezati s akutnom bolesti. Stoga pozitivan nalaz, posebno u endemskim područjima gdje postoji velika učestalost LB, može zavesti na krivi zaključak o dijagnozi.

MIKROBIOLOŠKA DIJAGNOSTIKA LAJMSKE NEUROBORELIOZE

Za dijagnozu LNB potrebno je definirati strategiju testiranja, isplanirati mikrobiološku dijagnostiku i uzeti uzorke. Općenito, za borelijsku infekciju karakteristična je dugotrajna prisutnost borelija u domaćinu. Stoga žive borelije možemo očekivati u SŽS-u bolesnika s LNB. Glavni mikrobiološki dijagnostički postupci općenito su usmjereni prema izdvajanju i izravnom dokazivanju uzročnika. Borelije se mogu uzgojiti na posebnim podlogama za kultivaciju i/ili dokazati metodama molekularne dijagnostike kao što je lančana reakcija polimerazom (engl. *polymerase chain reaction*, PCR). No, rutinska dijagnostika LB, pa tako i LNB najčešće se radi dokazivanjem specifičnih protutijela serološkim testovima. U bolesnika s LNB protutijela se očekuju u krvi i likvoru. Budući da je stvaranje protutijela dinamički proces, potrebno je određeno vrijeme pratiti imunosni odgovor. Pojavnost, količina i kretanje protutijela u krvi i likvoru te usporedba rezultata testiranja sukcesivnih seruma mogu dati jasniji uvid u tijek infekcije SŽS-a (1,5,8-10). Slika 1 prikazuje vremenski tijek razvoja kliničkih slika i optimalnu mikrobiološku dijagnostiku za definiranje LB i LNB.

Dijagnostika infekcije SŽS-a rutinski započinje analizom upalnih parametara u krvi i likvoru. Analiza likvora ključna je za LNB. Budući da je lumbalna punkcija invazivni postupak koji nije jednostavan za bolesnika, potrebno je dobro klinički procijeniti je li likvor nužno potreban za dijagnostiku, odrediti kada ga treba uzeti, koje analize treba napraviti i koliko je likvora za to potrebno. Likvor se analizira biokemijski, citološki i mikrobiološki, a ostatak se mora sačuvati za daljnje dodatne pretrage. Biokemijskom analizom de-



Slika 2. *Dijagnostički algoritam za lajmsku neuroboreliozu (LNB) (8).*

Bb, *Borrelia burgdorferi* s.l.; AI, *antibody index* (indeks likvorskih protutijela)

finiraju se osnovni parametri upalnog procesa, a citološkom količina i vrsta upalnih stanica. LNB je prije svega serozni meningitis koji prati pleocitoza. Obično se nađe 10-1000 leukocita/mm^3, uglavnom limfocita i plazma stanica. U perifernoj krvi pak rijetko se nađu povišene vrijednosti leukocita. Kod LNB povišena je razina albumina u likvoru što je znak oštećenja krvno–moždane membrane. Ako biokemijski i citološki nalazi upućuju na serozni meningitis, treba provesti mikrobiološku analizu za dokazivanje pretpostavljenog uzročnika. Među uzročnike seroznog meningitisa treba uključiti i borelije LB, posebno ako borelijsku infekciju podupiru epidemiološki i anamnestički podatci kao i osobitost kliničke slike. Razmotriti treba dostupnost različitih mikrobioloških postupaka i uzeti uzorke za kultivaciju, PCR i serologiju (8, 12). Dijagnostički algoritam za dokaz rane LNB prikazuje slika 2.

Kultivacija borelija

U LNB borelije su mogu pokušati izolirati iz likvora. U likvoru kao i u drugima tjelesnim tekućinama borelije su rijetke zbog čega količina uzorka mora biti što veća (npr. 2 mL likvora). Osim toga borelije su osjetljive bakterije i mogu brzo propasti zbog čega se preporuča uzeti likvor izravno u tekuću podlogu za rast borelija već za vrijeme lumbalne punkcije kako bi se povećala osjetljivost metode. Uspješnost izolacije borelija iz likvora ne prelazi 10vb% (1 5,7-10,13). Prema literaturnim podatcima o vrstama izoliranih borelija, LNB u europskih bolesnika najčešće uzrokuje *B. garinii*, a slijedi *B. afzelii* i rijetko *B. burgdorferi* sensu stricto. U Sjevernoj Americi jedini uzročnik LNB *B. burgdorferi* sensu stricto (1,5,7,9,13-17).

Borelije imaju dugo generacijsko vrijeme tako da rezultat kultivacije treba čekati tjednima. Za vrijeme višetjedne kultivacije uzorci se moraju presađivati na svježe podloge, centrifugirati i mikroskopirati, za što je potreban dobro izučeni kadar u opremljenom specijaliziranom laboratoriju. Borelije se najčešće dokazuju između drugog i trećeg tjedna kultivacije, ali treba čekati 9-12 tjedana prije nego li se zaključi da je kultura negativna. Za kliničare i bolesnike to je predugo vrijeme čekanja na nalaz. Stoga u današnje vrijeme suvremenih tehnologija i automatizacije, kultivacija kao dijagnostički postupak ostaje samo u referentnim laboratorijima (8,10,14).

Bez obzira na slabu osjetljivost, dugo čekanje rezultata i kompleksnu proceduru, kultivacija je neophodna za istraživanje borelija i LB. Jedino uzgojem spoznalo se da različite vrste borelija imaju različiti potencijal virulencije koji se povezuje s težinom kliničke slike i imunosnim odgovorom. Sposobnost prerastanja pojedine borelijske vrste u mješovitoj kulturi u tekućim podlogama (*B. burgdorferi* sensu stricto preraste *B. garinii*, a ova *B. afzelii*) ekvivalentna je s njenim potencijalom za nastanak i manifestacije infekcije (7,11,13,16,18-20).

Zahvaljujući kultivaciji dokazalo se da borelije nakon infekcije mogu vrlo brzo ući u SŽS (rana diseminacija) i potom se u njemu dugo zadržati. Osim toga borelije mogu preživjeti antibiotsku terapiju, a mogu opstati u organizmu (i u SŽS-u) unatoč snažnom imunosnom odgovoru. Uzastopnom izolacijom borelija iz likvora potvrđeno je da je moguće više puta u životu oboljeti od LNB (1,5).

Analizom izoliranih borelijskih sojeva pokušava se spoznati mehanizme patogenosti, faktore virulencije, organotropizam borelija te objasniti kliničke prezentacije infekcija, npr. različiti tijek LNB odnosno LB u europskih i američkih bolesnika (1,5,16,18,19). Temeljem stečenog znanja više centara u svijetu pokušava napraviti primjereno cjepivo, ali još uvijek bez uspjeha (21).

Molekularna dijagnostika (PCR) za lajmsku neuroboreliozu

Molekularna dijagnostika se već tridesetak godina pokušava implementirati u dijagnostiku LNB. Izvodi se iz istih uzoraka kao i kultivacija koja je preduvjet za

dizajniranje molekularnih testova temeljem istraženih karakteristika izoliranih borelija. Amplifikacijom specifičnih dijelova borelijskog genoma postiže se visoka specifičnost (93–100 %) (1,5,8-10,14,22). Za dokazivanje LNB likvor je najbolji uzorak, iako se PCR može raditi i iz krvi ili bioptata kože uz *erythema migrans*. Kao i kod kultivacije, bolji rezultati dobit će se analizom što većega uzorka, pa se preporuča analiza najmanje 1-2 mL likvora (22,23). Uspješnost dokazivanja borelija PCR-om u likvoru uspješnija je u američkih (25-96 %) nego u europskih (9-32 %) bolesnika, ponovno zbog različitosti uzročnika - *B. burgdorferi* sensu stricto u Sjevernoj Americi, a *B. garinii* ili neka druga borelija u Europi (1,5,15,16).

Velika prednost metode PCR je u brzini dobivanja rezultata koji su gotovi u istom danu. Automatizacija postupka omogućila je bolju standardizaciju i dostupnost metode, a premostila je ranije probleme, kao što su kontaminacija uzorka (lažno pozitivni rezultati), vrijeme čekanja nalaza ili inhibicija reakcije. Zbog malog broja borelija u tkivu, što je posebno značajno u Europi, PCR može biti češće lažno negativan nego što se očekuje. Za interpretaciju značenja rezultata PCR-a osim poznavanja potencijala metode i validacije rezultata, potrebno je znanje i iskustvo, jer za definiranje dijagnoze LNB treba sagledati sve kliničke, ostale laboratorijske te anamnestičke i epidemiološke podatke. Stoga PCR još uvijek nije uobičajena metoda za LB u laboratorijskoj praksi (23).

Serološka dijagnostika lajmske neuroborelioze

Određivanje specifičnih protutijela najčešći je način dokazivanja LNB kao i LB općenito. Provodi se u mnogim mikrobiološkim laboratorijima iako se radi o dijagnostici kod koje rezultati nisu jednoznačni i interpretacija rezultata je izazov. Borelije posjeduju brojne proteine koji sadrže različite antigenske determinante koje mogu potaknuti imunosni odgovor. Antigenska heterogenost borelija potiče stvaranje specifičnih protutijela koja možemo dokazati u krvi, likvoru i sinovijskoj tekućini (1,5,7-10,13,14). Neki borelijski antigeni kao što su npr. adhezini, flagelin, stresni proteini, membranski proteini, slični su ili jednaki, tj. zajednički s antigenima drugih bakterija, pa protutijela prema njima često križno reagiraju i nisu pouzdana za dijagnostiku LB. S druge strane, postoje specifični antigeni za borelije LB, kao što su OspA, OspB, OspC, p100, p39, p18 ili VlsE koje ne nalazimo kod drugih bakterija, pa je njihovo dijagnostičko značenje drugačije. Dokaz protutijela protiv ovih antigena puno sigurnije potvrđuje LB, iako i tu postoji mogućnost nespecifične reaktivnosti zbog mimikrije borelijskog proteina s proteinima domaćina (1,14). Važno je stoga poznavati karakteristike serološkog testa i antigene koje koriste. Smatra se da su najpouzdaniji serološki testovi koji sadrže rekombinantne specifične borelijske antigene i izražavaju rezultate kvantitativno što omogućava praćenje dinamike protutijela i izračunavanje indeksa intratekalne sinteze potrebnog za dijagnozu LNB.

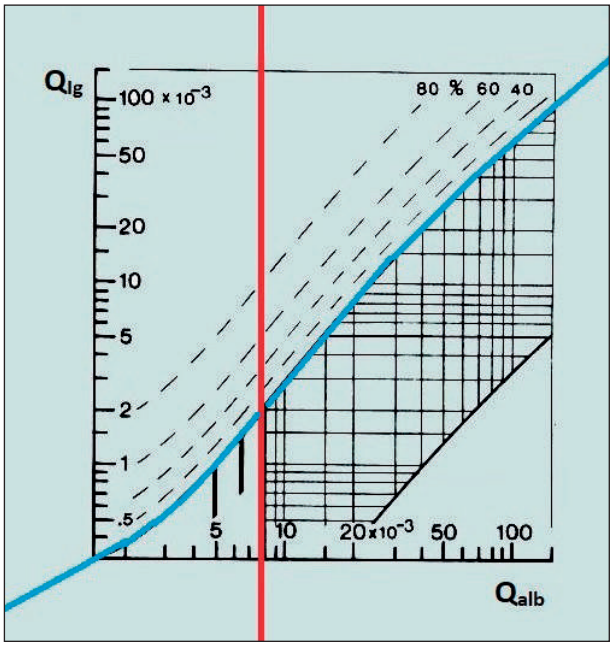
LNB nastaje nakon prodora borelija u SŽS. U SŽS se umnožavaju i potiču lokalni imunosni odgovor i u likvoru sintezu specifičnih protutijela. Iako bi se moglo zaključiti da nalaz pozitivnih protutijela u likvoru automatski potvrđuje LNB, to ipak nije jednoznačno. SŽS funkcionalno je odvojen krvno-moždanom membranom čija je fiziološka uloga održavati ravnotežu albumina i imunoglobulina između krvi i likvora. U upalnim bolestima fiziologija krvno-moždane membrane se poremeti. Najbolji biljeg funkcije krvno-moždane membrane je albumin (65 kD) koji se stvara izvan SŽS i difuzijom ulazi u likvor. Omjer količine albumina između likvora i krvi (likvorski albumin / serumski albumin, Q_{alb}) ima kod zdravih ljudi konstantnu vrijednost, iako se razlikuje prema dobi, i potvrđuje intaktnost krvno-moždane membrane. Kroz krvno-moždanu membranu u likvor osim albumina prolaze i serumski imunoglobulini (Ig). Difuzija imunoglobulina ovisi o razredu imunoglobulina zbog različite molekularne mase (IgM 970 kD, IgG 150 kD i IgA 160 kD) i koncentracije u krvi. Omjeri koncentracija pojedinog razreda imunoglobulina između likvora i krvi (ukupni imunoglobulini razreda M, G ili A u likvoru / ukupni imunoglobulini M, G ili A u krvi; Q_{Ig}) kod intaktne krvno-moždane membrane zdravog čovjeka su konstantni (12,24,25).

Borelijska protutijela mogu se zbog propusnosti krvno-moždane membrane prenijeti pasivno difuzijom iz krvi u SŽS. Osim toga pri uzorkovanju i lumbalnoj punkciji moguć je arteficijalni unos protutijela zbog oštećenja krvne žile i miješanja likvora i krvi. Stoga sama prisutnost borelijskih protutijela u likvoru nije nužno znak LNB-a. Borelije koje su prodrle u SŽS potiču lokalnu sintezu protutijela, dok upalni proces općenito povećava propusnost krvno-moždane membrane što zajednički utječe na lokalno povećanu količinu protutijela. Zato je za potvrdu LNB-a nakon procjene funkcionalnosti krvno-moždane membrane potrebno dokazati intratekalnu sintezu protutijela određivanjem indeksa protutijela, *antibody index* (AI). Za određivanje AI nužno je da se uzorci krvi i likvora uzmu u razmaku od najviše tri sata, da bolesnik u međuvremenu nije dobio infuziju ni transfuziju te da se uzorci krvi i likvora obrade istovremeno u istim serijama testiranja (8-10,12-14,24-27). Najprije se odredi količina ukupnih (total-IgM/IgG) i specifičnih borelijskih (Bb-IgM/IgG) protutijela u likvoru i serumu; nakon toga se izračuna omjer između likvorskih i serumskih ukupnih (Q_{Ig} = *total*-Ig-likvor / *total*-Ig-serum) i specifičnih protutijela (Q_{spec} = Bb-Ig-likvor / Bb-Ig-serum). Da

bi se definirao AI potrebno je izračunati odnos između omjera specifičnih i omjera ukupnih protutijela (Q_{spec}/ Q_{Ig}) što se prikazuje kao numerička vrijednost AI. Zbog fiziološke difuzije specifičnih Bb-Ig u SŽS kroz intaktnu krvno-moždanu membranu njihov AI morao bi biti 1,0 (uz SD 1,3 - 1,5). Sve vrijednosti AI veće od 1,0 ukazuju na intratekalnu sintezu. Što je veća vrijednost AI, intenzivnija je intratekalna sinteza specifičnih Bb-Ig u SŽS (8,10,26,28,29).

Ako je krvno moždana membrana oštećena zbog neke druge bolesti kao što su autoimune bolesti, multipla skleroza, sindrom Guillian-Barre ili drugo, procjenu funkcionalnosti krvno-moždane membrane (Q_{lim}) potrebno je provesti pomoću složenog matematičkog modela i Reiberove hiperbolične formule koja se temelji na koncentraciji albumina Q_{alb} i korelaciji albumina i imunoglobulina (slika 3). Nakon definiranja Q_{lim} pomoću Reiberove formule, AI se izračunava na standardni način usporedbom s vrijednostima za specifična borelijska protutijela (12,25,26).

Primarna detekcija likvorskih protutijela ovisi o osjetljivosti i specifičnosti serološkog testa, a značenje nalaza i procjena intratekalne sinteze obavezno se dokazuje izračunavanjem AI (7,13,27). Prema literaturnim podatcima, osjetljivost testova za dokaz rane LNB prosječno je za IgM 54 %, a za IgG 60 %, dok je za kasnu LNB osjetljivost za IgM 6 %, a za IgG do 100 %. Specifičnost primarnih testova načelno je visoka budući da se temelje na rekombinantnim specifičnim borelijskim antigenima, a specifičnost nalaza protutijela u primarnom serološkom testu može se potvrditi imunoblotom ili alternativnim rekombinantnim testom (8,10,28,30,31).



Slika 3. *Reiberov dijagram na kojem je prikazan omjer količina albumina (Q_{alb}) i ukupnih imunoglobulina (Q_{Ig}) u likvoru i krvi. Q_{alb} desno od crvene crte predstavlja oštećenu krvno-moždanu membranu, a Q_{Ig} iznad plave crte intratekalnu sintezu ukupnih protutijela pojedinog razreda (12, 25, 26).*

POSEBNOSTI SEROLOŠKE DIJAGNOSTIKE LAJMSKE NEUROBORELIOZE

Preporuke europskih i američkih stručnih društava za LB zastupaju potrebu potvrde rezultata primarnog serološkog testa western / imuno blotom ili alternativnim rekombinantnim testom. To je obavezno ako se za primarno probirno serološko testiranje (EIA ili IFA) koriste testovi koji sadrže brojne pročišćene borelijske proteine odnosno cijelu boreliju što je bila karakteristika prve generacije testova (EIA i IFA) (10,14,28,32). Druga generacija primarnih testova sadržavala je pročišćene borelijske proteine s ponekim rekombinantnim proteinom, dok se testovi treće generacije temelje na izabranim rekombinantnim specifičnim borelijskim antigenima (28). U endemskim područjima s visokom prevalencijom, primarni testovi s rekombinantnim specifičnim borelijskim antigenima dovoljno su osjetljivi i specifični, pa je potvrđivanje rezultata rijetko potrebno što smanjuje troškove i skraćuje vrijeme do rezultata. Međutim, neuobičajena klinička slika koja nije sukladna s pozitivnim borelijskim rezultatom mora potaknuti sumnju u pozitivan serološki rezultat i nalaz je potrebno dodatno evaluirati i provjeriti prije odluke o terapiji. Zato je laboratorijska dijagnostika LNB poseban izazov i odgovornost (8,10,14,28,30,31).

Općenito, specifična protutijela nastaju relativno sporo, količina im se povećava s duljinom trajanja infekcije čime raste broj serološki pozitivnih nalaza. Nije neobično da se u ranoj LNB, uz prisutnu pleocitozu, protutijela u likvoru ne nađu. Kasnu fazu LNB u pravilu prati jaki lokalni imunosni odgovor uz pleocitozu i češće pozitivni AI. Protutijela u likvoru mogu perzistirati mjesecima. S vremenom se likvor normalizira i pleocitoza se više ne nalazi, ali dugo mogu zaostati prisutna likvorska protutijela uz pozitivan AI. Zato je poželjno pratiti serološki odgovor u krvi i likvoru od dana kada se bolesnik javi zbog simptoma, pa zatim nakon jednog, tri, šest i dvanaest mjeseci radi procjene i korelacije nalaza i bolesti (8,10,14,28,30,31).

U dijagnostici LNB nisu rijetka odstupanja od očekivanih rezultata. Primjerice, kod kliničke sumnje na diseminiranu LB i mogući meningitis, zbog vrlo rane diseminacije borelija u SŽS, likvor bolesnika može biti uredan (jer se infekcija tek razvija), no u likvoru se mogu dokazati borelije kulitvacijom ili PCR-om. Dugotrajna prisutnost EM ili podatak o nedavno prebo-

ljenom EM kojeg može pratiti (ali ne mora) prisutnost borelijskih protutijela u krvi, zahtijeva praćenje zbog mogućeg razvoja borelijskog meningitisa koji će se tek kasnije moći dokazati prema imunosnom odgovoru i likvorskoj pleocitozi. Rjeđe, može se dogoditi da borelije iz kože vrlo brzo prodru u SŽS i razvije se lokalna upala i LNB s pleocitozom, pozitivnim likvorskim protutijelima, pozitivnom kultivacijom ili PCR-om iz likvora, a da se u krvi specifična protutijela ne moraju detektirati - kao što ni EM ne mora biti klinički vidljiv. Stoga i negativni serološki nalaz u krvi treba kritički sagledati i pratiti imunosni odgovor u krvi i likvoru.

S druge strane, protutijela u krvi nakon LB prisutna su u različitim koncentracijama dugo, pa i doživotno nakon simptomatske ili asimptomatske infekcije. To je posebno važno percipirati u endemskim područjima s visokom seroprevalencijom u zdravoj populaciji. Ako se kod osoba s pozitivnim borelijskim protutijelima razvije neka druga neurološka bolest, pozitivni serološki nalaz može zavesti u dijagnostici. Pozitivni serološki rezultati u krvi i likvoru sami za sebe ne potvrđuju LNB, pa i u bolesnika s ozbiljnim neurološkim simptomima, jer zbog pasivne difuzije kroz oštećenu krvno-moždanu membranu protutijela se mogu naći i u likvoru. Potrebno je sagledati kliničke, anamnestičke i epidemiološke podatke, duljinu trajanja simptoma, sve prethodne nalaze kao i provedenu terapiju. Ako su bolesnici uz to bez kliničkih i laboratorijskih znakova infekcije SŽS-a i ne nalazimo pleocitozu, koja gotovo uvijek ukazuje na akutni proces, LNB se može s velikom vjerojatnošću isključiti.

CXCL13 i lajmska neuroborelioza

S obzirom na složenost dijagnostike LNB rađene su brojne studije sa ciljem da se nađe specifičan infektivni biljeg (citokin ili kemokin) kao rani pokazatelj infekcije SŽS-a. Od svih ispitivanih biljega CXCL13 pokazao se najperspektivnijim (33). Nakon ulaza borelija u SŽS, lokalni monociti te dendritične stanice, mikroglija, endotelne stanice i limfociti T počinju izlučivati CXCL13 i brzo se poveća njegova razina u likvoru, prije nego li se potvrdi AI. Razina CXCL13 u likvoru povezuje se s količinom borelija u SŽS-u na koje se vežu lokalni monociti koji proizvode CXCL13. CXCL13 je kemoartaktant, potiče migraciju limfocita B u SŽS i time podupire humoralni imunosni odgovor. U ranoj fazi LNB, CXCL13 se nalazi u gotovo svim likvorima (100 %) i robustni je biljeg aktivne infekcije. Nakon liječenja brzo se normalizira (tijekom približno 4 mjeseca), mnogo prije nego što se normalizira AI (8,10,34).

CXCL13 nije specifičan za LNB. Može biti povišen u neurosifilisu, tuberkuloznom meningitisu, limfomima SŽS-a i drugim bolestima. Specifičnost za LNB je oko 63 % zbog čega se u rutinskoj dijagnostici ne primjenjuje široko (35). Ipak, budući da prati akutnu upalu, može biti dobar indikator za ranu LNB, iako ne i jedini. Posebno može biti koristan u diferencijalnoj dijagnozi, jer je npr. u virusnim meningitisima, pneumokoknom meningitisu, multiploj sklerozi negativan ili je prisutan u niskim koncentracijama (10,34,35).

ZAKLJUČAK

Dijagnoza LNB mora biti u skladu s kliničkom slikom i laboratorijskim nalazima. LNB je 1) sigurno potvrđena ako kliničku sliku prati pleocitoza i intratekalna sinteza specifičnih protutijela; 2) vjerojatna ako intratekalnu sintezu ne možemo potvrditi, ali su prisutna specifična protutijela u krvi bolesnika uz pozitivne epidemiološke i anamnestičke podatke; 3) LNB nije vjerojatna ako nema pleocitoze ili nema analize likvora, iako su prisutna specifična protutijela u krvi bolesnika, ali klinička slika i epidemiološka anamneza nisu specifični. Ako postoji mogućnost, potrebno je LNB potvrditi kultivacijom i molekularnom dijagnostikom. U dijagnostici LNB neophodne su biokemijske i citološke pretrage krvi i likvora. Za interpretaciju laboratorijskih i kliničkih nalaza potrebni su znanje i iskustvo, jer se informacije mogu prezentirati različito s obzirom na okolnosti i specifičnost bolesnika, tako da je svaki bolesnik poseban izazov.

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SUMMARY

SPECIFICITY OF LYME NEUROBORRELIOSIS DIAGNOSTICS

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Lyme neuroborreliosis (LNB) is caused by hematogenous spread of *Borrelia* into the central nervous system (CNS), but entry through a peripheral nerve has also been described. Aseptic meningitis develops with or without cranial nerve palsy, which is the predominant clinical presentation. Facial nerve is most frequently affected. Unlike North America, other cranial nerves can be affected in Europe, which is related to the prevalence of different species of *Borrelia. Borrelia (B.) garinii, B. bavariensis,* and less frequently *B. afzelii* are most common in Europe, while *B. burgdorferi* sensu stricto is the only North American strain. Meningoradiculitis or Bannwarth syndrome is a typical LNB presentation described only in Europe. The symptoms of LNB can resemble various neurological diseases, which makes the diagnosis of LNB difficult. The diagnosis of LNB must include analysis of cerebrospinal fluid (CSF) in which pleocytosis is significant to support aseptic meningitis, and the association with *Borrelia* must be proven. Microbiological diagnosis of LNB includes cultivation, a demanding and long-term method (9-12 weeks), which is performed exclusively in reference centers, and molecular (polymerase chain reaction, PCR) and serological diagnostics. PCR is often false-negative due to the low number of strains in the CSF. Thus, serological diagnosis remains crucial to confirm LNB. Serology is performed on simultaneously collected serum and CSF samples, from which specific IgM and IgG antibodies, total immunoglobulins and/or albumins need to be determined. Serum and CSF samples must be analyzed by the same method under the same conditions in order to assess the intrathecal synthesis of specific antibodies, i.e., to calculate the antibody index in CSF (antibody index, AI). In patients with Lyme borreliosis, specific antibodies are produced relatively slowly, and their quantity increases with the duration of the infection. In early LNB, antibodies in the CSF are not always detectable while pleocytosis is present. In late LNB, a strong immune response is present in the CSF, as well as pleocytosis, and a positive AI can be determined. Over time, CSF normalizes and pleocytosis is no longer detected, but CSF antibodies can remain present for a long period of time. Therefore, the immune response in the blood and CSF has to be monitored from the day the patient presented with symptoms, and then, for example, at one, three, six and twelve months to assess the correlation of laboratory findings with the disease. The diagnosis of LNB must be in accordance with clinical, epidemiological and history data and laboratory findings, especially in CSF. LNB is confirmed if the clinical picture is accompanied by pleocytosis and intrathecal synthesis of specific antibodies; LNB is probable if intrathecal synthesis is not confirmed while specific antibodies are present in the patient's blood; and LNB is unlikely if there is no pleocytosis or no CSF analysis, although specific antibodies are present in the patient's blood but the clinical picture and epidemiological history are not characteristic. If there is a possibility, LNB should be confirmed by culture and molecular diagnostics. CXCL13 is a marker that can be useful as an additional test, even though it is not specific for LNB as it is elevated in CSF and observed during acute inflammation. Interpretation of laboratory and clinical findings in LNB requires knowledge and experience. The findings should be interpreted in accordance with the circumstances and condition of the patient, and therefore each patient represents a special diagnostic challenge.

Key words: Lyme neuroborreliosis, *Borrelia burgdorferi,* microbiological diagnostics, serological diagnostics, cerebrospinal fluid, cerebrospinal fluid antibody index

DELAYED MANIFESTATION OF POST-COVID MYOCARDITIS

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Introduction: Myocardial involvement of coronavirus disease 2019 (COVID-19) varies and is considered to be more serious in patients with severe COVID-19 clinical presentation. Although myocarditis is usually recognized in the setting of acute SARS-CoV-2 infection, delayed manifestations are recognized as well. *Case report.* A 51-year-old male patient was admitted due to the clinical signs of congestive heart failure, two months after moderate clinical expression of COVID-19 pneumonia, treated as an outpatient. Transthoracic echocardiography (TTE) revealed dilated cardiomyopathy with the presence of diffuse left ventricular (LV) hypokinesia, severely reduced ejection fraction (EF 18%) and diastolic dysfunction with increased left atrial filling pressure. Baseline laboratory tests revealed elevated hs-troponin I, NT-proBNP. Diagnostic workout excluded coronary artery disease. Cardiac magnetic resonance imaging strongly pointed in the direction of unrecognized post-COVID myocarditis. The patient was treated according to current guidelines for heart failure with reduced EF. Eight months after discharge, the patient had no limitations of physical activity and TTE showed significant improvement in the systolic function of the left ventricle, EF was 47%, with normal LV filling pressure. *Conclusion:* Myocarditis is not an infrequent manifestation of COVID-19 infection, especially in hospitalized patients with severe clinical presentation, and commonly manifests within the first week after initial symptoms. Our case report represents an example that also patients with mild form of COVID-19 treated as outpatients can have delayed onset of heart failure as a consequence of COVID-19-induced myocarditis. Therefore, COVID-19 patients deserve a comprehensive approach with systematic clinical and echocardiographic follow-up in order to establish a timely diagnosis, provide appropriate treatment, and prevent serious complications.

Key words: COVID-19, myocarditis, dilated cardiomyopathy, magnetic resonance imaging

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INTRODUCTION

Myocarditis is an inflammation of the myocardium which usually follows a microbial infection, although it can be caused by various pathogens (1). It is a common cause of death in young subjects, as well as a relatively frequent underlying cause of dilated cardiomyopathy (1). It is estimated that around 20% of patients with myocarditis develop chronic dilated cardiomyopathy (2). Myocarditis is most commonly caused by a viral infection and until now, the most frequently detected pathogens are adenoviruses, enteroviruses (coxsackievirus A and B), parvovirus B19, hepatitis C virus, human immunodeficiency virus, human herpesvirus 6, influenza A and B viruses, and lately viruses from Coronaviridae family, particularly severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 (3-5).

Until now, the COVID-19 pandemic has caused loss of over 5 million lives around the globe (6). The disease might be asymptomatic, but the usual symptoms include fever, cough, nasal congestion, fatigue, and other signs of upper respiratory tract infections (6). The infection can progress to severe disease corresponding to pneumonia in more than 50% of patients (6). De-

spite the fact that respiratory symptoms predominate, COVID-19 infection shows a significant cardiovascular component (6).

Various studies that used cardiac magnetic resonance imaging (MRI) have shown variable cardiac involvement among COVID-19 patients (7). Most of them find the prevalence of myocardial injury underestimated. It is, however, considered higher in patients with severe COVID-19 clinical presentation (8). According to a recent meta-analysis of intensive care unit (ICU) hospitalized patients, heart failure as a complication of COVID-19 infection was identified in nearly 50% of patients who died and merely 3% of patients who recovered (9).

We report a case of a previously healthy male patient who presented with dilated cardiomyopathy with heart failure symptoms as a sequel of unrecognized delayed onset of SARS-CoV-2 myocarditis.

CASE REPORT

A 51-year-old, previously healthy male patient was admitted to ICU at the end of January 2021 due to the clinical signs of congestive heart failure. The symptoms began two weeks prior to admission with progressively worsened shortness of breath, fatigue and swelling of lower legs, ankle joints and feet. The patient denied having fever, chest pain or fainting.

In November 2020, he suffered COVID-19 caused pneumonia and he was treated as an outpatient. The patient reported having palpitations and occasional oppressions in the chest area during the acute phase of infection, but those symptoms had gradually declined and finally disappeared. The clinical expression was moderate and he was considered fully recovered.

At admission to ICU, physical examination revealed that the patient was hypertensive (blood pressure 140/100 mm Hg), tachycardic (heart rate 120/min), tachypneic (respiratory rate 30/min), without heart murmurs, but with late inspiratory crackles in the lower third of the chest, and with swollen ankles, New York Heart Association Class III (NYHA III).

Arterial blood gas analysis showed slight hypoxemia with SaO_2 95%, pO_2 68 mm Hg, pCO_2 38 mm Hg, pH 7.48, lactate 1.2 mmol/L, HCO_3^- 28.3 mmol/L, BE 4.6 mmol/L. A 12-lead electrocardiogram (ECG) revealed sinus tachycardia of 130 bpm without ST-segment changes (Figure 1).

Table 1. *Laboratory findings on admission*

Laboratory finding	Result	Reference range
Hemoglobin (g/L)	146	120.0-170.0
Leukocytes (x10^9/L)	7.9	4.0-10.0
Platelets (x10^9/L)	263	150-400
International normalized ratio (INR)	1.23	1.2
Urea (mmol/L)	9	2.5-7.5
Creatinine (μmol/L)	124	50.0-120.0
Uric acid (μmol/L)	538	150.0-420.0
AST (U/L)	96	10.0-37.0
ALT (U/L)	221	10.0-40.0
GGT (U/L)	199	2.0-55.0
Total proteins (g/L)	54	64-83
Albumin (g/L)	30	34-50
CRP (mg/L)	6	0.0-5.0
hsTroponin I (ng/L)	21.4	00-34.2
NT-proBNP (pg/mL)	4831	0.0-125.0
D dimer (ng/mL)	728	0.0-500.0

AST = aspartate aminotransferase; ALT = alanine transaminase; GGT = gamma-glutamyl transferase; CRP = C-reactive protein

Baseline laboratory tests showed elevated high-sensitivity troponin I (hs-troponin I, 21.4 ng/L), NT-proBNP (4831 pg/mL) and D-dimer (728 ng/mL). The real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 was negative. Baseline laboratory analysis are shown in Table 1.

Two-dimensional transthoracic echocardiography (TTE) revealed dilated left ventricle with end-systolic diameter of 5.3 cm, end-diastolic diameter of 6.0 cm, with the presence of diffuse left ventricular hypokinesia, severely reduced ejection fraction (EF 18%) and diastolic dysfunction with increased left atrial pressure (E/e'=15.3). There was also moderate mitral and tricuspid regurgitation, elevated right ventricular systolic pressure of 56 mm Hg, and dilated inferior vena cava (2.5 cm) without respiratory collapsibility (Figure 2).

The patient was initially treated with intravenous diuretics and nitroglycerin infusion (5 mcg/min), while angiotensin-converting enzyme inhibitor (ACE-I), mineralocorticoid receptor antagonist (MRA) eplerenone and beta blocker were introduced after satisfactory cardiac compensation.

In addition to chest x-ray, diagnostics was supplemented with computed tomography (CT) of the chest, which uncovered discrete scars in the apical part of the right lung, as well as linear atelectasis in S10 on the left (Figure 3).

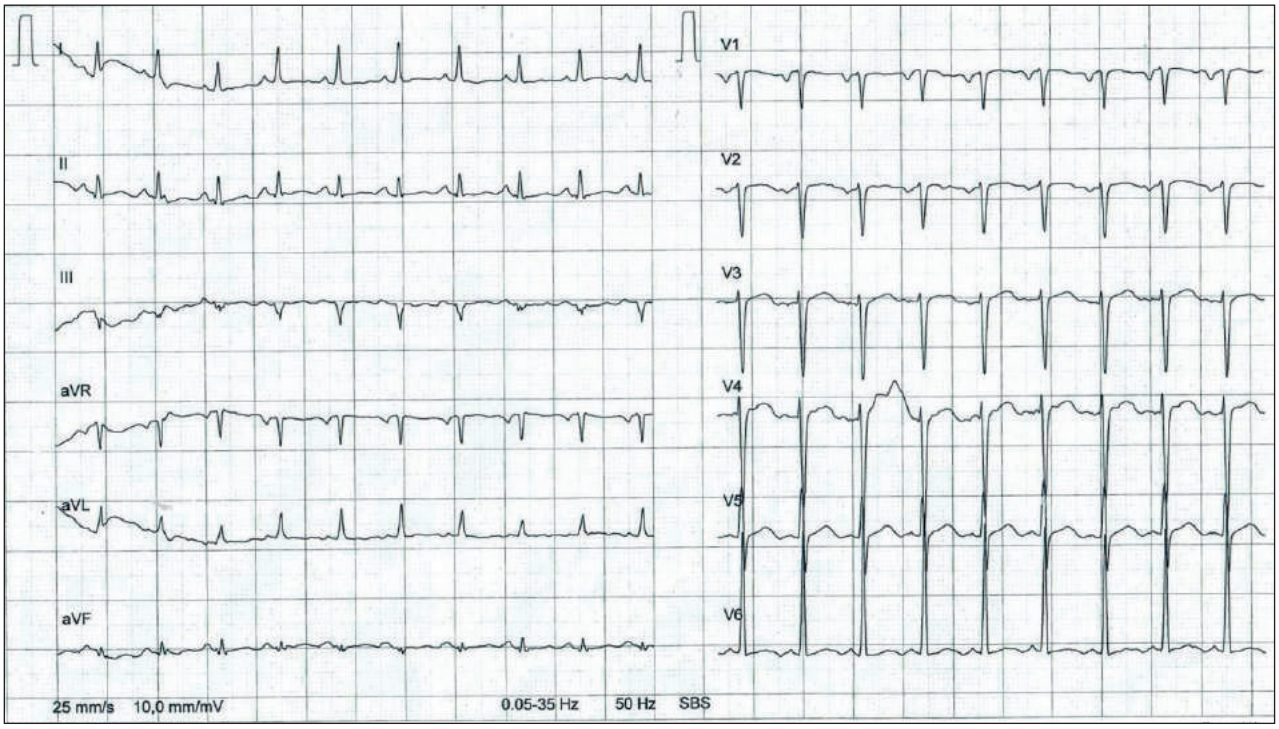

Figure 1. *Electrocardiogram: sinus tachycardia without ST-T changes.*

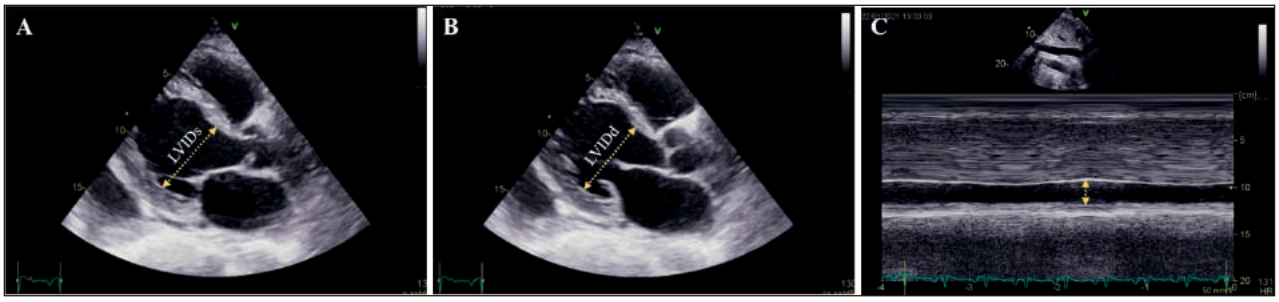

Figure 2. *Transthoracic echocardiography: (A) parasternal long axis-systole: LVIDs (left ventricular internal diameter end-systole) 5.3 cm (yellow dotted line); (B) parasternal long axis-diastole: LVIDd (left ventricular internal diameter end-diastole) 6.0 cm (yellow dotted line); (C) inferior vena cava without respiratory collapsibility (yellow line).*

To exclude coronary artery disease, CT coronarography was done and revealed nonsignificant stenosis of coronary arteries. A 24-hour Holter monitoring discovered two episodes of non-sustained ventricular tachycardia (both of 4 beats), thus the beta blocker was increased to a maximally tolerated dose.

In order to get to the underlying cause of heart failure, we performed serological tests that would detect potential cardiotropic viral infection. The patient tested negative for adenovirus, coxsackie virus, Epstein-Barr virus, cytomegalovirus, hepatitis B, human immunodeficiency virus (both IgG and IgM antibodies), influenza virus type A, influenza virus type B, and parainfluenza virus. Considering the x-ray result, we decided to exclude a possible atypical pneumonia causing microorganism. The patient's results were negative for *Coxiella burnetii, Chlamydia psittaci* and *Mycoplasma pneumoniae* infection.

Although the clinical presentation did not point in the direction of immune disease, we performed an essay in order to exclude the most common immune cause of heart failure (Table 2).

Cardiac magnetic resonance imaging (MRI) was done in order to establish if there were any signs of potential, previously unrecognized post-COVID myocarditis. Cardiac MRI revealed dilated left ventricle with moderately thickened myocardium, and globally and severely impaired kinetics. T2-weighted cardiovascular MRI showed increased intensity of the myocardi-

Table 2. *Immune assay results*

C3	C4	ANA	ASA	ASTO	Latex RF	CiC
Negative	Negative	Negative	Negative	Negative	Negative	Negative

C3 = complement component 3; C4 = complement component 4; ANA = antinuclear antibodies; ASA = anti-streptavidin antibodies; ASTO = antistreptolysin O test; RF = rheumatoid factor; CIC = Certification in Infection Control

um in comparison to the bone tissue. Myocardial late gadolinium enhancement (LGE) imaging highlighted the area with pathological coloring in the septal part of the mesocardium (mesocardial enhancement) (Figure 4), suggesting myocarditis as a possible cause of dilated cardiomyopathy.

The patient gradually recovered and after twelve days of hospital treatment, he was discharged with the following therapy: acetylsalicylic acid, beta-blocker, sacubitril/valsartan, furosemide and eplerenone. Laboratory tests on the day of discharge showed reference values (Table 3).

On the three-month follow-up, the patient had no limitations on ordinary physical activity although sustained effort was followed by discomfort, TTE showed increased EF to 30%, and 24-hour ECG demonstrated isolated ventricular ectopic beats without other heart rhythm abnormalities. The therapy continued without any significant changes, apart from the addition of dapagliflozin. We discussed the implantable cardioverter-defibrillator (ICD) placement but decided to temporarily give it up due to the EF increase.

On the second follow-up, eight months after discharge, the patient had no complaints or limitations of physical activity and TTE showed significant improvement in systolic function of the left ventricle, EF was 47%, with normal left atrial pressure (E/e' 7.0) and mild mitral and tricuspid insufficiency. Left ventricular global longitudinal strain showed slightly reduced values (peak global longitudinal strain was -15), mainly based on decreased values of regional strain for basal segments of anterior, anteroseptal and inferoseptal walls, and slightly decreased values for mid anterior, anteroseptal and inferoseptal segments (Figure 5). The medicamentous treatment was continued without changes.

Table 3. *Laboratory test results on the day of discharge*

Laboratory findings	Result	Reference range
Hemoglobin (g/L)	140	120.0-170.0
Leukocytes (x10^9/L)	7.4	4.0-10.0
Platelets (x10^9/L)	270	150-400
International normalized ratio (INR)	1	1.2
Urea (mmol/L)	6	2.5-7.5
Creatinine (μmol/L)	109	50.0-120.0
Uric acid (μmol/L)	379	150.0-420.0
AST (U/L)	20	10.0-37.0
ALT (U/L)	27	10.0-40.0
GGT (U/L)	40	2.0-55.0
Total proteins (g/L)	65	64-83
Albumin (g/L)	37	34-50
CRP (mg/L)	6	0.0-5.0
hsTroponin I (ng/L)	21.4	00-34.2
NT-proBNP (pg/mL)	300	0.0-125.0
D dimer (ng/mL)	<500	0.0-500.0

AST = aspartate aminotransferase; ALT = alanine transaminase; GGT = gamma-glutamyl transferase; CRP = C-reactive protein

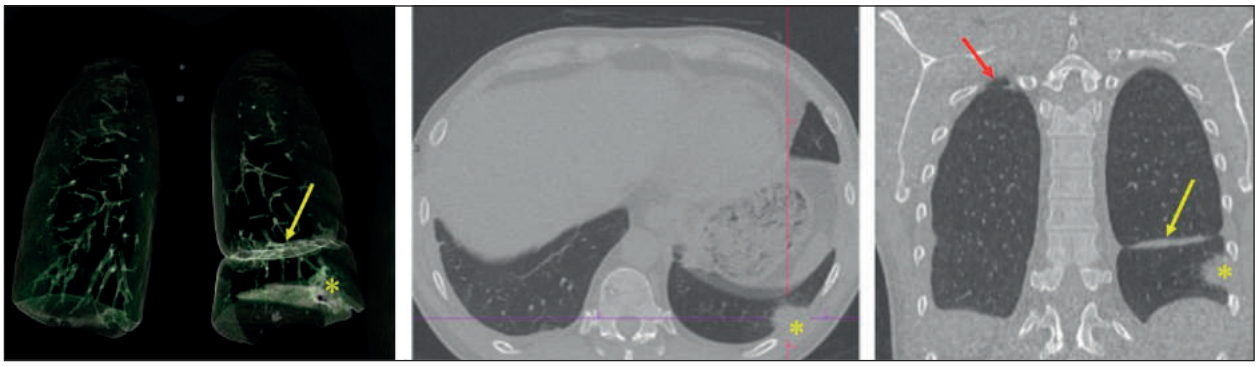

Figure 3. *Computed tomography of the chest: discrete scars in the apical part of the right lung (red arrow), subpleural nodule (asterisk), and linear atelectasis in S10 on the left (yellow arrow).*

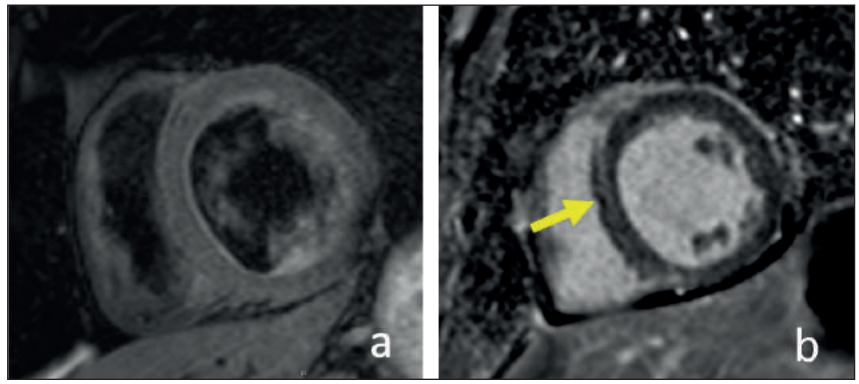

Figure 4. *Cardiac magnetic resonance imaging: short axis T2-weighted (A) and late gadolinium enhancement (B) images show areas of septal late gadolinium enhancement (mesocardial enhancement – yellow arrow), without visible edema.*

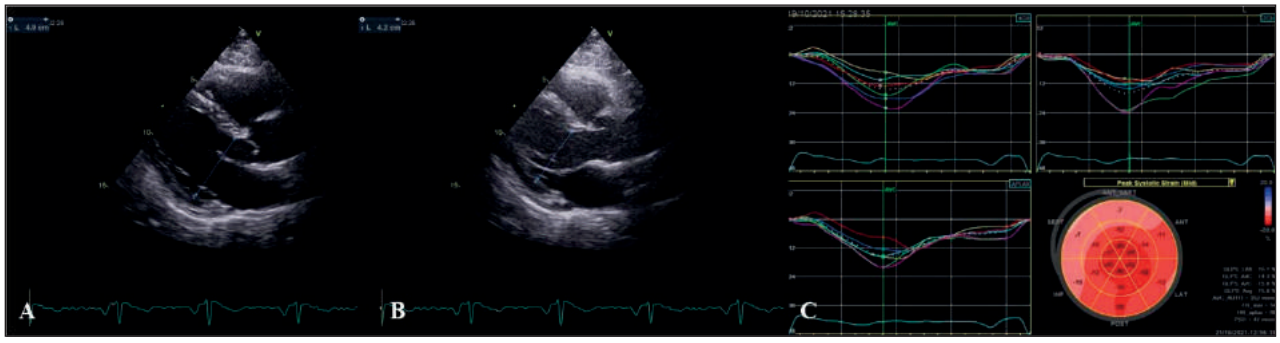

Figure 5. *Transthoracic echocardiography at eight-month follow-up: (A) parasternal long axis-diastole; (B) parasternal long axis-systole; (C) left ventricle global longitudinal strain.*

DISCUSSION

Myocarditis is a challenging diagnosis not only due to the heterogeneity of clinical presentations, but also because the endomyocardial biopsy as the diagnostic gold standard is not widely used (10). Myocarditis symptoms vary from mild chest pain and palpitations to cardiogenic shock and ventricular arrhythmia (10). Typically, myocarditis has a viral prodrome including fever, myalgias, and respiratory/gastrointestinal symptoms, but this can be exceedingly variable (11). It happens often, similar to the case we have chosen to describe, that the acute myocarditis symptoms remain unrecognized, and the patient first presents with the new onset of heart failure with corresponding symptoms (10).

Whenever myocarditis is suspected, it is required to exclude coronary artery disease and other cardiovascular or extra-cardiac non-inflammatory diseases that could explain clinical presentation (10). We did the same in our case, the patient was fully noninvasively examined and the coronary artery disease was excluded.

According to the current knowledge (8-10), endomyocardial biopsy (EMB) is the gold standard and should be done in order to set a definitive diagnosis. Being quite an invasive and highly specific method, it is not available in a vast number of centers (11). In circumstances where EMB cannot be obtained, cardiac MRI is described as a reasonable alternative (11-13). Furthermore, EMB is shown to be prone to a sampling error that can lead to false-negative results (14) and is not a routine clinical practice in our institution. We performed cardiac MRI in our patient. Cardiac MRI using Lake Louise criteria (that target three aspects of myocardial inflammation, i.e., edema, hyperemia, and necrosis and/or fibrosis) is shown to be highly specific and rather sensitive when it comes to diagnosing of myocarditis (11-13). Image interpretation relies upon analysis of signal intensities on T2-weighted, early gadolinium enhancement and late gadolinium enhancement (LGE) images (14). In our case, the T2 weighted sequence did show increased signal intensity of myocardium in comparison to the bone tissue, although not to the extent to diagnose myocardial edema (15). Myocardial LGE imaging did show changes in the septal part of the mesocardium that pointed strongly in the direction of possible myocarditis diagnosis (11,14,15), especially when combined with history data. We believe that the viral prodrome, constellation of symptoms, history of SARS-CoV-2 infection two months prior to admission, in relationship with MRI finding and myocardial recovery in the follow-up

period strongly support the diagnosis of delayed manifestation of post-COVID-19 myocarditis as the cause of heart failure in our patient.

The pathophysiology of COVID-19 caused myocardial injury is still elusive. It is thought to be a combination of direct viral injury and damage through the host's immune response (16). The presence of so-called cytokine storm with observed elevation of inflammatory cytokines (interleukin (IL)-2R, IL-6, IL-10, and tumor necrosis factor α) is so far considered to be crucial (11,16). Another theory implies that the virus uses the angiotensin-converting enzyme 2 *via* its S-spike to bind to the receptors, which becomes the entry point to the cell, cardiomyocytes as well (11). COVID-19 is recognized as a systemic vasculitis that affects not only the lung but all organs, such as the myocardium, which could be another possible mechanism of cardiac injury (17).

When it comes to treatment, patients with suspected COVID-19 myocarditis should be treated according to current guideline recommendations for heart failure and/or arrhythmia (11), the same as we did in our patient's case. Supportive care is also important. Some authors find that the routine use of immunosuppressive strategies did not show advantage when it comes to the course of COVID-19 myocarditis, although it may be helpful when it comes to treatment of COVID-19 pneumonia (11,16). Antiviral therapy has been used especially at the very beginning of the pandemic, but it did not show any benefit in mortality compared with placebo (11).

The high prevalence of post COVID-19 complications requires systematic clinical and echocardiographic follow-up of patients who developed COVID-19. Since we are dealing with a new clinical entity, there is still a lot of research to be done considering COVID-19 itself, as well as how it affects various organ systems, cardiovascular system included. By comprehensive approach, we aim to establish timely diagnosis, provide appropriate treatment, and prevent serious complications.

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S A Ž E T A K

ODGOĐENA MANIFESTACIJA MIOKARDITISA NAKON COVID-A

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Uvod: Zahvaćenost miokarda koronavirusnom bolešću 2019 (COVID-19) varira i smatra se ozbiljnijom u bolesnika s teškom kliničkom slikom COVID-19. Iako se miokarditis obično prepoznaje u okruženju akutne infekcije SARS-CoV-2, prepoznaju se i odgođene manifestacije. ***Prikaz slučaja:*** Bolesnik u dobi od 51 godine primljen je zbog kliničkih znakova kongestivnog zatajenja srca dva mjeseca nakon umjerene kliničke manifestacije pneumonije COVID-19, liječen ambulantno. Transtorakalna ehokardiografija (TTE) otkrila je dilatiranu kardiomiopatiju s prisutnošću difuzne hipokinezije lijevog ventrikla (LV), ozbiljno smanjenu ejekcijsku frakciju (18%) i dijastoličku disfunkciju s povećanim osjećajem tlaka u lijevom atriju. Osnovni laboratorijski testovi otkrili su povišen hs-troponin I, NT-proBNP. Dijagnostička vježba isključila je koronarnu bolest. Magnetska rezonancija srca snažno je pokazala u smjeru neprepoznatog miokarditisa nakon COVID-a. Bolesnik je liječen prema važećim smjernicama za zatajenje srca sa smanjenom ejekcijskom frakcijom. Osam mjeseci nakon otpusta bolesnik nije imao ograničenja tjelesne aktivnosti, a TTE je pokazao značajno poboljšanje sistoličke funkcije lijeve klijetke, ejekcijska frakcija je iznosila 47%, uz normalan tlak punjenja LV. ***Zaključak.*** Miokarditis nije rijetka manifestacija infekcije COVID-19, osobito u hospitaliziranih pacijenata s teškom kliničkom slikom, i obično se manifestira unutar prvog tjedna nakon početnih simptoma. Naš prikaz bolesnika je primjer da i pacijenti s blagim oblikom COVID-19 koji se liječe ambulantno mogu imati odgođeni početak zatajenja srca kao posljedicu miokarditisa izazvanog COVID-19. Stoga bolesnici s COVID-19 zaslužuju cjelovit pristup uz sustavno kliničko i ehokardiografsko praćenje kako bi se pravodobno postavila dijagnoza, omogućilo odgovarajuće liječenje i spriječile ozbiljne komplikacije.

Ključne riječi: COVID-19, miokarditis, dilatacijska kardiomiopatija, magnetska rezonancija

OUR EXPERIENCES IN THE TREATMENT OF CARCINOID NEOPLASMS OF THE GASTROINTESTINAL TRACT AT KARLOVAC GENERAL HOSPITAL – A RETROSPECTIVE STUDY

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Carcinoid is a slow-growing tumor of neuroendocrine origin from enterochromaffin APUD cells. About 2/3 of it arise in the digestive tract. Carcinoid tumors make up 1% of the cancers of the gastrointestinal tract and 50% of them are located in the area of the small intestine. They often are asymptomatic in the early stages of the disease, which makes them difficult to diagnose. It occurs most often in people at a mean age of 61.4 years. Epidemiological data show that the incidence is 2.47-4.48 *per* 100,000 and in the last 2-3 decades the incidence has been increasing. The cause of carcinoid tumors is unknown, but a genetic factor can play a role (it was observed in multiple endocrine neoplasia type 1, neurofibromatosis type 1, Von Hippel-Lindau disease) and inactivation of the tumor suppressor gene on the 11q chromosome. Carcinoids are hormonally active in about 10% of cases. A relative 5-year survival is 70%-90%. In our retrospective study conducted during the 2015-2019 period, we included 10 patients with carcinoid tumors of different locations who underwent surgical treatment with 5-year follow-up. The results of treatment were similar to those reported by other authors.

Key words: carcinoid, APUD cells, gastrointestinal tract

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INTRODUCTION

Carcinoid is a slow-growing tumor of neuroendocrine origin from enterochromaffin, amine precursor uptake and decarboxylation (APUD) cells. About 2/3 of carcinoid tumors arise in the digestive tract and make up 1% of the cancers of the gastrointestinal tract. The most common site is the area of the small intestine and appendix (more than 60%), then the rectum (15%), colon (5%-7%), stomach (2%-4%), liver (1%) and pancreas (2%-3%) (1-3). Carcinoid tumors are often asymptomatic until the late stage of the disease, and clinical presentation depends on the size, location, hormonal activity and presence of metastases. Obstruction occurs in about 1/3 of patients, followed by bleeding and appearance of carcinoid syndrome. The problems can last for years in the form of pain in the stomach and intermittent obstructions (4,5).

It most often occurs in people at a mean age of 61.4 years. Epidemiological data show that the incidence is 2.47-4.48 *per* 100,000 and in the last 2-3 decades the incidence has been increasing (1). The cause of carcinoid tumors is unknown, but a genetic factor can play a role (it was observed in multiple endocrine neoplasia (MEN) type 1, neurofibromatosis type 1, Von Hippel-Lindau disease) and inactivation of the tumor suppressor gene on the 11q chromosome (2).

About 10% of carcinoids are hormonally active and secrete excessive levels of hormones (the best known is serotonin 5-HT) (3).

Tumors smaller than 2 cm rarely metastasize (about 2%), unlike tumors larger than 2 cm (80%) (4,6). The metastatic disease that affects the liver can cause carcinoid syndrome, i.e., carcinoid crisis. The location of the tumor can be local, regional when the tumor has spread to the surrounding tissue or lymph nodes, metastatic when the tumor has spread to other parts of the body, and recurrent when the tumor has returned after treatment.

The diagnosis is based on clinical presentation, laboratory diagnostics (complete blood count, biochemical blood tests, 24-hour urine collection), gastrointestinal endoscopy, multi-slice computed tomography (MSCT), positron emission tomography, nuclear magnetic resonance, scintigraphy with somatostatin receptor, biopsy, angiogram (7).

Treatment and prognosis depend on the location of the tumor, size of the tumor, presence of metastases, and hormonal activity of the tumor (4). Treatment options for gastrointestinal carcinoids include surgery, radiation, chemotherapy, biological therapy, and hormone therapy. Relative 5-year survival is 70%-90% (1).

The prognosis of the disease is best in carcinoid of the appendix, and the prognosis of carcinoid of the small intestine is better than of carcinoid of the stomach and rectum (8).

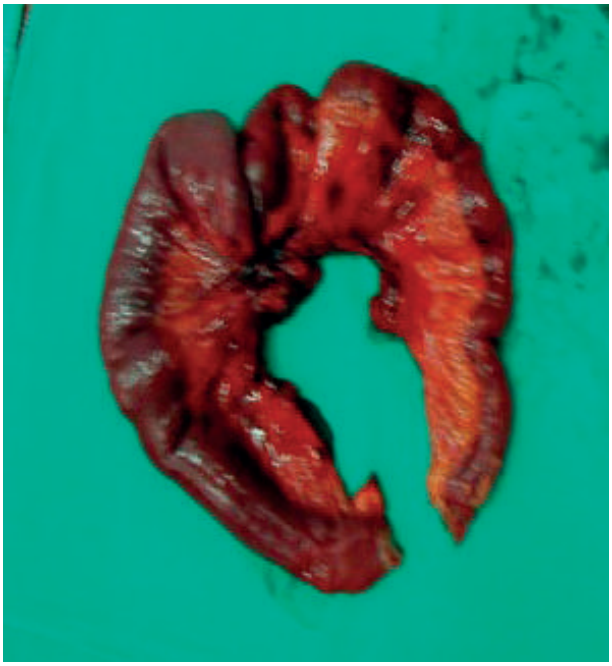

Figure 1. *Resected intestine with a tumor.*

MATERIALS AND METHODS

This retrospective study was conducted at Karlovac General Hospital, Department of Abdominal Surgery, during the 2015-2019 period. Data from medical records (history, letter of discharge) were evaluated. Data on 10 patients were included, 6 male patients in the age range of 55-69 years, and 4 female patients in the age range of 59-80 years. In 6 patients, carcinoid was diagnosed in the area of small intestine, in 3 patients in the area of stomach, and in 1 patient in the appendix. The disease was clinically manifested by bleeding from the digestive tract in 5 patients, obstruction of passage in 3 patients, subileus in 1 patient, and carcinoid syndrome in 1 patient.

The diagnostic methods we used were clinical presentation (gastrointestinal bleeding, passage obstruction, carcinoid syndrome), x-ray of the native abdomen (obstruction), gastrointestinal endoscopy, spiral MSCT, histopathologic diagnosis, immunohistochemical analysis (chromogranin, synaptophysin), and biochemical blood tests (serotonin, 5 HIAA in 24-hour urine).

Pathological macroscopic findings showed a 1.8-3 cm tumor growth, exophytic, partly ulcerated, mucosa with inflammatory changes of the epithelium, and preserved rough architecture (Figure 1). Histological findings showed solid clusters of trabecular and pseudo glandular formations, poorly differentiated uniform atypical epithelium with numerous mitoses, and hyperchromatic nuclei.

All patients underwent preoperative preparation, which included complete laboratory tests, x-ray of the heart and lungs, electrocardiogram, preoperative examination of the anesthesiologist (American Society of Anesthesiologists score) and were familiarized with treatment methods, possible complications, outcome and prognosis of the disease.

Operative procedures were performed by abdominal surgeons under general anesthesia, with early postoperative care in the intensive care unit and later on the abdominal surgery ward. Chemoprophylaxis was also carried out according to the scheme for abdominal surgery.

RESULTS

All 10 patients were operated on. In 6 patients, resection of the small intestine with a tumor, with formation of the side-to-side (LL) anastomosis was performed (Figures 2 and 3). In 3 patients, resection of the stomach with a tumor was performed according to the Billroth II method, and in one patient appendectomy was sufficient considering that the tumor size was less than 2 cm at the top of the apex of the appendix. In one patient, with the primary site in the stomach and metastatic liver disease, chemotherapy with Sandostatin was performed after gastric resection according to the Billroth II method.

All patients were monitored for 5 years. They had undergone MSCT of the abdomen and gastroscopy in pa-

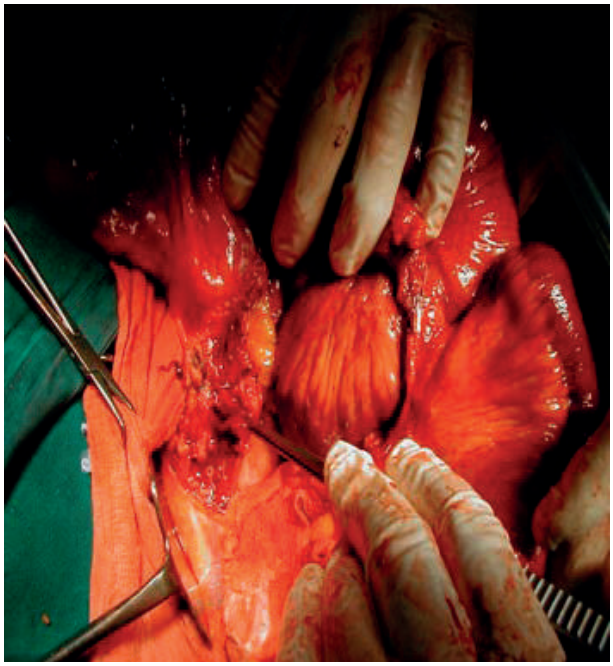

Figure 2. *Resection of the small intestine with a tumor.*

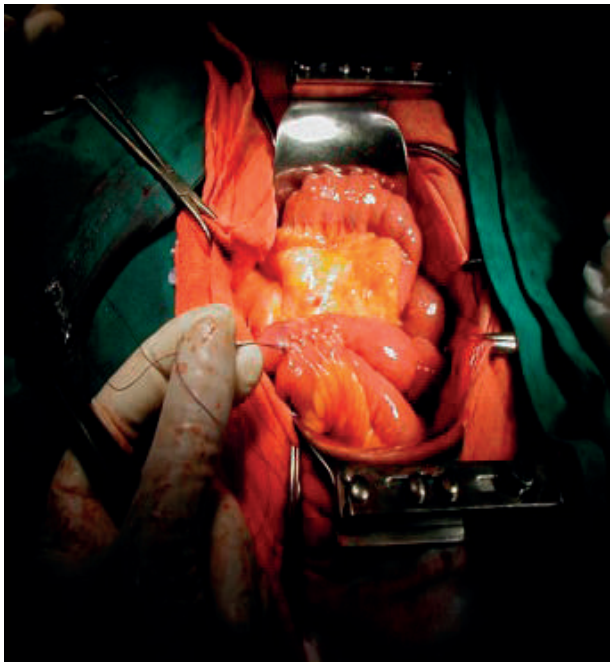

Figure 3. *Formation of the side-to-side (LL) anastomosis.*

tients with primary tumor sites in the stomach, with biochemical blood tests and determination of serotonin levels.

In 9 patients, there were no signs of the disease. In one of them, the disease relapsed in the stomach area with metastases in the liver two years after the operation; then, 6 cycles of chemotherapy with cisplatin and etoposide and Sandostatin were administered, after which the disease was under control. An 80-year-old female patient diagnosed with carcinoid of the stomach died within one year of gastric carcinoid diagnosis.

DISCUSSION

Carcinoid is a slow-growing tumor of neuroendocrine origin from enterochromaffin APUD cells. About 2/3 of carcinoid tumors arise in the digestive tract and make up 1% of the cancers of the gastrointestinal tract. The most common site is the area of small intestine and appendix (more than 60%), then the rectum (15%), colon (5%-7%), stomach (2%-4%), liver (1%) and pancreas (2%-3%) (1,3).

It most often occurs in people at a mean age of 61.4 years. Epidemiological data show that the incidence is 2.47-4.48 *per* 100,000 and in the last 2-3 decades the incidence has been increasing (1). The cause of carcinoid tumors is unknown, but a genetic factor can play a role (it was observed in MEN type 1, neurofibromatosis type 1, Von Hippel-Lindau disease) and inactivation of the tumor suppressor gene on the 11q chromosome (2). Carcinoids are hormonally active in about 10% of cases.

Gastrointestinal carcinoid tumors are clinically presented by obstruction, gastrointestinal bleeding, and secretion of excessive levels of hormones, the best known of which is serotonin 5-HT. Clinical presentation depends on the size, location, hormonal activity and presence of metastases (4,5). Tumors smaller than 2 cm rarely metastasize (2%), unlike tumors larger than 2 cm (80%) (4).

In our study, we showed that the mortality in non-metastatic disease was extremely low and that adjuvant chemotherapy in metastatic disease could have a significant effect on prolonging life.

CONCLUSION

Gastrointestinal carcinoid is a slow-growing tumor originating from neuroendocrine APUD cells and occurs most often in the small intestine and appendix, more than 60% of cases. Surgical treatment is the method of choice for non-metastatic disease, while lymphadenectomy and wide excision are unnecessary for localized disease. In the case of extended and metastatic disease, in addition to surgical treatment, adjuvant chemotherapy is also considered, which greatly prolongs median survival.

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SAŽETAK

NAŠA ISKUSTVA U LIJEČENJU KARCINOIDNIH NOVOTVORINA GASTROINTESTINALNOG TRAKTA, OPĆA BOLNICA KARLOVAC – RETROSPEKTIVNO ISTRAŽIVANJE

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Karcinoid je sporo-rastući tumor neuroendokrinog podrijetla iz enterokromafinih APUD stanica. Oko 2/3 karcinoida nastaje u probavnom traktu. Čine oko 1% raka u probavnom traktu, a primarno sijelo u 50 % karcinoida je u području tankog crijeva. U ranom stadiju bolesti bolesnici su često bez simptoma, što otežava postavljanje dijagnoze. Najčešće se javlja u osoba srednje dobi od 61,4 godine. Epidemiološki podatci pokazuju da je incidencija 2,47-4,48 na 100 000 stanovnika, a posljednjih 2-3 desetljeća incidencija je u porastu. Uzrok karcinoidnih tumora je nepoznat, ali ulogu može imati genetski čimbenik (uočeno je kod MEN tip 1, neurofibromatoze tip 1, Von Hippel-Lindau bolesti) te inaktivacija tumor supresorskog gena na kromosomu 11q. Karciniodi su u oko 10 % hormonski aktivni. Relativno petogodišnje preživljavanje je 70 %-90 %. U našoj retrospektivnoj studiji u razdoblju 2015.-2019. godine prikazujemo 10 bolesnika s karcinoidnim tumorom različitog sijela, koji su kirurški liječeni te praćeni tijekom 5 godina. Rezultati liječenja su slični podatcima koje iznose drugi autori.

Ključne riječi: karcinoid, stanice APUD, probavni trakt

TRAHEOTOMIJA U LIJEČENJU ORTOPNEJE KAO POSLJEDICE MADELUNGOVE BOLESTI

STJEPAN GRABOVAC[1,3], ĐURĐICA GRABOVAC[3], GORDANA KESIĆ VALPOTIĆ[2,3]

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Cilj rada je prikazati traheotomiju kao terapijsku opciju kod teške ortopneje uzrokovane Madelungovom bolesti. ***Prikaz slučaja:*** U radu smo prikazali bolesnika s teškom ortopneom kao posljedicom uznapredovale Madelungove bolesti. Bolesnik godinama spava u sjedećem položaju. Kao mjera liječenja predložena je traheotomija. Bolesnik je zadovoljan jer nakon više od 20 godina mirno, bez straha diše i spava u ležećem položaju što mu poboljšava kvalitetu života. ***Rasprava:*** Madelungova bolest vrlo je rijedak klinički entitet nepoznate etiologije. Karakterističan izgled bolesnika posljedica je prekomjernog, neprestanog i nekontroliranog nakupljanja neinkapsuliranog masnog tkiva ispod kože vrata, ramena i gornjeg dijela trupa. Nakupine masti rastu sporo, godinama, i početni simptomi su uglavnom estetske prirode. Zbog pritiska masnih naslaga mogu se javiti kompresivni simptomi. Dijagnoza je laka i potvrđuje se već na temelju anamneze i kliničkog pregleda, a kompjutorizirana tomografija i magnetska rezonancija utvrđuju distribuciju masnog tkiva. Liječenje je uglavnom kirurško. ***Zaključak:*** Dugotrajna Madelungova bolest kao jedan od simptoma ima i smetnje disanja, a kao najteži smatra se ortopneja kada bolesnik može disati samo u uspravnom položaju. Kako ne bi došlo do razvoja ortopneje, bolesnika treba na vrijeme upozoriti na moguće posljedice ako se Madelungova bolest na vrijeme ne liječi.

Ključne riječi: traheotomija, ortopneja, Madelungova bolest

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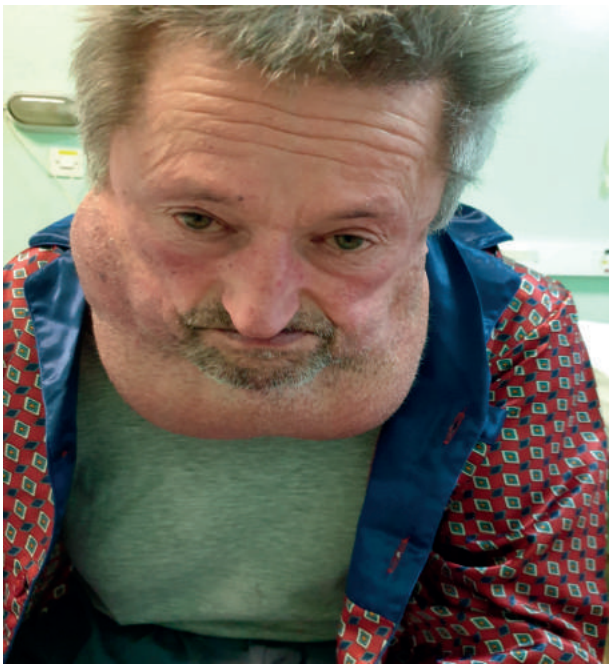
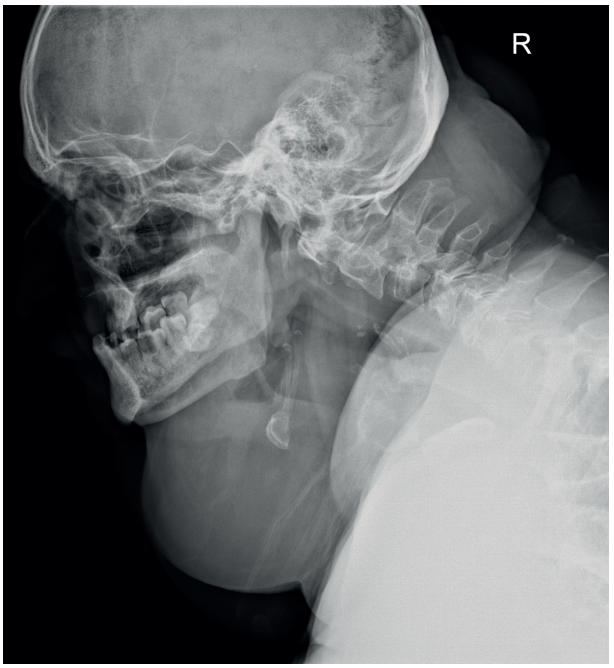
UVOD

Prvi opis Madelungove bolesti potječe još od sredine devetnaestog stoljeća (1-4). Klinički tijek je karakteriziran s dvije faze rasta. Ranijom, brzom, progresivnom i kasnijom, sporom fazom koja traje desetljećima. Prvi simptomi su uglavnom kozmetske naravi dok se u kasnijem tijeku mogu javiti i smetnje više zbog pritiska a manje zbog direktnog prodora masnog tkiva u okolne organe (4). Opisano je samo 7 slučajeva direktnog prodora masti u dušnik ili jednjak (4-7). Histološki se radi o zrelom masnom tkivu, iako je opisana i maligna degeneracija u miksoidni liposarkom (8). U liječenju se predlažu razne dijetetske mjere a kao kirurško liječenje radi se uglavnom redukcija masnog tkiva ponajprije u slučajevima pritiska ili jačih psihičkih smetnji uzrokovanih vanjskim izgledom (1). Nažalost, recidivi su neizbježni, a količina odstranjenog masnog tkiva unatoč trudu kirurga nikad nije dovoljna (8,9). Većina pacijenata s uznapredovalom Madelungovom bolesti ima probleme s disanjem poglavito u određenom položaju glave i tijela (5-7). Ortopneju kao jednu od najtežih respiratornih smetnji kod koje disanje uopće nije moguće osim u uspravnom položaju, nismo našli opisanu kod Madelungove bolesti. Zbog ortopneje, preoperacijske radiološke pretrage poput magnetske rezonancije i kompjutorizirane tomografije teško je napraviti u budnom stanju. Velike količine masnog tkiva u predjelu glave i vrata gotovo imobiliziraju vratnu kralježnicu stavljajući je u položaj koji je uvjetovan količinom i rasporedom masnog tkiva. U slučaju potrebe za medicinskim zahvatima koji zahtijevaju intubaciju, taj zahvat može biti otežan zbog nemogućnosti zabacivanja glave unatrag ili ograničenog otvaranja usta zbog obilnog masnog tkiva. Nazočnost masnog tkiva u predjelu ždrijela i grkljana može praviti anatomsku zapreku klasičnoj intubaciji. Ako liječenje bolesnika s Madelungovom bolešću zahtijeva intubaciju, neophodna je mogućnost korištenja alternativnih metoda poput intubacije pomoću fleksibilnog bronhoskopa (10-14).



Sl. 1. *Prijeoperacijski izgled bolesnika s Madelungovom bolesti.*

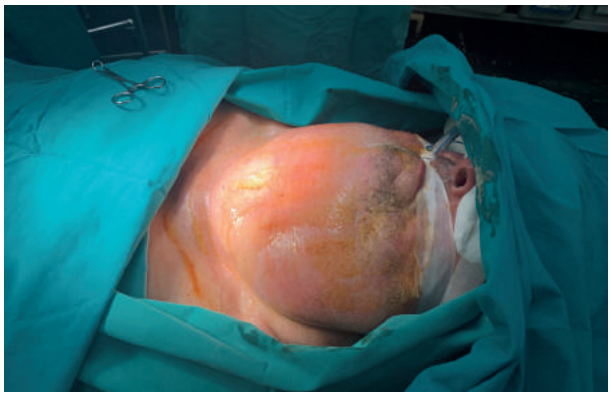


Sl. 2. *Profilna snimka vratne kralježnice. Strijelice pokazuju mekotkivna zasjenjenja koja odgovaraju lipomatoznim masama prednje i stražnje regije vrata.*

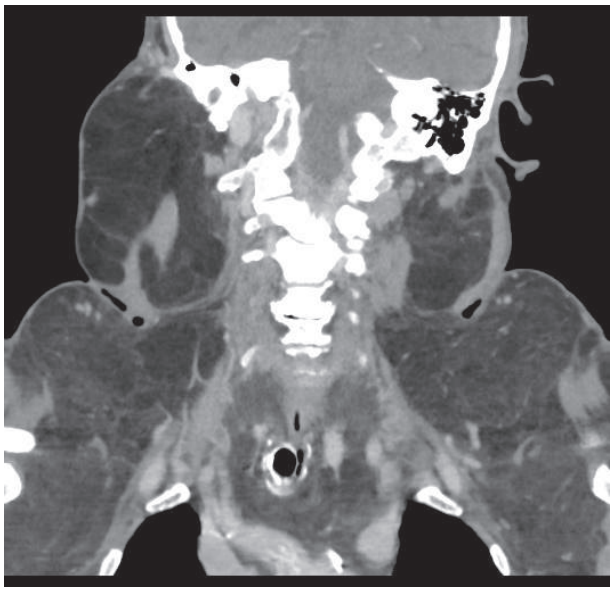
PRIKAZ BOLESNIKA

Muškarac u dobi od 69 godina javio se u otorinolaringološku ambulantu zbog poteškoća s disanjem. Navodi kako godinama spava u fotelji, a cijeli dan je u uspravnom položaju jer čim legne, nema zraka. Obavljanje osnovnih životnih aktivnosti izrazito mu je teško. Čitav izgled bolesnika bio je impresivan. Sve regije vrata, zaušne regije lica, ramena i prsa zauzete su velikim mekanim tvorbama koje klinički odgovaraju masnom tkivu. Lipomatozna masa spuštala se na prsa, a cijela glava je bila pognuta naprijed bez mogućnosti retrofleksije (slika 1). Nakupljanje masnog tkiva započelo je još prije 40 godina, nije teže bolovao i nije alkoholičar. Pri pregledu diše mirno s uzdignutom glavom, dok je pri promjeni položaja prisutan inspiratorni stridor. Fleksibilnom nazofaringolaringoskopijom uz bazu jezične strane epiglotisa bile su vidljive dvije okruglaste tvorbe oko 15 mm, intaktne površine koje ne zatvaraju dišni put, glasiljke su pomične, prostor između dostatan za disanje, a vidljivi dio dušnika slobodan. Od radiološke dijagnostike napravljena je snimka prsnog koša i vratne kralježnice u stojećem stavu na kojoj su vidljive sjene mekih tkiva bez suženja dišnog puta (slika 2).

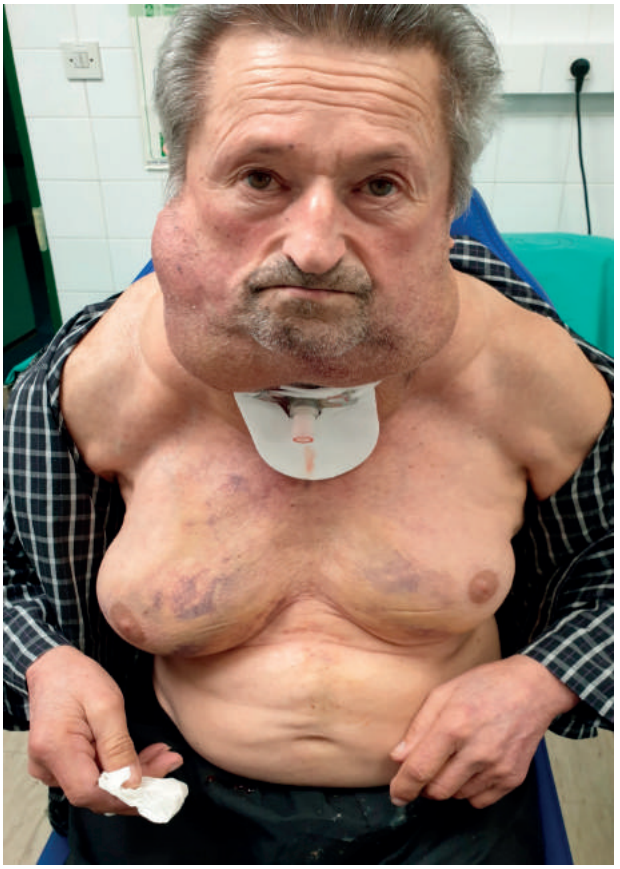
Sve radiološke pretrage koje bi zahtijevale ležeći položaj nije bilo moguće napraviti. Već prvu noć nakon prijma disanje se pogoršava, bolesnik postaje smeten, umoran je zbog kroničnog nespavanja i stalno sjedi pognute glave što dovodi do gušenja. Pulsnom oksimetrijom zasićenje krvi kisikom variralo je od lake, 9 kPa do teške 7,2 kPa hipoksemije, što je u prvom redu ovisilo o položaju glave. Kako se stanje nije moglo poboljšati konzervativnim mjerama, bolesniku je predložena traheotomija. Zbog anatomskih prepreka, ponajprije ogromne lipomatozne mase u sredini vrata te nemogućnosti retrofleksije glave zahvat nije bilo moguće izvesti u lokalnoj anesteziji. Anesteziolog je pregledao bolesnika i zaključio da je bolesnik apsolutno ortopnoičan te je odlučeno da se učini intubacija pomoću fleksibilnog bronhoskopa u budnom stanju i u sjedećem položaju. Bolesnik je bio suradljiv. Objašnjeno mu je o kakvom se postupku radi. Anestezirali smo sluznicu nosa i nazofarinksa 0,1 %-tnim sprejem lidokaina, kroz nos napravili orijentacijsku endoskopiju te kroz radni kanal bronhoskopa aplicirali epimukozni anestetik na glasnice i u dušnik. Između zuba postavili smo Heisterov otvarač te kroz usta uz pomoć bronhoskopa na koji smo postavili endotrahealni tubus broj 7,5 intubirali bolesnika i uveli ga u balansiranu endotrahealnu anesteziju uz relaksaciju, minimalnu analgosedaciju i puni monitoring. Učinili smo horizontalni rez cijelom širinom sredine vrata te odstranili preko 2000 grama masnog tkiva srednje regije vrata kako bismo došli do dušnika i napravili traheotomiju (slika 3). Patohistološki se radilo o zrelom masnom tkivu. Već prvu noć bolesnik je spavao mirno na leđima što nije mogao godinama. Nakon traheotomije bolesnika smo uputili na kompjutoriziranu tomografiju gdje je vidljivo izrazito umnoženo masno tkivo svih regija vrata koje razmiče vratnu muskulaturu. Masno tkivo prati se od razine nepčanih lukova, parafaringealno i reducira lumen



Sl. 3. *„Ispunjen" vrat od brade do ispod prsne kosti. Na koži je označena linija reza.*



Sl. 4. *Kompjutorizirana tomografija u koronarnoj projekciji: tamnija područja pokazuju distribuciju masti u parotidnim regijama, bočnim regijama vrata i oko dušnika (bijele strijelice).*



Sl. 5. *Poslijeoperacijski izgled bolesnika.*

ždrijela. U predjelu epiglotisa i ventrikularnih nabora vidljive su nodozne promjene koje sužuju lumen grkljana (slika 4). Konačan dekanilman zbog lokalnog nalaza za sada ne dolazi u obzir, bolesnik uredno govori sa začepljenom kanilom, a o daljnjem postupanju glede lipomatoze za sada nismo odlučili (slika 5).

RASPRAVA

Velike proširene masne nakupine u predjelu glave i vrata prvi je opisao Sir Benjamin Brodie 1846., a 1888. godine Otto Madelung opisuje seriju od 33, a 1889. Launois i Banasaude od 65 bolesnika sa sličnim vanjskim izgledom i kroničnim alkoholizmom kao zajedničkom karakteristikom (2-4). Do sada je opisano nešto više od 200 bolesnika s Madelungovom bolesti (1,5). Kako je izgled bolesnika dojmljiv, postoje brojni sinonimi koji ga u svom nazivu opisuju, kao što su: simetrična adenolipomatoza, cefalotorakalna lipodistrofija, masni vrat, multipla simetrična lipomatoza no kao najupečatljiviji zadržao se Madelungova bolest (1,5,14,15). Danas se opisuju dva fenotipa Madelungove bolesti: tip 1. koji se javlja isključivo kod muškaraca u dobi između 30 i 70 godina uz nakupljanje masti u predjelu glave i vrata te gornjeg dijela trupa i tip 2. koji se može naći i kod osoba ženskog spola gdje se nakupine masti nalaze i u predjelu donjih udova. Bolest se uglavnom javlja sporadično, češće u zemljama mediterana i istočne Europe (1,4). Ima pojava bolesti i unutar obitelji gdje masno tkivo za razliku od sporadičnih slučajeva zahvaća i donje ekstremitete. Iako se većinom javlja u odrasloj dobi, opisano je nekoliko slučajeva Madelungove bolesti kod djece (5,16). Većinom se radi o uznapredovalim slučajevima s obilnim masnim naslagama. Nakupine masti daju bolesniku karakterističan izgled koji se uspoređuje s vratom poput bizona, konjskim ovratnikom ili ako su naslage izražene u predjelu parotidne regije govori se o obrazima kao kod hrčka. Mast

prodire u susjedna tkiva, inkorporira krvne žile, mišiće i živce a kasnije može doći do razvoja kompresivnog sindroma zbog pritiska na dušnik i jednjak (1,5,7). Madelungova bolest često je udružena s hiperlipidemijom, endokrinim bolestima, bolestima jetre i polineuropatijom. Iako postoji nekoliko hipoteza nastanka, etiologija Madelungove bolesti je i dalje nepoznata (1,17-19). U liječenju se koriste razne dijetetske mjere: izbjegavanje alkohola, davanje multivitaminskih pripravaka, hormona štitnjače pa čak i agonista ß 2 adrenergičnih receptora (5,7,9). Jedino liječenje s djelomičnim rezultatima je kirurško odstranjenje masnih naslaga pazeći pri tome na vitalne strukture u regijama lica i vrata koje je zbog njihove uklopljenosti u masno tkivo ponekad teško identificirati. Kako bi se izbjegli veliki kirurški rezovi radi se i liposukcija vrata pod kontrolom ultrazvuka (20,21). Kompresivne simptome kao što je ortopneja trebalo bi preduhitriti i bolesnika ranije uputiti u ustanovu u kojoj mu se može pomoći (22,23). Ako se pristupa kirurškom liječenju posljedica Madelungove bolesti neophodna je dobra endoskopska procjena dišnog puta kao i mogućnost intubacije uz pomoć fiberoptičkog bronhoskopa poglavito u slučaju oropneje (23-26).

ZAKLJUČAK

Madelungova bolest osim kozmetskih simptoma može imati i za život opasne posljedice kao što je ortopneja. Traheotomija u slučaju teške ortopneje ne samo da spašava život bolesniku već mu značajno poboljšava i kvalitetu života. Madelungovu bolest svakako treba promatrati s aspekta otežanog dišnog puta te bolesnika treba puno ranije upozoriti na moguće komplikacije koje mu u konačnici mogu ugroziti život.

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SUMMARY

TRACHEOTOMY IN THE TREATMENT OF ORTHOPNEA AS A CONSEQUENCE OF MADELUNG'S DISEASE

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The aim of this paper is to present tracheotomy as a therapeutic option in severe orthopnea caused by Madelung's disease. This paper presents a patient with severe orthopnea as a consequence of advanced Madelung's disease. The patient had been sleeping in a sitting position for years. Tracheotomy was suggested as a treatment measure. He was satisfied because after more than 20 years, he could breathe calmly and without fear and could sleep in a lying position, which improved his quality of life. Madelung's disease is a very rare clinical entity of unknown etiology. The characteristic appearance of the patient is a consequence of the excessive, continuous and uncontrolled accumulation of unencapsulated adipose tissue under the skin of the neck, shoulders and upper trunk. Fat deposits rise slowly over years and the initial symptoms are generally of aesthetic nature. Pressurized fat deposits may cause compressive interference. Diagnosis is easy and confirmed on the basis of medical history and clinical examination, and computed tomography and magnetic resonance imaging determine the distribution of adipose tissue. The treatment is mainly surgical. One of the symptoms of long-term Madelung's disease are breathing problems, and orthopnea is considered most severe when the patient can only breathe in upright position. In order to prevent the development of orthopnea, patients should be warned in time about the possible consequences that can occur if Madelung's disease is not treated on time.

Key words: tracheotomy, orthopnea, Madelung's disease

CUTANEOUS TUBERCULOSIS IN AN IMMUNOCOMPETENT TWENTY-ONE-YEAR-OLD MAN

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Cutaneous tuberculosis is a rare form of tuberculosis. Considering the numerous skin manifestations that differ not only in clinical features but also in the way the infection reaches the skin, cutaneous tuberculosis is a great challenge for diagnosis. We present a case of cutaneous tuberculosis in a 21-year-old male migrant from Pakistan, hospitalized at the Department of Lung Diseases and Tuberculosis, Sarajevo University Clinical Center due to recurrent abscesses, furuncles and carbuncles of the left thigh and right forearm, that persisted for the past year and were treated with various antibiotics. Pus obtained by abscess incision, wound swabs of the left thigh, and skin tissue obtained by biopsy were sent for microbiological and *Mycobacterium (M.) tuberculosis* analysis. Wound swabs and skin tissue culture were positive for *M. tuberculosis* on solid (Löwenstein) and liquid (MGIT) medium. Sputum smear and culture for *M. tuberculosis* were negative. Chest x-ray also was without active pathomorphological changes. Antituberculosis therapy was started with four drugs (isoniazid, ethambutol, pyrazinamide, rifampicin). In addition to antituberculosis therapy, the wound of the left thigh required surgical treatment. The patient was successfully treated with antituberculosis therapy along with surgical treatment of skin changes and their healing. The diagnosis of cutaneous tuberculosis in our patient was established after a long period and after the failure of antibiotic treatment. Proper diagnosis of cutaneous tuberculosis is very important since it can be well treated with common antituberculosis therapy.

Key words: cutaneous tuberculosis, diagnosis, treatment

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INTRODUCTION

Cutaneous tuberculosis is a rare form of tuberculosis, occurring in less than 1% of cases, especially in developing countries (1). Considering the numerous skin manifestations that differ not only in clinical features but also in the way the infection reaches the skin, cutaneous tuberculosis is a great challenge for diagnosis (2). *Mycobacterium (M.) tuberculosis* complex, including *Mycobacterium bovis*, as well as Bacillus Calmette-Guérin (BCG) vaccine, are the most common causes of skin changes (3). Mycobacteria are slow-growing aerobic bacilli with a high concentration of lipids in their cell wall, which makes them impermeable to many colors. Because of their ability to retain their color and survive decolorization with acid and alcohol, they are labelled as acid-fast bacilli (AFB) (4). Numerous factors are associated with an increased risk of getting sick with tuberculosis, the most important of which are the patient's immune status, living conditions, history of previous illnesses, environmental factors, and nutrition (5,6).

CASE REPORT

We present a case of cutaneous tuberculosis in a 21-year-old man, a migrant from abroad, without prior immunocompromising conditions or comorbidities. The patient was admitted to the Department of Lung Diseases and Tuberculosis, Sarajevo University

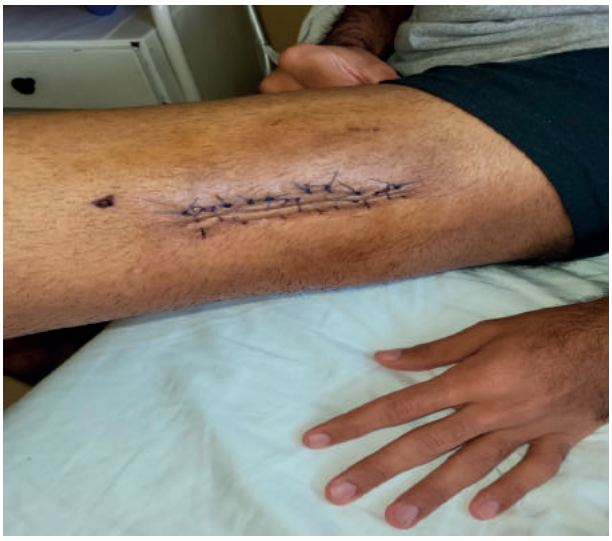

Figure 1. *Skin defect with sutures.*

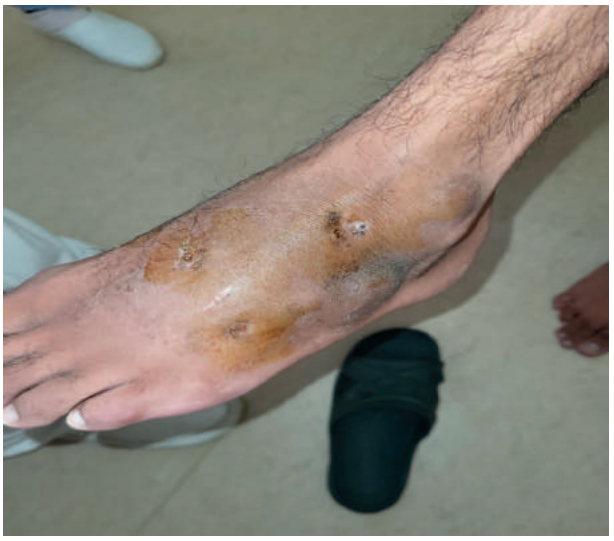

Figure 2. *Crusts on the skin after furuncle secretion.*

Clinical Center, due to recurrent abscesses, furuncles and carbuncles of the left thigh and foot, as well as the left and right forearm, that had lasted for the past year, along with osteomyelitis of the left foot, which had been treated for a year with various antibiotics. For the previous two years, the patient lived in large refugee camps in different countries with a low level of hygiene, without proper nutrition, and was in contact with tuberculosis patients. On admission, he was communicative, moved with the help of crutches, average osteomuscular constitution, malnourished (body mass index 18.2), afebrile, eupneic, with normal auscultatory findings over the lungs and heart, and hypotensive. BCG vaccination scar was visible on his left upper arm. On examination, in the area of the lateral side of the left thigh, there was a skin defect, neat edges, with sutures present, and slightly moist (Figure 1). There was a visible scar tissue 10 cm long on the right elbow, as well as scar tissue on the left forearm and crusts on the skin of the dorsal part of the left foot after furuncle secretion (Figure 2). Extremities were without edema, regularly palpable pulsations over arteries.

DIAGNOSTIC METHODS

Upon admission, complete laboratory findings were obtained, which indicated a normal number of leukocytes and erythrocytes, with a drop in hemoglobin and hematocrit, slightly elevated D-dimer values, and high C-reactive protein. In differential blood count, a reduced number of lymphocytes and monocytes was found, along with a normal number of neutrophils. Electrolyte status was normal.

Sputum smear and culture for AFB (*M. tuberculosis*) were negative. Chest x-ray was normal, without active pathomorphological changes, and so was bronchoscopic examination. As the bronchoscopic findings and chest radiograph were normal, we decided not to perform computed tomography scan of the chest.

On standard x-rays of the left foot in the dorsoplantar and profile projection, the bone structure and mineralization were reduced, with emphasized reduction of the bone structure and mineralization of the base of the metatarsal III and IV, that according to x-ray characteristics, may have corresponded to osteomyelitis changes (Figure 3).

Pus obtained by abscess incision, wound swabs of the left thigh, and skin tissue obtained by biopsy were sent for microbiological and *M. tuberculosis* analysis. There was no isolation of the usual microbiological agents in the biological samples of our patient. Wound swabs and skin tissue culture were positive for AFB on solid (Löwenstein) and liquid (MGIT) (mycobacteria growth indicator tube) medium. Hepatitis markers were negative. VIKIA and COMBO HIV were negative. Interferon Gamma Release Assay (IGRA) analysis for *M. tuberculosis* was performed. The positive result was obtained by Quantiferon-TB Gold In-Tube (QFT-GIT) analysis conducted at the Biochemical Laboratory, Sarajevo University Clinical Center.

Antituberculosis treatment was administered and started with four drugs (isoniazid, ethambutol, pyrazinamide, and rifampicin) for nine months. General condition of the patient was improving, but there was no adequate healing of the existing wound despite regular wound treatment and antituberculosis therapy admin-

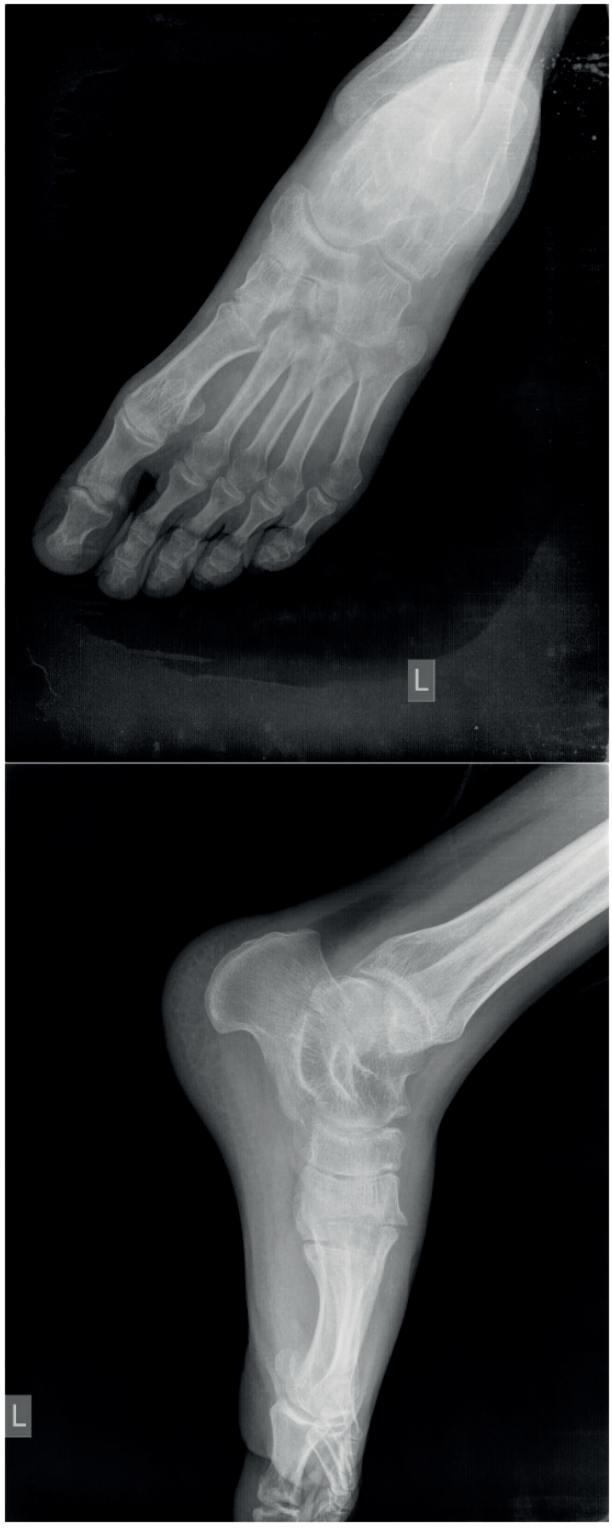

Figure 3. *X-ray of the left foot consistent with osteomyelitis.*

istered. Wound swabs were positive for AFB on liquid (MGIT) medium after 40 days of the antituberculosis therapy prescribed. Wound dehiscence occurred (Figure 4) and an open wound was bandaged daily with sterile dressings.

Figure 4. *Wound dehiscence despite regular wound treatment and antituberculosis therapy.*

In addition to antituberculosis therapy, the wound on the left thigh also required surgical treatment. After consultation with an orthopedist, the patient was transferred to the Department of Orthopedics due to the need of surgical treatment of the existing wound. After the wound healing, the patient was discharged for further treatment at the refugee center under the supervision of a resident doctor. Antituberculosis therapy was carried out for 9 months. The patient was successfully treated with antituberculosis therapy along with surgical treatment of skin changes and their healing**.** The patient recovered, and the wounds and abscesses were healed.

DISCUSSION

Tuberculosis is an infectious disease caused by *M. tuberculosis*, which predominantly affects the lungs, while lymph node involvement is the most common form of extrapulmonary tuberculosis (EPTB), which occurs in 20%-60% of all EPTB cases. Tuberculosis can affect the bones (6% of cases) and central nervous system (3% of cases), while the skin is very rarely involved, occurring in less than 1% of cases (1).

According to the latest data from the World Health Organization, a total of 1.6 million people died from tuberculosis in 2021, including 187,000 people infected with human immunodeficiency virus (HIV). Globally, tuberculosis was the 13th leading cause of death and the second leading infectious cause, right behind COVID-19 and ahead of HIV/AIDS in 2021. As many as 10.6 million people were affected by tuberculosis, of

which 6 million were men, 3.4 million women, and 1.2 million children. In addition, tuberculosis is present in all countries and age groups. However, the 30 most affected countries accounted for 87% of cases. Globally, tuberculosis incidence is decreasing by around 2% annually, which is an overall decline of 11% since 2015 and 2020, but which compares to a 20% decline in the incidence over the same period, according to the End Tuberculosis Strategy, almost half as much as predicted (7).

Factors such as malnutrition, alcoholism, drug addiction, disorders of the immune response, and even poor housing conditions can cause tuberculosis (8). An additional challenge are patients suffering from HIV infection, where *M. tuberculosis* is the most virulent opportunistic pathogen among HIV patients (9,10).

Clinical signs and symptoms of cutaneous tuberculosis may include joint pain, regional lymphadenopathy, painful nodular, and later ulcerating lesions, with or without symptoms of pulmonary tuberculosis (10). Cutaneous tuberculosis can occur as a result of exogenous inoculation, continuous spread from the focus of infection, or hematogenous spread from a distant focus. An additional useful concept of dividing cutaneous tuberculosis is based on the 'bacterial load', where cutaneous tuberculosis is classified into multibacillary and paucibacillary forms (11). Primary tuberculosis inoculation occurs when mycobacteria enter the skin or, less commonly, the mucosa of a person who has not previously been infected or vaccinated against *M. tuberculosis*. As AFB cannot penetrate the normal, intact skin barrier, some form of injury is required to initiate the infection. The entry point for AFB is usually through minor skin scratches, nail wounds, impetigo or ulcers (12). In patients with pre-existing immunity to tuberculosis, post-primary skin inoculation usually occurs, with the development of a hyperkeratotic papule, which eventually becomes a true wart (5,11,12).

Enlargement of the lymph glands as a result of inoculation is seen in patients with primary lesions, while it is absent in persons who have been vaccinated or previously treated for tuberculosis (11). Cutaneous tuberculosis infection may also occur by continuous spread to the skin from a subcutaneous focus (most commonly tuberculous lymphadenitis or tuberculosis of the bones and joints), or may be secondary to tuberculous epididymitis (5,13). In the past, the term scrofuloderma was used to describe this condition. The most commonly affected organs are cervical lymph nodes; children are affected more often than adults (5,11,12).

Cutaneous tuberculosis can be the result of autoinoculation of the mucosa and adjacent openings, which occurs when tubercle bacilli are directly coughed up or transmitted to immunocompromised patients. Then the tubercle bacilli invade tissue that is normally resistant to infection. In the past, the term orofacial tuberculosis was used to describe this condition (2,5,12).

Lupus vulgaris is an example of cutaneous tuberculosis caused by the hematogenous spread of the causative agent. It is a special type of chronic cutaneous tuberculosis that occurs in individuals previously immunized against *M. tuberculosis*. Occasionally, lupus vulgaris can appear precisely at the sites of primary contact with tuberculosis, such as on the scars of scrofuloderma, or at the sites of repeated BCG vaccination.

The disease presents clinically differently, from psoriasiform lesions, nasal ulceration, and nasal cartilage destruction to widespread hematogenous dissemination, which is why the disease is often misdiagnosed. In 10.5% of cases, the disease can change malignantly in terms of the development of squamous cell or basal cell cancer up to 25-30 years after infection (2).

A less common, fulminant form of cutaneous tuberculosis, previously known as *TB cutis miliaris disseminata,* occurs in infants or children after acute hematogenous dissemination of *M. tuberculosis*, which is often fatal. Increasingly, it can also be found in individuals with weakened cellular immunity, such as patients with advanced HIV (14). Cutaneous hematogenous dissemination of *M. tuberculosis* can often occur in the form of soft tissue abscesses (in the past the term gumma was used) and nodules (2).

Such presentation of cutaneous tuberculosis was present in our patient, who was not immunocompromised, but was a malnourished person on the move, constantly exposed to poor hygienic conditions and contact with other people suffering from tuberculosis.

Both scrofuloderma and gumma are forms of cutaneous tuberculosis often associated with the involvement of bones and joints (5,11,12) as it was also the case in our patient.

Cases of chronic cutaneous miliary tuberculosis have also been described, a very often forgotten entity, which is also caused by hematogenous spread of the bacilli into the skin, and which is characterized by numerous erythematous, confluent papules and plaques, prone to peeling (14). A group of skin changes that appear in the presence of tuberculosis but do not contain culturable AFB are designated as tuberculids (5,11). Seen histopathologically, they were previously considered an allergic reaction. These conditions included erythema induratum, papulonecrotic tuberculides, and lichen scrofulosorum. They are considered paucibacillary forms of cutaneous tuberculosis infection (5,8,11).

Because most patients with cutaneous tuberculosis have an active systemic infection, treatment consists of standard two months of 4 antituberculosis drugs and then four months of two antituberculosis drugs (15).

Considering that our patient had bone involvement by tuberculosis as well, we administered antituberculosis therapy according to the nine-month regimen, namely isoniazid, rifampicin, ethambutol, and pyrazinamide for two months, followed by another seven months of isoniazid, rifampicin.

CONCLUSION

Taking into account a number of predisposing factors that favor the development of tuberculosis, such as diabetes mellitus, HIV infection, immunocompromising conditions, previous malignant diseases, malnutrition, but also information about previous tuberculosis or immunization against it (BCG vaccine) that can affect the clinical tuberculosis presentation, the importance of taking thorough history data and insight into previous history of the patient's illness is emphasized in order to raise suspicion of this disease.

In the vast majority of cases of cutaneous tuberculosis, the diagnosis of this disease was established only after a long period and after failure of the usual antibiotic regimens for the treatment of skin lesions, as was the case in our patient. Only proper diagnosis established on time and initiation of antituberculosis therapy can prevent further progression of skin changes.

Recurrent abscesses, and the absence of isolation of the usual microbiological agents in the biological samples of our patient ultimately raised suspicion of a cutaneous form of tuberculosis.

Although rare, accounting for less than 1% of cases, cutaneous tuberculosis has a good treatment response rate to common antituberculosis therapy regimens.

Ultimately, this work aims to draw attention to the quite unusual manifestations of tuberculosis and enable patients to have timely access to treatment of this disease.

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S A Ž E T A K

TUBERKULOZA KOŽE KOD IMUNOKOMPETENTNOG DVADESETJEDNOGODIŠNJEG MUŠKARCA

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Kožna tuberkuloza je rijedak oblik tuberkuloze. S obzirom na brojne kožne manifestacije koje se razlikuju ne samo po kliničkim karakteristikama, nego i po načinu na koji infekcija dospijeva na kožu, kožna tuberkuloza veliki je izazov za dijagnosticiranje. Prikazujemo slučaj kožne tuberkuloze kod 21-godišnjeg migranta iz Pakistana, hospitaliziranog na Klinici za plućne bolesti i tuberkulozu, Klinički centar Univerziteta u Sarajevu zbog ponavljajućih apscesa, furunkula i karbunkula lijeve natkoljenice i desne podlaktice, koji su trajali jednu godinu i liječeni su različitim antibioticima. Gnoj dobiven incizijom apscesa, brisevi rane lijevog bedra, tkivo kože dobiveno biopsijom poslani su na mikrobiološku analizu i analizu na *Mycobacterium (M.) tuberculosis.* Brisevi rana i kulture kožnog tkiva bili su pozitivni na *M. tuberculosis* na čvrstom (Löwenstein) i tekućem (MGIT) mediju. Razmaz sputuma i kultura na *M. tuberculosis* bili su negativni. Rentgenogram prsnog koša bio je također bez aktivnih patomorfoloških promjena. Antituberkulozna terapija započeta je s četiri lijeka (izonijazid, etambutol, pirazinamid, rifampicin). Uz antituberkuloznu terapiju ukazala se potreba i za kirurškim liječenjem rane lijeve natkoljenice. Bolesnik je uspješno liječen antituberkuloznom terapijom uz kirurško liječenje kožnih promjena i njihovo cijeljenje. Dijagnoza kožne tuberkuloze kod našeg bolesnika postavljena je nakon dužeg vremena i nakon neuspjeha antibiotskog liječenja. Pravilna dijagnoza kožne tuberkuloze vrlo je važna, jer se može dobro liječiti uobičajenom antituberkuloznom terapijom.

Ključne riječi: tuberkuloza kože, dijagnoza, liječenje

STAVOVI LIJEČNIKA I MEDICINSKIH SESTARA/TEHNIČARA SPLITSKO DALMATINSKE ŽUPANIJE PREMA CIJEPLJENJU PROTIV COVID-19

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Uvod: Stavovi zdravstvenih djelatnika prema cijepljenju protiv bolesti COVID-19 su važan čimbenik u provođenju cijepljenja i pridobijanju neodlučnih građana koji se oklijevaju cijepiti. Medicinske sestre su veći dio svog radnog vremena od liječnika u kontaktu s pacijentima, pa su njihovi stavovi prema cijepljenju jako važni. ***Cilj rada*** bio je analizirati stavove zdravstvenih djelatnika o cijepljenju protiv COVID-19 prema spolu, dobi, stručnoj spremi/obrazovanju i obrazovanju roditelja na području Splitsko-dalmatinske županije. ***Metode:*** Korišten je anonimni, dobrovoljni anketni upitnik na koji je odgovorilo 396 zdravstvenih djelatnika. Ispitanici su podijeljeni u dvije skupine: liječnike i medicinske sestre. U uzorku su dominirale medicinske sestre kojih je bilo 319 (80,55 %). Za procjenu stavova korištena je Likertova ljestvica. Razlike između medicinskih sestara/tehničara i liječnika prema promatranim obilježjima ispitane su $\chi 2$ testom. Hipoteze su testirane T-testom i Anova testom s LSD *post hoc* testom, dok je normalnost razdiobe prethodno ispitana Kolmogorov-Smirnovim testom. Zavisnost je testirana $\chi 2$ testom. ***Rezultati i zaključak:*** Potvrđena je pretpostavka da medicinske sestre/tehničari imaju negativnije stavove prema cijepljenju od liječnika. Od svih ispitivanih čimbenika (životna dob, stupanj obrazovanja ispitanika, obrazovanje roditelja, strah od cijepljenja) jedino obrazovanje ispitanika ima utjecaja na pozitivan stav prema cijepljenju. Iako se činilo da obrazovanje oca i majke također ima utjecaja, naknadnim, *post hoc* testiranjem ta mogućnost nije potvrđena.

Ključne riječi: COVID-19; stavovi o cijepljenju; zdravstveni djelatnici

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UVOD

Krajem 2019. godine pojavio se novi, neidentificirani virus u Kini. Nitko nije mogao pretpostaviti ni tijek ni razvoj bolesti koja se tada evidentirala kao upala pluća, a ni razmjer koji će doseći u narednih nekoliko mjeseci. Nije se trebalo dugo čekati na pojavnost virusa u europskim zemljama i u SAD-u. Svjetska zdravstvena organizacija – SZO (engl. *World Health Organization,* WHO) je 11. ožujka 2020. godine proglasila je SARS-CoV-2 globalnom pandemijom, a bolest koju virus izaziva nazvana COVID-19 (1).

Početkom pandemije sve države svijeta su se udružile u nastojanju što uspješnijeg suzbijanja prijenosa i širenja SARS-CoV-2 uvođenjem različitih mjera. Na taj se način pokušao smanjiti broj oboljelih, a samim tim i broj umrlih osoba. Svi odgovorni čimbenici društva surađivali su na što bržem razvoju terapijskih i preventivnih mjera (1-6). Kako se infekcija uglavnom širila putem aerosola i kapljičnim putem, neke od preventivnih mjera koje su se uvele svugdje u svijetu pa tako i u Hrvatskoj bile su: poticanje fizičkog distanciranja, redovita higijena ruku, nošenje maski posebno u zatvorenim prostorima, zabrana okupljanja većeg broja ljudi, te određivanje mjera samoizolacije i izolacije (1-6).

Prvi zabilježeni slučaj u Hrvatskoj bio je u Zagrebu 25. veljače 2020. (7).

Veliki broj asimptomatskih kliconoša, kapljični put prijenosa i prenošenje indirektnim kontaktom osim osobina virusa (RNA virus, velika sklonost mutacijama, relativna otpornost u vanjskoj sredini) razlog su, osim društvenih razloga, poteškoćama u suzbijanju pandemije (5,6,8,9).

Vlada Republike Hrvatske i Hrvatski zavod za javno zdravstvo (HZJZ) organizirali su informativno edukativnu kampanju pod nazivom „Misli na druge - cijepi se" (10). Cilj kampanje bio je ukazati na činjenice da je cijepljenje jedini uspješni način da se u skoroj budućnosti umanji utjecaj virusa COVID-19 na naše živote. Učinak kampanje nije opravdao očekivanja, udio cijepljenih građana u Hrvatskoj nije dostigao željeni obuhvat (11).

Mnogo je istraživanja tijekom aktualne pandemije provedeno o stavovima zdravstvenih djelatnika prema cijepljenju protiv bolesti COVID-19. Iako većina studija navodi da zdravstveni djelatnici imaju pozitivan stav prema cijepljenju protiv COVID-19, dio studija spominje negativne stavove prema korištenju cjepiva što može rezultirati propuštenim prilikama ili izazovima na lokalnoj i globalnoj razini te naporima usmjerenim na suzbijanje pandemije (12-26).

CILJ RADA

Cilj rada bio je utvrditi postoji li razlika u stavovima između liječnika i medicinskih sestara/tehničara o cijepljenju protiv bolesti COVID-19.

Ispitali smo stavove medicinskih sestara/tehničara i usporedili ih sa stavovima liječnika. Željeli smo saznati imaju li medicinske sestre/tehničari negativniji stav prema cijepljenju u odnosu na liječnike. Ispitali smo učinak životne dobi, obrazovanja roditelja, obrazovanje ispitanika te utjecaj straha od cijepljenja protiv COVID-19.

ISPITANICI I METODE

Anketa je provedena od 1. travnja do 31. svibnja 2022. godine.

Anketni upitnik je podijeljen putem društvenih mreža te putem elektroničke pošte na adrese liječnika i medicinskih sestara/tehničara čije je sudjelovanje bilo dobrovoljno i anonimno. Istraživanje je odobreno od Etičkog povjerenstva KBC-a Split i Doma zdravlja Splitsko–dalmatinske županije.

Ispunjavanjem upitnika smatrali smo da su ispitanici pristali na anketiranje.

Pri izradi anketnog upitnika korištena su dva upitnika koja su prethodno ispitivala stavove studenata o cijepljenju (27,28). Isti anketni upitnik korišten je za liječnike i medicinske sestre/tehničare.

Upitnik je ispunilo 396 zdravstvenih djelatnika. Na području Splitsko–dalmatinske županije na dan 1. lipnja 2022. godine prema evidenciji Hrvatske komore medicinskih sestara (HKMS) bili su 3641 zaposlenih medicinska sestra/tehničar (29), a prema evidenciji Hrvatske liječničke komore (HLK) bilo je 1676 radno aktivnih liječnika (30). Anketi je pristupilo 77 liječnika (4,5 %) i 319 (8,7 %) medicinskih sestara/tehničara od ukupnog broja u županiji.

Za procjenu stavova korištena je Likertova ljestvica. Najmanji mogući ukupni rezultat na ljestvici je 1, a najveći 5 pri čemu veći rezultat ukazuje na pozitivniji stav.

Razlike između medicinskih sestara/tehničara i liječnika prema promatranim obilježjima ispitane su $\chi 2$ testom. Hipoteze su testirane T-testom i Anova testom s LSD *post hoc* testom, dok je normalnost razdiobe prethodno ispitana Kolmogorov-Smirnovim testom. Zavisnost je testirana $\chi 2$ testom.

Analiza je rađena u statističkom softwareu STATISTICA 12, Tibco, Kalifornija. Statistička značajnost postavljena je na razinu $p = 0,05$.

REZULTATI

Struktura ispitanika s obzirom na zanimanje prikazana je na tablici 1. Ispitivanjem je utvrđena statistički značajna razlika u zastupljenosti ispitanika s obzirom na skupinu ($\chi 2=530,95$; $p<0,001$).

Ispitanici su kategorizirani u dvije skupine – liječnike i medicinske sestre/tehničare. Testiranjem je utvrđeno kako su medicinske sestre/tehničari zastupljeniji u ukupnom broju ispitanika ($\chi 2=147,89$; $p<0,001$) (tablica 2).

Glede spolne strukture, u obje promatrane skupine veći je broj žena u odnosu na muškarce. U skupini medicinskih sestara/tehničara dominiraju žene (n=291; 91,22 %), a u skupini liječnika zastupljenost žena je niža (n=40; 51,95 %). Testiranjem je utvrđena statistički značajna razlika u zastupljenosti zdravstvenih djelatnika s obzirom na spol ($\chi 2=108,81$; $p<0,001$) (tablica 2).

Tablica 1. *Struktura ispitanika s obzirom na zanimanje*

	n	%	χ2	p*
Doktor medicine	32	8,08	530,95	<0,001
Specijalist obiteljske medicine	2	0,51		
Doktor dentalne medicine	11	2,78		
Specijalist kliničke medicine	29	7,32		
Specijalist preventivne medicine	3	0,76		
Medicinska sestra/tehničar srednje stručne spreme	126	31,82		
Medicinska sestra/tehničar prvostupnica/ik sestrinstva	165	41,67		
Medicinska sestra/tehničar magistra/ar sestrinstva	28	7,07		

*χ2 test

S obzirom na obrazovanje roditelja, najvećem broju medicinskih sestara/tehničara obrazovanje oca je srednja školska sprema (n=217; 68,03 %), dok je najvećem broju liječnika obrazovanje oca visoka školska sprema (n=33; 42,86 %) – tablica 3.

U obje promatrane skupine, najvećem broju ispitanika obrazovanje majke je srednja školska sprema. U skupini liječnika veća je zastupljenost majki s višom (15,58 %) i visokom stručnom spremom (36,36 %). U ovom uzorku očevi i majke liječnika su obrazovaniji, više ih ima završen fakultet (p=0,001). Najveći broj ispitanika (308; 96,55 %) zaposlen je u gradskim sredinama (tablica 3).

Stavovi zdravstvenih radnika prema cijepljenju protiv COVID-19 bolesti i utjecaj životne dobi na stav

Srednja razina stava prema cijepljenju protiv COVID-19 za 0,46 bodova veća kod liječnika u odnosu na medicinske sestre/tehničare (slika 1).

Tablica 2. *Struktura uzorka obzirom na zanimanje i spol*

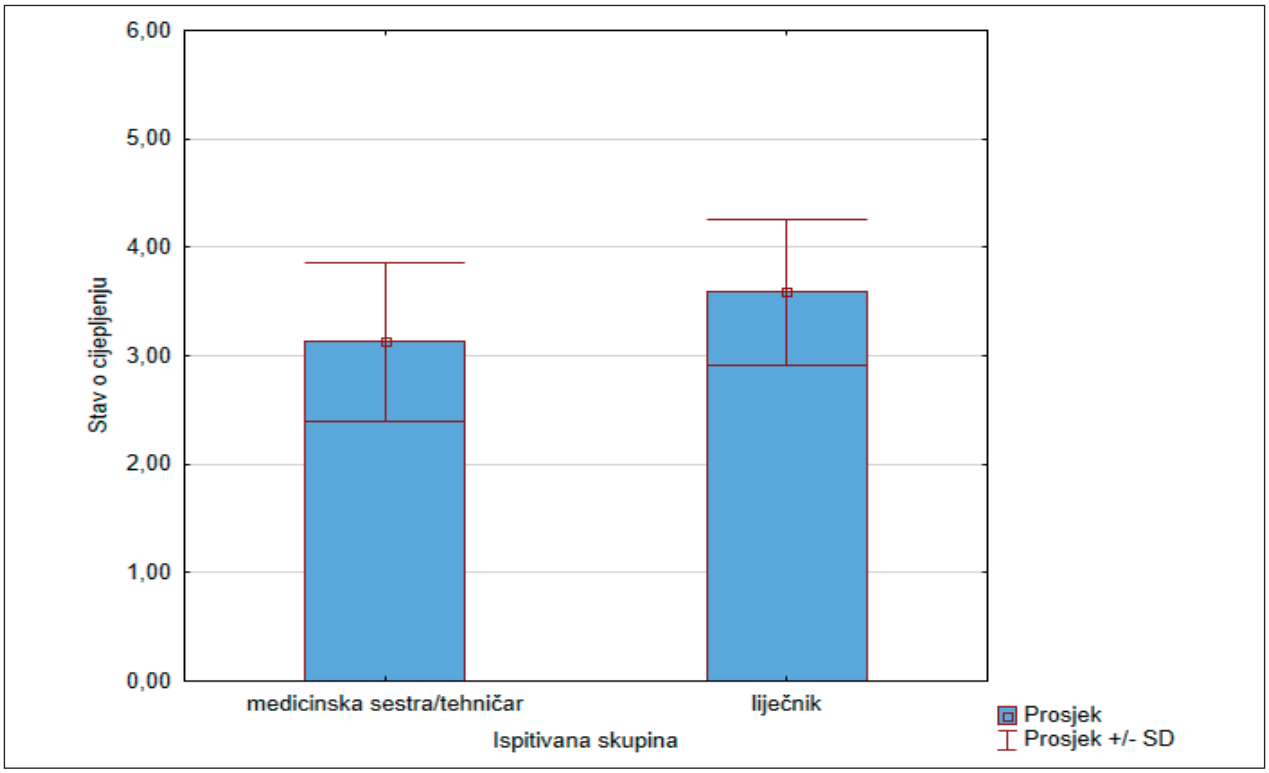
Zanimanje/spol	Ukupno		Muški		Žene		χ2	p*
	N	%	N	%	N	%		
Liječnici	77	19,44	37	48,05	40	51,95%	69,74	<0,001
Medicinske sestre/tehničari	319	80,56	28	8,78	291	91,22%	147,89	<0,001
Ukupno	396	100,00	65	16,41	331	83,59	108.81	<0,001

*χ2 test

Tablica 3. *Struktura ispitanika s obzirom na radnu sredinu i obrazovanje roditelja*

		Medicinska sestra/tehničar		Liječnik			
		n	%	n	%	χ2*	p
Radna sredina	grad	308	96,55	72	93,51	n/a	n/a
	selo	6	1,88	4	5,19		
	otok	5	1,57	1	1,30		
Obrazovanje oca	nezavršena osnovna škola	3	0,94	0	0,00	69,66	<0,001
	završena osnovna škola	40	12,54	4	5,19		
	srednja školska sprema	217	68,03	25	32,47		
	viša škola	32	10,03	15	19,48		
	fakultet	27	8,46	33	42,86		
Obrazovanje majke	nezavršena osnovna škola	4	1,25	1	1,30	63,31	<0,001
	završena osnovna škola	56	17,55	3	3,90		
	srednja školska sprema	217	68,03	33	42,86		
	viša škola	20	6,27	12	15,58		
	fakultet	22	6,90	28	36,36		

*χ2 test



Slika 1. *Srednja razina stava prema skupinama*

Nakon testiranja utvrđena je statistički značajno niža razina stava o cijepljenju među medicinskim sestrama/tehničarima u odnosu na liječnike (t=5,00; p<0,001) (tablica 4).

Temeljem testiranja donosi se zaključak da se hipoteza kojom se pretpostavlja da medicinske sestre/tehničari imaju negativnije stavove prema cijepljenju protiv COVID-19 od liječnika prihvaća kao istinita.

U odnosu na životnu dob, srednja razina stava prema cijepljenju protiv COVID-19 je za 0,03 bodova veća kod zdravstvenih djelatnika u dobi 35 - 45 godina u odnosu na zdravstvene djelatnike ostalih dobnih skupina.

Testiranjem nije utvrđena statistički značajna razlika u razini stava o cijepljenju među zdravstvenim djelatnicima promatranih dobnih skupina (t=0,28; p=0,777). Pretpostavka da stariji zdravstveni djelatnici, bez obzira na zanimanje, imaju pozitivnijie mišljenje o cijepljenju nije potvrđena (tablica 4).

Utjecaj obrazovanja roditelja na stav zdravstvenih djelatnika prema cijepljenju

Testiranjem je utvrđena prisutnost statistički značajne razlike (F=2,95; p=0,020) – tablica 5.

Testiranjem razlike među skupinama utvrđena je statistički značajno viša razina pozitivnog stava među zdravstvenim djelatnicima čiji očevi imaju završen fakultet u odnosu na zdravstvene djelatnike čiji očevi imaju završenu srednju školu (p=0,008), kao i među zdravstvenim djelatnicima koji imaju završenu osnovnu školu u odnosu na zdravstvene djelatnike koji imaju završenu srednju školu (p=0,013) (tablica 5).

Prema dobivenim rezultatima, obrazovanje roditelja ima pozitivan učinak na stavove ispitanika o cijepljenju. Međutim, naknadnom *post hoc* analizom ova hipoteza nije potvrđena (tablica 6).

Tablica 4. *Stav o cijepljenju prema zanimanju i dobi ispitanika*

Zanimanje/životna dob	Stav o cijepljenju			
	Prosjek	SD	t*	p
Liječnici	3,59	0,67	5,0	<0,001
Medicinske sestre/tehničari	3,13	0,73		
Životna dob				
35-45 godina	3,24	0,79	0,28	0,777
Ostale dobne skupine	3,21	0,72		

*χ2 test

Tablica 5. *Stav o cijepljenju prema obrazovanju oca*

Obrazovanje oca	N	Prosjek	SD	F	p*
Nezavršena osnovna škola	3	3,33	0,61	2,95	0,020
Završena osnovna škola	44	3,42	0,65		
Srednja školska sprema	242	3,12	0,72		
Viša škola	47	3,28	0,83		
Fakultet	60	3,41	0,77		

Tablica 6. *Stav o cijepljenju prema obrazovanju oca – post hoc ispitivanje**

	Srednja školska sprema	Fakultet	Završena osnovna škola	Viša škola
Fakultet	0,008			
Završena osnovna škola	0,013	0,902		
Viša škola	0,184	0,375	0,348	
Nezavršena osnovna škola	0,624	0,866	0,835	0,902

* Least Significant Difference (LSD) test

Tablica 7. *Stav ispitanika o cijepljenju prema obrazovanju majke*

Obrazovanje majke	N	Prosjek	SD	F	p*
Nezavršena osnovna škola	5	3,30	0,45	0,83	0,505
Završena osnovna škola	59	3,30	0,65		
Srednja školska sprema	250	3,17	0,76		
Viša škola	32	3,33	0,69		
Fakultet	50	3,31	0,81		

Tablica 8. *Zdravstveni djelatnici prema razlozima necijepljenja*

Razlog ne cijepljenja	Strah od cjepiva		Ostali razlozi		$\chi 2$	p*
Skupina	n	%	n	%		
Medicinska sestra/ tehničar	94	74,02%	33	25,98%	0,92	0,336
Liječnik	6	60,00%	4	40,00%		

* $\chi 2$ test
** strah od cjepiva = necijepljenje zbog mogućih nuspojava, smatram da cjepivo nije dovoljno istraženo

Najviša pozitivna razina stava o cijepljenju utvrđena je među zdravstvenim djelatnicima čije majke su imale završenu višu školu. Međutim, testiranjem se nije pokazala statistički značajna razlika (F=0,83; p=0,505), pa se se hipoteza da viši stupanj obrazovanja roditelja ima utjecaj na pozitivniji stav ispitanika prema cijepljenju odbacuje kao neistinita (tablica 7).

Utjecaj straha od cijepljenja na stavove o cijepljenju protiv COVID 19

Iako je strah od cijepljenja 1,23 puta učestaliji među medicinskim sestrama/tehničarima u odnosu na liječnike, testiranjem nije utvrđena statistički značajna razlika ($\chi 2$=0,92; p=0,336) pa se pretpostavka da medicinske sestre/tehničari imaju veći strah od cijepljenja/cjepiva od liječnika odbacuje kao neistinita (tablica 8).

Utjecaj obrazovanja ispitanika na stav prema cijepljenju protiv COVID-19

Najviša razina pozitivnog stava o cijepljenju utvrđena je među zdravstvenim djelatnicima s obrazovanjem „C“ i za 0,40 bodova je veća u odnosu na zdravstvene djelatnike kod kojih je utvrđena najmanja razina stava. Testiranjem je utvrđena prisutnost statistički značajne razlike (F=7,11; p<0,001) (tablica 9).

Naknadnom provjerom, *post hoc* testiranjem LSD testom utvrđena je statistički značajno veća razina pozitivnog stava među zdravstvenim djelatnicima stupnja obrazovanja kategorije „C“ u odnosu na zdravstvene djelatnike stupnja obrazovanja „A“ (p<0,001), dok testiranjem nije utvrđena statistički značajna razlika (p=0,251) među zdravstvenim djelatnicima stupnjeva obrazovanja A (srednja stručna sprema) i B (prvostupnici sestrinstva).

Tablica 9. *Stav o cijepljenju prema obrazovanju ispitanika*

Obrazovanje	N	Prosjek	SD	F	p*
A	126	3,10	0,66	7,11	<0,001
B	197	3,20	0,76		
C	73	3,50	0,74		

b=doktor medicine
c=specijalist obiteljske medicine
c=doktor dentalne medicine
c=specijalist klinicke medicine
c=specijalist preventivne medicine
a=medicinska sestra/tehnicar srednje strucne spreme
b=medicinska sestra/tehnicar prvostupnica/ik sestrinstva
c=medicinska sestra/tehnicar magistra/ar sestrinstva

Nakon provedenog testiranja zaključuje se da viši stupanj obrazovanja ispitanika utječe na pozitivniji stav prema cijepljenju protiv COVID-19.

RASPRAVA

Stav zdravstvenih djelatnika prema cijepljenju od najveće je važnosti za promicanje njegovog prihvaćanja (14). Ako zdravstveni djelatnik sam ne vjeruje u učinkovitost i svrsishodnost cijepljenja, teško će uvjeriti druge u potrebu cijepljenja, a njegov negativan stav odbit će od cijepljenja one koji su u dilemi. Jedan od razloga zašto je napravljen ovaj rad je procjena stavova zdravstvenih djelatnika prema cijepljenju i čimbencima koji utječu na te stavove. U stručnoj literaturi i medijima primijećeno je da pozitivnije stavove prema cijepljenju imaju liječnici (12-26).

Željeli smo provjeriti kakav je stav zdravstvenih djelatnika prema cijepljenju protiv COVID-19 na području Splitsko-dalmatinske županije. Iz dobivenih rezultata vidljivo je da medicinske sestre/tehničari, unatoč zdravstvenom obrazovanju, imaju negativnije mišljenje i stavove prema cijepljenju od liječnika. Jedini čimbenik koji ima utjecaja na stavove prema cijepljenju protiv COVID-19 među našim ispitanicima je njihovo obrazovanje. Potvrđena je hipoteza kako viši stupanj obrazovanja utječe na pozitivan stav o cijepljenju. Slične rezultate, ali općenito o cijepljenju, dobili su i *Šalamun* i suradnici koji su 2017. godine proveli istraživanje o stavovima i znanju zdravstvenih djelatnika o cijepljenju u općoj bolnici u Vukovaru i na Veleučilištu u Bjelovaru. Polovica ispitanika smatrala je nepotrebnim cijepljenje protiv bolesti koje su praktički eliminirane, dok je samo trećina ispitanika smatrala cijepljenje najvećim medicinskim uspjehom 20. st., te da je obvezno cijepljenje u Republici Hrvatskoj opravdano (13).

U sjeverozapadnoj Hrvatskoj je provedeno slično istraživanje, a rezultati su nalik našima: medicinske sestre pokazuju više oklijevanja i imaju negativnije stavove prema cijepljenju (14). Medicinska sestra/tehničar je često prvi i zadnji zdravstveni profesionalac s kojim korisnici zdravstvene skrbi komuniciraju. Vrijeme trajanja, posebnosti komunikacije temeljene na povjerenju i doživljaju profesionalnog iskustva medicinskih sestara nemjerljivo su važni pri formiranju stava. Stoga je edukacija medicinskih sestara/tehničara ključan čimbenik formiranja zdravstvene kulture i prihvaćanja novih intervencija.

Problem stavova zdravstvenih djelatnika općenito prema cijepljenju i prema cijepljenju protiv COVID-19 prisutan je među svim zdravstvenim djelatnicima i laicima u svijetu, ali zastupljenost odnosno proporcija onih koji imaju negativne stavove varira od zemlje do zemlje. Mnogo autora istraživalo je ovu pojavu želeći saznati razlog i poboljšati mjere prevencije promjenom stavova (12-26). Zabrinutost zbog nuspojava cijepljenja najčešći je razlog oklijevanja među ispitanicima u istraživanju koje su proveli Solis Arce JS i suradnici (31). Istraživanje voljnosti za cijepljenje protiv COVID-19 provedeno je anketnim upitnikom među zdravstvenim djelatnicima u deset zemalja Južne Amerike, Afrike i Azije (zemlje niskog BDP-a). Rezultate su usporedili s rezultatima iz SAD-a i Rusije. U tim zemljama zdravstveni djelatnici su izrazili spremnost za primanje cjepiva protiv COVID-19 (prosjek 80,3 %; medijan 78 %) u usporedbi sa SAD (prosjek 64,6 %) i Rusijom (prosjek 30,4 %). Prihvaćanje cjepiva protiv COVID-19 u zemljama s niskim BDP-om ponajprije se objašnjava osobnim interesom za zaštitu od bolesti, a razlog oklijevanju je strah od nuspojava (31).

U Republici Hrvatskoj se cijepljenje protiv COVID-19 preporučuje za sve građane, a obavezno je za određene kategorije djelatnika, uključujući i zdravstvene djelatnike. Preporuke su se mijenjale ovisno o epidemiološkoj situaciji (11). Bilo je mnogo različitih mišljenja i dilema u svezi primijenjenih mjera. Situaciju su dodatno zakomplicirale antivakserske skupine, teorije zavjera i uporne akcije preko medija i društvenih mreža (32).

Neka istraživanja ukazuju da je voljnost za cijepljenje protiv gripe povezana s voljnošću cijepljenja protiv COVID-19 (14,22,26).

U Njemačkoj je u veljači 2021. godine provedena online anketa na temu prihvaćanja cijepljenja protiv COVID-19 među zdravstvenim djelatnicima. Prikupljeno je ukupno 4500 anketa od čega je 91,7 %. ispitanika potvrdno odgovorilo kako prihvaća cijepljenje ili je već bilo cijepljeno. Čimbenici koji su najčešće navedeni kao razlozi za neprihvaćanje cijepljenja bili su nedostatak povjerenja u vlasti, zdravstvenu politiku i farmaceutske tvrtke, brzi proces odobravanja i razvoja cjepiva, neažuriranje cijepljenja, strah od dugotrajnih i kratkoročnih nuspojava te nedostatak povjerenja u cjepiva, a takvi ispitanici su češće prikupljali informacije o cjepivima protiv COVID-19 putem internetskih video platformi i bili su slabiji na ispitu znanja (17).

Kanadska skupina autora provela je istraživanje u prosincu 2020. godine, neposredno nakon dolaska cjepiva protiv COVID-19 na tržište u *Centre Intégré Universitaire de Santé et de Services Sociaux Centre-Ouest-de-Montréal* (CIUSSS COMTL) u Quebecu nastojeći doznati razloge zbog kojih zdravstveni djelatnici, prije svega liječnici i medicinske sestre/tehničari, odbijaju primiti cjepivo protiv COVID-19. Anketi je pristupio 2761 ispitanik, a 2233 je prihvatilo cjepivo, odnosno 80,9 %, dok ih je 528 odnosno 19,1 % odbilo primiti

cjepivo. Kao najvažniji razlog odbijanja cijepljenja navedena je zabrinutost da se radi o novom cjepivu (56 %), želja da se navedenim cjepivom najprije cijepi netko drugi (53 %) te manjak dostupnih informacija o cjepivu (47 %) (22).

Negativan stav prema cijepljenju je možda više nego što očekujemo povezan s zdravstvenom politikom i nepovjerenjem u strukture vlasti te odnosom prema farmaceutskoj industriji. Pretraživanjem podataka često odabiremo izvore koji će potvrditi naše već formirane stavove. Ovaj problem je istražen na velikom broju ispitanika u Njemačkoj te nam zaključci istraživača naglašavaju multikauzalnost negativnih stavova prema provjerenim zdravstvenim intervencijama (17).

Uspoređujući podatke iz njemačkog, francuskog i istraživanja provedenog u Izraelu (19) te istraživanja u drugom dijelu Hrvatske (14) s podatcima dobivenim u ovom istraživanju može se zaključiti kako su u svim istraživanjima liječnici imali pozitivniji stav prema cijepljenju od medicinskih sestara/tehničara, te da je viši stupanj obrazovanja utjecao na taj pozitivan stav (22-26).

Ovim istraživanjem smo pokušali istražiti učinke do sada znanih čimbenika na formiranje stava o cijepljenju. Nismo pronašli povezanost negativnog stava i mlađe životne dobi koja se često navodila kao jedan od bitnih čimbenika niske procijepljenosti u našoj sredini. Nezainteresiranost i negativan stav prema cijepljenju povezan je s blažom kliničkom slikom kod mladih, rjeđom pojavom komplikacija u toj životnoj dobi. Našim istraživanjem potvrdili smo znanje kao ključan čimbenik koji utječe na formiranje stava. Pristup mladima je od iznimno važan zbog njihove društvene uključenosti i lakšeg usvajanja znanja, te mogućnosti promjene stava temeljem provjerenih činjenica.

Zakonski okvir je jasan, jer je propisivanje cijepljenja protiv izrijekom propisanih zaraznih bolesti usmjereno na eliminaciju bolesti iz ukupne populacije čime se ostvaruje pozitivna obveza države (33-35).

Neodlučnost i nepristajanje na cijepljenje ostaje prepreka potpunoj imunizaciji stanovništva i stvaranju kolektivnog imuniteta kod pojave visoko zaraznih bolesti kao što je to slučaj s aktualnom pandemijom COVID-19. Poboljšanje sadržaja o cijepljenju u programima edukacije budućih zdravstvenih djelatnika, olakšavanje pristupa pouzdanim informacijama za korištenje tijekom konzultacija te razvoj i validacija instrumenata za mjerenje negativnih stavova zdravstvenih djelatnika prema cijepljenju ključne su za poboljšanje (29).

ZAKLJUČCI

Medicinske sestre/tehničari imaju negativnije stavove prema cijepljenju od liječnika.

Od svih ispitivanih čimbenika (životna dob, stupanj obrazovanja ispitanika, obrazovanje roditelja, strah od cijepljenja) jedino obrazovanje ispitanika ima utjecaja na pozitivan stav prema cijepljenju. Iako se činilo da obrazovanje oca i majke također ima utjecaja, naknadnim, *post hoc* testiranjem ta mogućnost nije potvrđena.

Mogući nedostatak istraživanja je manja zastupljenost liječnika u apsolutnom i relativnom broju/postotku. To može biti rezultat „cehovske solidarnosti", jer je voditeljica istraživanja apsolventica diplomskog studija sestrinstva.

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SUMMARY

ATTITUDES ABOUT VACCINATION AGAINST COVID−19 AMONG MEDICAL DOCTORS AND NURSES IN SPLIT-DALMATIA COUNTY

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Background: Attitudes of healthcare workers towards vaccination against COVID-19 represent an important factor in performing the vaccination, dealing with vaccine hesitancy and motivating the hesitant population. Medical nurses spend more working time than medical doctors in direct contact with patients, so their vaccination attitudes are very important. *Objective* of the study was to analyze the attitudes of healthcare workers in Split-Dalmatia County, Croatia, towards vaccination against COVID-19, according to their occupation, gender, age, educational level, and educational level of their parents. *Methods:* An anonymous, voluntary survey questionnaire was completed by 396 health workers. The respondents were divided into two groups of medical doctors and nurses. The sample was predominated by medical nurses, with a total of 319 (80.55%) nurses. Likert scale was used for assessing the attitudes. Differences between medical nurses/technicians and doctors regarding the observed features were examined by use of the χ^2-test. The hypothesis was validated through T-test and ANOVA test with the Least Significant Difference *post hoc* test, whereas the Kolmogorov-Smirnov test was previously used to test normal data distribution. The dependence was checked through χ^2-test. *Results and Conclusion:* The hypothesis was confirmed, i.e., medical nurses/technicians have more negative attitudes towards vaccination than doctors.

Of all included parameters (age, gender, educational level, parents' educational level, fear of injections), only the respondents' educational level positively affected the attitude towards vaccination. Although it initially seemed that the educational level of the respondents' parents had effects on the vaccination attitudes, subsequent *post hoc* testing did not prove this possibility.

Key words: COVID-19, attitudes towards vaccination, health workers

PROSTITUTION IN LAIBACH IN 1888

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This short review presents the state of prostitution in public health medical historiography of the then Laibach in the year 1888. All prostitutes were registered in the *Evidenz Protocol and* Ljubljana city physicist performed regular examinations of prostitutes three times a week and issued medical certificates of medical fitness and a report on the state of prostitution in the city of Ljubljana.

Key words: prostitution, Ljubljana, 19th century, history of medicine

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In the Austro-Hungarian Monarchy, prostitution was strictly legally public health and police controlled in the late 19th and early 20th centuries based on the Criminal Code of 1852, which regulated the issue of prostitution, so that jurisdiction over prostitutes and prostitution belonged to the city authorities, i.e., *Mestni magistrat* (Figure 1) (1,2). Then there were laws and bylaws based on the mentioned Criminal Code, such as the Decree on Prostitution of September 12, 1874, issued in Linz *(Vorschriften der Prostitution)*, and in Ljubljana in 1888 the decree according to the *Regulativ für der Prostitution* issued in Graz was implemented as of April 3, 1877, which was used in all cities where there were bordellos (3-5). All prostitutes were registered in the *Evidenz Protokoll* under the ordinal number with generals, first and last name, age and place of birth, and items 1 and 2 contain a translation of the Regulation: "how to control prostitution in Ljubljana" (5):

1) "Public women" or "unclean women" are all those women whose bodies perform unclean trades.

2) Every "public woman" has to report in person to the police department of the city magistrate, which describes her in a special book, the so-called main record (*Haupt Protokoll*). Only one woman is entered on one side of the minutes, and the adjectives are liked; serial number, class, first and last name, age, status, place of birth and homeland, previous job, apartment, will and propensity to prostitution. At each entry, the woman was questioned about the above-mentioned matters.

Arriving at the service in the brothel, the prostitute had to report to the city police where she received a prostitute's card, which recorded her generals and medical examinations as proof of her health or illness (1,6,7). The scope of work of the city physicist, among many others, included medical control of the health of prostitutes. In 1878-1888, Dr. Avelin Roblek, the then Ljubljana city physicist, performed regular examinations of prostitutes and issued medical certificates of medical fitness and a report on the state of prostitution in the city of Ljubljana (8). Hygienic-epidemiological inspection of the prostitute was performed once a month, and examination of the prostitute three times a week and entered in the Prostitute's Card (1,3,5). Thus, at the beginning of 1888, according to records, four

prostitutes were recorded to be on duty in Ljubljana. During January 1888, they were examined as follows: Ana aged 21 eight times; a 50-year-old prostitute born in Trento four times; Josefa aged 20, born in Fiume (Rijeka), eight times; and Sofia aged 18, born in Prague, six times (6). Their health status was monitored by use of special tables, with generals about prostitutes, their age, duration of prostitution turnover, date of illness and recovery, diagnosis (illness) in Latin and then German, with consequences, post-convalescence status, and treatment outcome and procedure. Thus, during 1888, four prostitutes aged 24, 19, 30 and 22 fell ill and were diagnosed with *ulcus specificum (lues primarium), condylomata lata et blenorrhoea vaginae* (lues degree II with gonorrhea), *abortus et metrorrhagia* (later inflammation of the uterus, *Gebärmuttercatthar*) and pleuritis (Figure 2). These diseases and conditions of prostitutes were directly related to sexually transmitted diseases and unwanted pregnancies, and fornication was often considered the source of any sexually transmitted disease, including the most common syphilis and gonorrhea (blenorrhea), so the 19-year-old prostitute was diagnosed with both entities. Their recovery and exclusion from the fornication service lasted for about a month, and prostitutes with chronic uterine catarrh were not allowed further work (5,6).

Figure 1. *Front page of the Ljubljana Magistrat, 1888.*

If treatment was needed in the Ljubljana Hospital *(Mestni* špital*),* 16 crowns were allocated for treatment at the city expense (6). If a prostitute did not want to come for treatment as recommended by a doctor, they were forcibly taken to the hospital for treatment, with the help of the police (1). The extent of fornication was

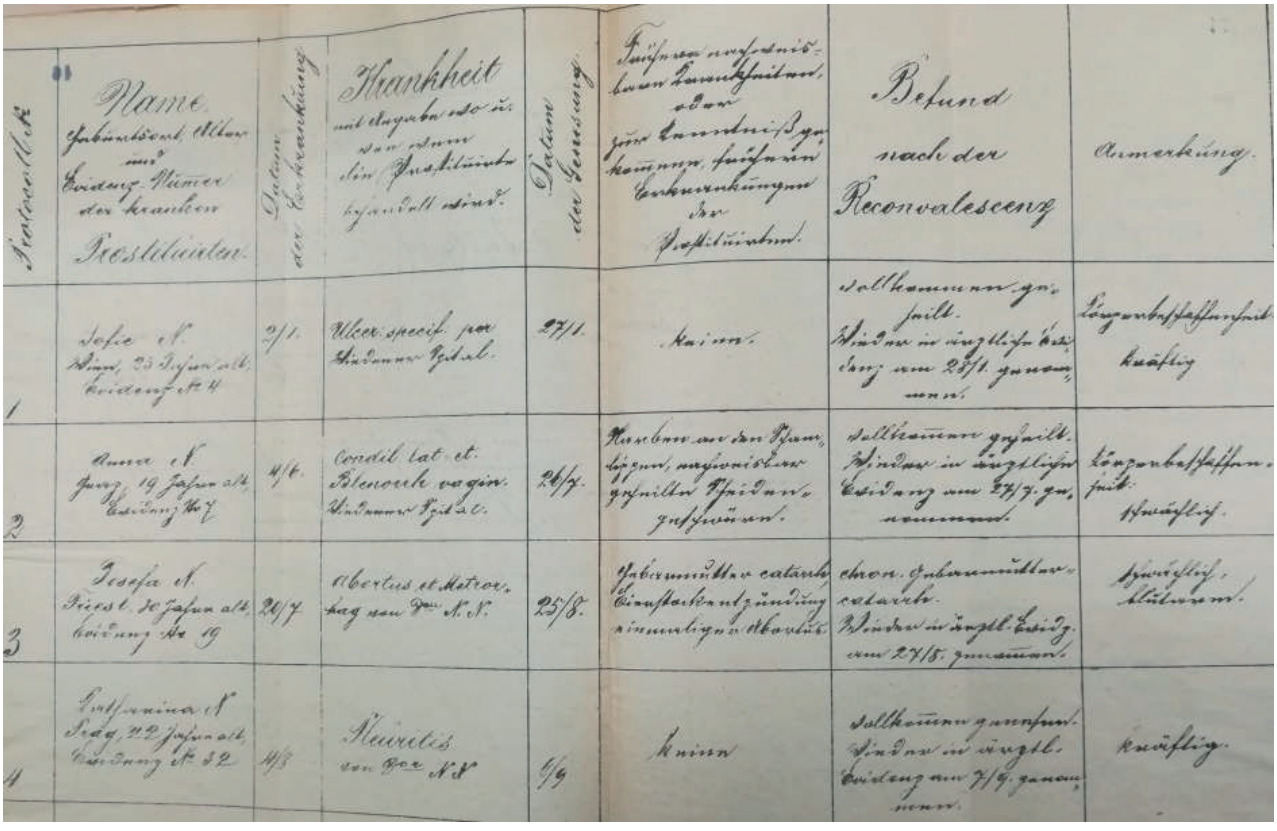

Figure 2. *From the Evidenz Protokoll 1888 (source: Mestni Arhiv Ljubljana. Lju 489 Reg I, 1-744).*

a public health and hospital problem in the then Monarchy, as shown by the example from 1888, when 8% of the total number of hospitalized patients in Bjelovar County Hospital were syphilitic, and in 1889 as many as 13.8% (1,7). From that time, there are no historical data on brothels in Bjelovar where Dr. Avelin Roblek served before serving in Ljubljana. However, according to his own previous research, there were three Bjelovar brothels before the Great War (1), which still operated under the same regular public health guides and police oversight. This short review is the result of the original research in the City Archives of Ljubljana as part of the work of the Ljubljana local physicist in 1888 and a contribution to the then public health medical historiography of the then Laibach.

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SAŽETAK

PROSTITUCIJA U LJUBLJANI U 1888. GODINI KRATKO PRIOPĆENJE (IZ MEDICINSKE PROŠLOSTI)

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Ovaj kratki pregled prikazuje stanje prostitucije u povijesti javnoga zdravstva Ljubljane (Laibach) 1888. godine. Sve prostitutke bile su upisivane u *Evidenz Protokoll,* a mjesni fizik grada Ljubljane obavljao je redovite preglede prostitutki tri puta na tjedan, izdavao uvjerenje o njihovu zdravstvenom stanju i podnosio izvješće o stanju prostitucije u Ljubljani.

Ključne riječi: prostitucija, Ljubljana, 19. stoljeće, povijest medicine

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