

UDK 61

ISSN 1848-641X (Online)

ISSN 1330-5301 (Tisak)

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HRVATSKE AKADEMIJE
ZNANOSTI I UMJETNOSTI

522

MEDICINSKE ZNANOSTI

41



ZAGREB, 2015.

RAD HRVATSKE AKADEMIJE ZNANOSTI I UMJETNOSTI
KNJIGA 522

UDK 61

ISSN 1848-641X (Online)
ISSN 1330-5301 (Print)

R A D

CROATIAN ACADEMY
OF SCIENCES AND ARTS

522

MEDICAL SCIENCES

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RAZRED ZA MEDICINSKE ZNANOSTI
CROATIAN ACADEMY OF SCIENCES AND ARTS
DEPARTMENT OF MEDICAL SCIENCES

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EDITORIAL

Dear readers.

It is a real pleasure to present Volume 41 (2015) of the journal RAD of the Croatian Academy of Sciences and Arts – Medical Sciences. The present Volume introduces papers from two symposia, which were co-organised by the Department of Medical Sciences of the Croatian Academy, and held at the Academy Palace in Zagreb.

Every calendar year, around fifty symposia, scientific meetings, congresses, round tables, and book and monograph promotions, are organized, co-organized or held under the patronage of the Department of Medical Sciences of the Croatian Academy of Sciences and Arts. When two summer months are excluded, the fact remains that nearly every week, one event involving the Department of Medical Sciences takes place. The Editorial Board of the journal RAD has so far, depending on the topicality and actuality of the content of individual symposia, and as agreed with symposia leaders, published papers tackling symposia statements. In our journal RAD, we have usually published papers from one symposium; Volume 41 however comprises papers from two symposia, both of major importance. Each of these symposia was the first to have tackled the topics involved, held within not only the Croatian Academy, but also Croatia.

The intention of the *First Croatian Symposium on Lung Transplantation*, which was held in November 2014, was to present and emphasize the most important indications and updated status of the lung transplantation programme in Croatia. As a part of this Symposium, state-of-the-art lectures regarding indication, pre-evaluation, transplantation surgical procedures, post-transplantation care, immunosuppressive treatment, and pulmonary rehabilitation were presented. The Department of Medical Sciences of the Croatian Academy of Sciences and Arts and the Croatian Thoracic Society organized this Symposium jointly. The chairs of the Symposium were Academy members, Professors Zvonko Kusić and Marko Pećina on behalf of the Croatian Academy; and Professors Walter Klepetko and Miroslav Samaržija on behalf of the Croatian Thoracic Society. Speakers at the Symposium were distinguished experts from Croatia, Austria and Slovenia.

To emphasize the complexity regarding the Colorectal Cancer (CRC) incidence; pathogenesis; and the complexity in diagnosis and treatment, the 1st scientific meeting entitled *Tumours of the Gastrointestinal Tract: Colorectal Cancer* was organized on December 21st, 2014 by the Department of Medical Sciences of the Croatian Academy of Sciences and Arts, and the Department of Clinical Oncology of the Faculty of Medicine, University of Zagreb. The Organizing Committee included the following members of the Department of Clinical Oncology: Prof. Lidija Beketić-Orešković, MD, PhD; Prof. Nikola Đaković, MD, PhD; Prof. Antonio Juretić, MD, PhD; Academy President Prof. Zvonko Kusić, MD, PhD; and Prof. Fedor Šantek, MD, PhD.

On behalf of the Editorial Board of our journal, I cordially thank our guest editors – Prof. Miroslav Samaržija, MD, PhD; and Ass. Prof. Marko Jakopović, MD, PhD (Editors for the Lung Transplantation Symposium); as well as Prof. Antonio Juretić, MD, PhD (Editor for the Colorectal Cancer Symposium) – for their great effort in compiling the symposium papers.

Dear readers, it is with great pleasure that I announce RAD 42 (2015), which will include papers from the Symposium held in March 2015. in Rijeka “*Developmental Biology of Platelets, Trombocytopenia and Myeloproliferative Neoplasms*” and led by Academy member Daniel Rukavina, who will act as guest editor of this upcoming volume of RAD. We will thus publish two volumes of RAD in 2015, both tackling major issues. I believe a wide audience will find them both useful and interesting.

Marko Pećina

Symposium

FIRST CROATIAN SYMPOSIUM ON
LUNG TRANSPLANTATION

Guest Editors:

Miroslav Samaržija, Marko Jakopović

PREFACE

Lung transplantation is one of the greatest achievements in modern medicine. In comparison with transplantation of other solid organs, transplantation of the lung is the most demanding and that was the reason why fully implementation of this procedure was the latest accomplished goal in transplantation medicine. Lung transplantation offers new hope to patients with severe respiratory diseases and significantly improves survival and quality of life. In majority of patients, bilateral lung transplantation is standard procedure, but in some cases, unilateral lung transplantation or lung and heart transplantation is indicated.

The most common indications for lung transplantation are: chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, cystic fibrosis and pulmonary arterial hypertension. Lung transplantation has become standard treatment option for end-stage of these diseases in experienced centers. Lung transplantation program in Croatia started in 2003, and full implementation was accomplished in 2011 through co-operation with University Hospital AKH in Vienna.

The intention of *First Croatian Symposium on Lung Transplantation* which was held in November 2014 was to present and emphasize the most important indications and updated status of lung transplantation program in Croatia. As a part of this Symposium state-of-the art lectures regarding indication, pre-evaluation, transplantation surgical procedures, post-transplantation care, immunosuppressive treatment and pulmonary rehabilitation were presented.

Symposium was organized jointly by Department of Medical Sciences of Croatian Academy of Sciences and Arts and Croatian Thoracic Society. The chairpersons of the symposium were academician Zvonko Kusić and academician Marko Pećina on behalf of the Academy and professor Walter Klepetko and professor Miroslav Samaržija on the behalf of Croatian Thoracic Society. Speakers in the Symposium were distinguished experts from Croatia, Austria and Slovenia.

On behalf of the Organizing Committee:
Miroslav Samaržija and Marko Jakopović

LUNG TRANSPLANTATION PROGRAMME IN CROATIA

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Summary

Lung transplantation was first performed in 1963, but the real increase in performed transplantations occurred in the last twenty years. Leading indications for lung transplantation are: chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary arterial hypertension, and sarcoidosis. Careful selection of donors is extremely important for prognosis after transplantation. Physicians should consider lung transplantation as a therapeutic option and follow the guidelines on the indications for transplantation so patients could be sent on time to a referral center for all the necessary examinations and preparation for recruiting to the waiting list. Department for Lung Diseases Jordanovac, University Hospital Centre Zagreb, is a referral center for lung transplantation in Croatia. The Department is performing processing and preparing patients for transplantation as well as follow them during post-transplantation period.

Keywords: transplantation; lung; referral center.

Introduction

Lung transplation is today indicated like last therapeutic option for patients with end-stage lung diseases in which all conservative options are exhausted. First lung transplantation was performed in 1963. [1]. However, further twenty years were needed to get some modest clinically significant survival benefit after the procedure. With these encouraging results, in the last twenty years significant increase in number of lung transplantation was noticed. Today, more than 32 000 procedures were performed in the World [2]. Lung transplantation from rare medical procedure has become a standard of care in patients with terminal stage of lung disease.

Indications for lung transplantation

In the last decade indications for lung transplantation significantly broadened and include large spectrum of airway, lung parenchymal and pulmonary vasculature diseases. Nowadays, most common indication for lung transplantation is chronic obstructive pulmonary disease (COPD) and represents around one third of all procedures [3], (*Figures 1 and 2*).



Figure 1. X ray of COPD patient before transplantation



Figure 2. X ray of COPD patient after transplantation

Idiopathic pulmonary fibrosis (IPF) is the second most common indication, with constant increase in number of transplanted patients. IPF has become the most common reason for lung transplantation in the United States [4], (*Figures 3 and 4*).

Cystic fibrosis (CF) is responsible for 15% of all transplantations. Less common indications are emphysema of the lung due to α_1 – antitrypsin deficiency, sarcoidosis, bronchiectasis and lymphangioleiomyomatosis (LAM), [3]. Idiopathic pulmonary arterial hypertension (IPAH) used to be the most common indication for lung transplantation, but today is cause of only 2% of all transplantations [2]. Significant decrease in number of transplantations in these patients is clear indicator of great improvement in conservative treatment of patients with IPAH (*Figures 5 and 6*).

Some indications are still largely debatable. Lung transplantation in patients with lung diseases associated with connective tissue diseases can lead to significant post – transplantation mortality because of a large spectrum of extra – pulmonary



Figure 3. X –ray of patient with idiopathic pulmonary fibrosis before transplantation



Figure 4. X –ray of patient with idiopathic pulmonary fibrosis after transplantation



Figure 5. X ray of patient with pulmonary arterial hypertension before transplantation



Figure 6. X ray of patient with pulmonary arterial hypertension after transplantation

manifestations in these patients. After thorough medical checkup, in selected patients with little or no extra-pulmonary manifestation lung transplantation can be performed [5]. Attempts to treat very early stages of lung cancer resulted in high rate of recurrence. Therefore, lung cancer is nowadays considered to be a contraindication for lung transplantation [6].

Contraindications for lung transplantation

There is surprisingly small number of absolute contraindications for lung transplantation. Absolute contraindications are: recent malignancy (except carcinoma of the skin), active hepatitis B and C with liver damage, active smoking, alcohol and drugs abuse, severe psychiatric disorder, non – compliance with medical procedures, poor social status and HIV infection [3]. Controlled diabetes, osteoporosis and coronary artery disease are not considered absolute contraindications [6].

In majority of transplantation centers, upper age limit for the procedure is 65 years. In some centers, attempts are done to move the upper limit according to functional status and not only age. Greatest improvement is achieved in the United States where almost 20% of all transplanted patients were older than 65 [4].

Mechanically ventilated patients were for a long time considered not to be candidates for lung transplantation due to high post-transplantation morbidity and mortality. However, improvements in technology and introduction of extra – corporeal membranous oxygenation (ECMO) have removed that as contraindication and patients on oxygenation support can be successfully transplanted in experienced centers [8].

Chronic infections of sinuses and lower respiratory tract are great problem in patients with cystic fibrosis. Chronic infections with vast majority of pathogens (like *Pseudomonas aeruginosa*) are nowadays not a contraindication for transplantation. Only chronic infection with *Burkholderia* is the contraindication for transplantation due to high post – operative mortality [3,9].

When to list patients for transplantation?

When to put patient on transplantation waiting list is not easy to determine. Patients became candidates for transplantation in the late stage of their disease when survival is more likely better with transplantation comparing to conservative treatment [3]. But it is extremely important to estimate a moment when patients are still capable of undergoing the procedure. It is really important to sensitize patients, their families and physicians not to hesitate to refer patients to expert center to estimate the right indication and ideal timing for putting patients on the waiting list [3].

Criteria for referring patient to referral center are shown on *Table 1* [10].

Table 1. Criteria for lung transplantation in most common indications

Chronic obstructive pulmonary disease
BODE index 7-10
Frequent exacerbations
pCO ₂ >50mmHg at rest
Oxygen dependent
Pulmonary hypertension, cor pulmonale
FEV ₁ <20%, DLCO <20%, emphysema
6-MWT 300 m
BMI
Pulmonary arterial hypertension
NYHA III-IV
Progression regardless of treatment
Walking distance <300 m on 6-MWT
Cardiac index <2L/min/m ²
Right atrial pressure >15mmHg
Cystic fibrosis and bronchiectasis
FEV ₁ <30% or fast decrease in FEV ₁ , especially in younger age
Exacerbations, ICU days
Frequency of exacerbations, antibiotic treatment
Pneumothorax
Haemoptysis
BMI
Oxygen dependent
Hypercapnia
Pulmonary hypertension
Idiopathic pulmonary fibrosis
UIP – histological or radiological finding
DLCO <35%
Decrease in FVC >10% in the last 6 months
SHbO ₂ <88% during 6-MWT
Honeycombing HRCT (fibrosis score > 2)
Histological NSIP:
- DLCO < 35%
- FVC of 10% or DLCO of 15%

Lung transplantation programme in Croatia

First lung transplantation was performed in Croatia in year 2003. After that, programme wasn't continued due to lack of specific surgical equipment. That was the reason why we started co-operation with Professor Walter Klepetko from University Hospital AKH in Vienna. He is recognized as one of the World leading surgeons in the field of thoracic surgery, and especially lung transplantation. Until now, his center performed around 3000 transplantations with one year survival of more than 90%, and 5-year survival of around 70%. Our collaboration started formally in 2011 and consisted of four different procedures: lung transplantation, lung transplantation with reconstructive cardiac surgery, lung and heart transplantation and pulmonary endarterectomy (PEA), (*Figure 7*).



Figure 7. Surgical specimen obtained by pulmonary endarterectomy

AKH Vienna is responsible for surgical procedures, early post-operative care and regular post – transplantation evaluations. Head of post-transplantation evaluation team in Vienna is dr Peter Jaksch. Croatian part of the programme is located at Department for Respiratory Diseases Jordanovac, University Hospital Centre Zagreb. Head of that part of the programme is Professor Miroslav Samaržija, his team consists of 5 physicians and 3 nurses who are responsible for screening, pre-transplantation evaluation, recruitment of patients to Vienna and Euro-Transplant active list, transport of patients, rehabilitation and comprehensive post-transplantation care.

After start of collaboration until now 157 patients were enrolled in the programme. Lung transplantation was performed in 34 patients, lung and heart transplantation in two patients and PEA in 8 patients. Detailed number of patients in the programme is shown in *Table 2 and Table 3*.

Table 2. Number of patients in lung transplantation programme

Total number - 118
Performed - 34 (1 re-LuTx)
Waiting list – 5
Accepted from AKH – 10
Patients in screening – 26
Screening failures - 7
Patients died after transplantation - 6
Patient died on waiting list – 1
Patients died in screening period – 18

Table 3. Number of patients in pulmonary endarterectomy programme

Total number - 29
Performed – 8
Patients in screening – 6
Screening failure – 3
Died after PEA – 2
Died in screening period – 2

Survival and rate of complications of Croatian patients is similar to whole Vienna cohort. Huge improvement was done when ECMO was introduced as essential part of pre- and post-transplantation support. Indications for ECMO are shown in *Table 4*.

Table 4. Indication for extracorporeal life support

CO ₂ removal
Bridge to recovery
Bridge to transplantation (lung ± heart)
Post transplantation support

Another step forward was done in last year when first successful re-transplantation of the lung was done in patient with chronic rejection. Programme is also expanded to children with end-stage lung diseases.

Conclusion

Lung translation program which started 4 years ago is fully implement in regular medical care of severe respiratory diseases. This program significantly reduced mortality and improved quality of life of these patients. At the same time development of this programme enables introduction of new diagnostic and therapeutic methods and improved general knowledge in the field of respiratory medicine, especially in the treatment of critically and severely ill patients. The collaboration with AKH enables Croatian physicians to get specific education as well as to improve scientific collaboration between the two institutions.

References

- [1] Hardy JD, Webb WR, Dalton ML Jr, Walker GR Jr. Lung homotransplantation in man. *JAMA*. 1963;186:1065–74.
- [2] Christie JD, Edwards LB, Kucheryavaya AY, Aurora P, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Hertz MI. The registry of the International Society for Heart and Lung Transplantation: Twenty-Seventh Official Adult Lung and Heart-Lung Transplant Report-2010. *J Heart Lung Transplant*. 2010;29:1104-18.
- [3] Kotloff RM, Thabut G. Lung transplantation. *Am J Respir Crit Care Med*. 2011;184:159-71.
- [4] Yusef RD, Shearon TH, Qian Y, Kotloff R, Barr ML, Sweet S, Dyke DB, Murray S. Lung transplantation in the United States, 1999–2008. *Am J Transplant*. 2010;10:1047-68.
- [5] Saggarr R, Khanna D, Furst DE, Belperio JA, Park GS, Weigt SS, Kubak B, Ardehali A, Derhovanessian A, Clements PJ, Shapiro S, Hunter C, Gregson A, Fishbein MC, Lynch Iii JP, Ross DJ, Saggarr R. Systemic sclerosis and bilateral lung transplantation: a single centre experience. *Eur Respir J*. 2010;36(4):893-900.
- [6] de Perrot M, Chernenko S, Waddell TK, Shargall Y, Pierre AF, Hutcheon M, Keshavjee S. Role of lung transplantation in the treatment of bronchogenic carcinomas for patients with end-stage pulmonary disease. *J Clin Oncol*. 2004;22:4351-6.
- [7] Grannas G, Neipp M, Hoeper MM, Gottlieb J, Lück R, Becker T, Simon A, Strassburg CP, Manns MP, Welte T, Haverich A, Klempnauer J, Nashan B, Strueber M. Indications for and outcomes after combined lung and liver transplantation: a single-center experience on 13 consecutive cases. *Transplantation*. 2008;85(4):524-31.
- [8] Mason DP, Thuita L, Nowicki ER, Murthy SC, Pettersson GB, Blackstone EH. Should lung transplantation be performed for patients on mechanical respiratory support? The US experience. *J Thorac Cardiovasc Surg*. 2010;139:765-73.
- [9] Murray S, Charbeneau J, Marshall BC, LiPuma JJ. Impact of Burkholderia infection on lung transplantation in cystic fibrosis. *Am J Respir Crit Care Med*. 2008;178:363-71.

- [10] Orens JB, Estenne M, Arcasoy S, Conte JV, Corris P, Egan JJ, Egan T, Keshavjee S, Knoop C, Kotloff R, Martinez FJ, Nathan S, Palmer S, Patterson A, Singer L, Snell G, Studer S, Vachieri JL, Glanville AR; Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. International guidelines for the selection of lung transplant candidates: 2006 update--a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2006;25(7):745-55.

Sažetak

Transplantacija pluća u Hrvatskoj

Transplantacija pluća prvi puta je učinjena 1963. godine, no pravi uzlet doživjela je tek unazad dvadeset godina. Vodeće indikacije za transplantaciju pluća su: kronična opstruktivna plućna bolest, idiopatska plućna fibroza, cistična fibroza, plućna arterijska hipertenzija te sarkoidoza. Pažljiv odabir donora iznimno je važan za prognozu i ishod postupka. Liječnici bi trebali razmišljati o transplantaciji pluća kao terapijskoj opciji te pratiti smjernice o indikacijama za transplantaciju kako bi bolesnike na vrijeme uputili u referalni centar radi pripreme i obrade. Klinika za plućne bolesti Jordanovac, Kliničkog bolničkog centra Zagreb, referalni je centar za transplantaciju pluća u Hrvatskoj. U Klinici se provodi obrada i priprema bolesnika za transplantaciju te posttransplantacijsko liječenje i praćenje.

Ključne riječi: transplantacija; pluća; referalni centar.

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PULMONARY HYPERTENSION – DIAGNOSTIC AND THERAPEUTIC OPTIONS

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Summary

Pulmonary hypertension is a complex group of diseases characterized by a common denominator: elevated pressure in the pulmonary circulation. The etiology of pulmonary hypertension can be very various. The first step in successful treatment of pulmonary hypertension is early detection of this serious and potentially fatal disease. Very important is the suspect about the disease and send patient to additional diagnostic procedures. The most effective diagnostic method for screening today is echocardiogram, which can indirectly measure pressures in the pulmonary circulation and evaluate function of the right ventricle. The final diagnostic method proving pulmonary hypertension is right heart catheterization. In the treatment of pulmonary hypertension different medications are used: calcium channel antagonists, prostaglandins, endothelin antagonists and inhibitors of phosphodiesterase. In patients whom medical therapy is not effective lung transplantation remains a final step.

Keywords: pulmonary hypertension; pulmonary pressure; treatment; idiopathic.

Introduction

Pulmonary hypertension is chronic disorder of pulmonary circulation which is characterized by elevated mean pulmonary artery pressure over 25 mmHg [1]. Disease of pulmonary vasculature leads to elevated pulmonary vascular resistance and elevated pulmonary artery pressure which consequently causes symptoms: exercise intolerance, dyspnea, weakness, presyncope and syncope and clinically meaningful right heart failure [2,3].

In the last decade, novel insights in pathogenesis and pathophysiology of pulmonary hypertension has thought us that pulmonary hypertension is a heterogeneous group of disorders. Thus, according to the etiology of the disease pulmonary hyper-

tension is divided into five groups: pulmonary arterial hypertension, pulmonary hypertension due to left heart disease, pulmonary hypertension due to lung disease and/or chronic hypoxemia, chronic thrombo-embolic pulmonary hypertension and pulmonary hypertension due to unclear and/or multifactorial mechanisms [4].

Symptoms and signs of pulmonary hypertension

Due to the fact that symptoms of the disease usually develop gradually, patients visit physicians due to the symptoms many years after pathophysiologic process which causes the diseases started. Typical symptoms which are reason for referring to physician are: exercise intolerance, shortness of breath, weakness, presyncope or syncope, dry cough and chest tightness. In the more advanced disease patient usually have peripheral edema, cyanosis and finger clubbing [5,6]. Most common form of pulmonary hypertension, pulmonary hypertension due to left heart diseases, usually is manifested in worsening of respiratory function in supine position, orthopnea and paroxysmal night dyspnea, whereas, such symptoms are not present in pulmonary arterial hypertension [6].

Detailed family medical history is essential in inherited forms of pulmonary arterial hypertension [7,8]. History of cocaine, amphetamine and alcohol abuse, as well as smoking history, can be very useful in determining the etiology of pulmonary vascular disease [9,10].

Typical, but not specific signs of pulmonary hypertension can be found at detailed physical examination. Neck veins distension, positive hepato - jugular reflex, ascites, peripheral edema and finger clubbing can be found, as well as parasternal drifting due to tricuspid regurgitation. Accentuated pulmonary component of second heart tone (S₂), usually narrowly torn, can be heard during auscultation. In advanced pulmonary hypertension, holosystolic murmur of tricuspid regurgitation can be heard.

Classification and pathogenesis of pulmonary hypertension

World Health Organization in 1973 classified pulmonary hypertension in two groups: primary and secondary. Development in diagnostic procedures, and better understanding has lead to increasing knowledge about epidemiology, pathogenesis, diagnostic and treatment of pulmonary hypertension has lead to Venice classification in 1998 of pulmonary hypertension into five groups. Further understanding lead to latest classification from Nice in 2013 which is shown in *table 1*.

Regardless of the etiology of pulmonary hypertension, pulmonary hypertension is characterized by prolonged vasoconstriction, thickening and remodeling of

medium size pulmonary arteries and arterioles [2,3]. Prolonged vasoconstriction occurs due to endothelial dysfunction and misbalance between synthesis of vasoconstrictors and vasodilators. Prolonged vasoconstriction leads to increased pulmonary vascular resistance which then leads to increased right ventricular afterload resulting in concentric hypertrophy of right ventricle. Remodeling of pulmonary vasculature leads to fibrosis and obliteration of pulmonary vessels which additionally increases pressure in pulmonary artery and additionally increases afterload of the right ventricle. Increase pulmonary vascular resistance leads to decreased perfusion of pulmonary vasculature and decreased oxygenation of the blood resulting in hypoxemia.

Pathogenesis of pulmonary venous hypertension due to left heart diseases is completely different. In this form of pulmonary hypertension there is no remodeling and obstruction of pulmonary vessels. Hypertension develops due to over-volume of pulmonary vessels due to systolic and/or diastolic dysfunction of the left ventricle or due to valvular disease of the left heart [11].

In pulmonary hypertension which develops in patients with chronic lung diseases hypoxia is main pathophysiologic mechanism. Chronic hypoxia leads to prolonged vasoconstriction of pulmonary vessels and then to remodeling of vessels which is similar to remodeling in pulmonary arterial hypertension [12].

Recurrent embolism of pulmonary vessels leads to obstruction of pulmonary vasculature, increase of pulmonary vascular resistance and to chronic thrombo – embolic pulmonary hypertension (CTEPH). CTEPH is a form of pulmonary hypertension which can be successfully surgically treated with a procedure named pulmonary thromb-endarterectomy (PEA), [13].

Separate group in classification of patients with pulmonary hypertension is pulmonary hypertension due to multifactorial and/or unclear mechanisms. These forms of pulmonary hypertension are rare and represent great challenge even in specialized pulmonary hypertension centers [14].

Diagnosis

Medical history and signs and symptoms are described earlier. Pulmonary arterial hypertension, especially idiopathic, has onset in younger age so disease usually remains unrecognized for several years. Average time from first onset of symptoms to right diagnosis is more than two years.

Table 1. Classification of pulmonary hypertension

1. Pulmonary arterial hypertension (PAH)	
1.1	idiopathic PAH
1.2	heritable
1.2.1	BMPR2
1.2.2.	ALK1, ENG, SMAD9, CAV1, KCNK3
1.2.3.	unknown
1.3	drugs and toxins induced
1.4	associated with:
1.4.1	connective tissue diseases
1.4.2	HIV infection
1.4.3	portal hypertension
1.4.4	congenital heart diseases
1.4.5	schistosomiasis
1'	pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary hemangiomatosis (PCH)
1''	persistent PH of the newborn
2. Pulmonary hypertension due to left heart disease	
2.1	LV systolic dysfunction
2.2	LV diastolic dysfunction
2.3	valvular disease
2.4	congenital/acquired left heart inflow/outflow tract obstruction and congenital cardiomyopathies
3. PH due to lung diseases and/or hypoxia	
3.1	COPD
3.2	interstitial lung disease
3.3	other pulmonary diseases with mixed restrictive and obstructive pattern
3.4	Sleep-disordered breathing
3.5	Alveolar hypoventilation disorders
3.6	Chronic exposure to high altitude
3.7	Developmental abnormalities
4. Chronic thromboembolic pulmonary hypertension (CTEPH)	
5. PH with unclear and/or multifactorial mechanisms	
5.1	hematological disorders: chronic hemolytic anemia, myeloproliferative disorders, splenectomy
5.2	systemic disorders, sarcoidosis, pulmonary histiocytosis, lymphangioleiomyomatosis
5.3	metabolic disorders: glycogen storage disease, Gaucher disease, thyroid disorders
5.4	others: tumoral obstruction, fibrosing mediastinitis, chronic renal failure on dialysis, segmental PH

Electrocardiogram can show us signs right heart axis and enlargement and hypertrophy of right atrium and ventricle [14,15,16]. On the chest X – ray you can see enlarged right heart, dilated pulmonary arteries and reduces peripheral pulmonary vasculature [14,15,16].

Basic screening procedure is echoradiography. With echocardiography you can stated that pulmonary hypertension is likely, possible and unlikely by looking at pulmonary artery systolic pressure and tricuspid regurgitation velocity [14,17], (*table 2*).

Table 2. Likelihood of pulmonary hypertension on echocardiography

PH unlikely: systolic PA ≤ 36 mmHg, TR velocity ≤ 2.8 m/sec

PH possible: systolic PA 37 – 50 mmHg, TR velocity 2.9 – 3.4 m/sec

PH likely: systolic PA > 50 mmHg, TR velocity > 3.4 m/sec

Patients with likely or possible pulmonary hypertension should be referred to expert center where further diagnostic should be performed. Right heart catheterization is fundamental diagnostic procedure for confirmation of pulmonary hypertension with hemodynamic parameters and vasoreactivity testing. Vasoreactivity can be performed with prostanoids, adenosine and NO. Vasoreactivity testing should be done before starting any specific treatment of pulmonary hypertension [14,18].

After confirming pulmonary hypertension further test should be done to clarify etiology of pulmonary hypertension like: CT pulmonary angiography, ventilation and perfusion lung scintigraphy, pulmonary function tests, laboratory tests, genetic testing, liver test, HIV testing [14,18].

Treatment of pulmonary hypertension

Treatment starts with the treatment of underlying disease if known and general measures: oxygen, oral anticoagulants, diuretics and if needed digoxin. Specific treatment depends on vasoreactivity testing. In group of patients with pulmonary arterial hypertension specific treatment is available. In patients with positive test (unfortunately only about 10% patients) initial treatment should be calcium channel blockers [19,20,21,22]. In that group of patients, normalization of pulmonary artery pressure is expected and these patients have good prognosis. In majority of patients with negative vasoreactivity testing treatment with vasodilators should be initiated. Vasodilators can be divided into four groups according to mechanisms of action: prostacyclin analogues, endothelin antagonists, phosphodiesterase 5 inhibitors and activators of soluble guanylate – cyclase. Vasodilators are shown in *table 3*.

Treatment usually starts with monotherapy and in patients with insufficient effects combination of two or three vasodilators of different mechanisms of action is recommended [14].

In patients with failure of conservative treatment surgical procedures like ECMO support, thromb-endarterectomy, lung and/or lung and heart transplantation are indicated in selected patients [14].

Table 3. Specific vasodilators in pulmonary hypertension

Endothelin receptor antagonists	bosentan, ambrisentan, macitentan
PDE – 5 inhibitors	sildenafil, tadalafil
Prostacyclin analogues	epoprostenol, iloprost, treprostinil, beroprost
Activators of soluble guanylate – cyclase	riociguat

Follow – up

Follow up of patients is usually done with estimation of functional class, echocardiography, proNT-BNP and six-minute walking test distance. Improvement or deterioration in the distance walked in the test is today crucial for estimation of treatment efficacy [23].

Conclusion

Pulmonary hypertension is devastating, usually fatal disorder which is characterized by elevated pulmonary artery pressure and pulmonary vascular resistance which can lead to right heart failure and consequently death. Early diagnostic is crucial to improve outcome of these patients. Novel treatment options like specific pulmonary vasodilators significantly improved outcome of patients with pulmonary hypertension. Surgical procedures can be life – saving options in selected patients in whom conservative treatment has failed.

References

- [1] Simonneau G, Robbins I, Beghetti M, Channick RN, Delcroix M, Denton CP, Elliott CG, Gaine S, Gladwin MT, Jing ZC, Krowka MJ, Langleben D, Nakanishi N, Souza R. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* 2009;54:S43-S54.
- [2] Pietra GG, Capron F, Stewart S, Leone O, Humbert M, Robbins IM, Reid LM, Tudor RM. Pathologic assessment of vasculopathies in pulmonary hypertension. *J Am Coll Cardiol.* 2004;43:S25-S32.

- [3] Tuder RM, Abman SH, Braun T, Capron F, Stevens T, Thistlethwaite PA, Haworth S. Pulmonary circulation: development and pathology. *J Am Coll Cardiol.* 2009;54:S3-S9.
- [4] Simonneau G, Galie` N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, Gibbs S, Lebrec D, Speich R, Beghetti M, Rich S, Fishman A. Clinical classification of pulmonary hypertension. *J Am Coll Cardiol.* 2004;43:S5-S12.
- [5] Forfia PR, Trow TK. Diagnosis of pulmonary arterial hypertension. *Clin Chest Med.* 2013;34(4):665-81.
- [6] Ewert R, Olschewski H, Ghofrani HA, Opitz CF. Early diagnosis and therapy in pulmonary hypertension--aspects of a vision. *Pneumologie.* 2013;67(7):376-87.
- [7] Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, Humbert M. Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am J Respir Crit Care Med.* 2008;177: 1377-83.
- [8] Machado R, Eickelberg O, Elliott CG, Geraci M, Hanoaka M, Loyd J, Newman J, Phillips JA, Soubrier F, Trembath R, Chung WK. Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol.* 2009;54:S32-S42.
- [9] Chaouat A, Bugnet AS, Kadaoui N, Schott R, Enache I, Ducolone A, Ehrhart M, Kessler R, Weitzenblum E. Severe pulmonary hypertension and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2005;172:189-94.
- [10] Al-Naamani N, Roberts KE. Portopulmonary hypertension. *Clin Chest Med.* 2013;34(4):719-37.
- [11] Oudiz RJ. Pulmonary hypertension associated with left-sided heart disease. *Clin Chest Med.* 2007;28:233-41.
- [12] Nathan SD, Hassoun PM. Pulmonary hypertension due to lung disease and/or hypoxia. *Clin Chest Med.* 2013;34(4):695-705.
- [13] Marshall PS, Kerr KM, Auger WR. Chronic thromboembolic pulmonary hypertension. *Clin Chest Med.* 2013;34(4):779-97.
- [14] Galie` N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, Beghetti M, Corris P, Gaine S, Gibbs JS, Gomez-Sanchez MA, Jondeau G, Klepetko W, Opitz C, Peacock A, Rubin L, Zellweger M, Simonneau G; ESC Committee for Practice Guidelines (CPG). Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J.* 2009;30(20):2493-537.
- [15] Fisher MR, Forfia PR, Chamera E, Houston-Harris T, Champion HC, Girgis RE, Corretti MC, Hassoun PM. Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. *Am J Resp Crit Care Med.* 2009; 179:615-21.

- [16] Mukerjee D, St George D, Knight C, Davar J, Wells AU, Du Bois RM, Black CM, Coghlan JG. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. *Rheumatology*. 2004;43:461-66.
- [17] Tunariu N, Gibbs SJR, Win Z, Gin-Sing W, Graham A, Gishen P, Al-Nahhas A. Ventilation-perfusion scintigraphy is more sensitive than multidetector CTPA in detecting chronic thromboembolic pulmonary disease as a treatable cause of pulmonary hypertension. *J Nucl Med*. 2007;48:680-4.
- [18] Hoepfer MM, Lee SH, Voswinckel R, Palazzini M, Jais X, Marinelli A, Barst RJ, Ghofrani HA, Jing ZC, Opitz C, Seyfarth HJ, Halank M, McLaughlin V, Oudiz RJ, Ewert R, Wilkens H, Kluge S, Bremer HC, Baroke E, Rubin LJ. Complications of right heart catheterization procedures in patients with pulmonary hypertension in experienced centers. *J Am Coll Cardiol*. 2006;48:2546-52.
- [19] Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med*. 1992;327:76-81.
- [20] Weitzenblum E, Sautegau A, Ehrhart M, Mammosser M, Pelletier A. Long-term oxygen therapy can reverse the progression of pulmonary hypertension in patients with chronic obstructive pulmonary disease. *Am Rev Respir Dis*. 1985; 131:493-8.
- [21] Rich S, Seidlitz M, Dodin E, Osimani D, Judd D, Genthner D, McLaughlin V, Francis G. The short-term effects of digoxin in patients with right ventricular dysfunction from pulmonary hypertension. *Chest*. 1998;114:787-92.
- [22] Fuster V, Steele PM, Edwards WD, Gersh BJ, McGoon MD, Frye RL. Primary pulmonary hypertension: natural history and the importance of thrombosis. *Circulation*. 1984;70:580-7.
- [23] Oudiz RJ, Barst RJ, Hansen JE, Sun XG, Garofano R, Wu X, Wasserman K. Cardiopulmonary exercise testing and six-minute walk correlations in pulmonary arterial hypertension. *Am J Cardiol*. 2006;97:123-6.

Sažetak

Plućna hipertenzija – dijagnostičke i terapijske mogućnosti

Plućna hipertenzija složena je skupina bolesti koje karakterizira zajednički nazivnik: povišen tlak u plućnoj cirkulaciji. Etiologija plućne hipertenzije je različita. Prvi korak u uspješnom liječenju plućne hipertenzije rano je otkrivanje ove teške, i potencijalno smrtonosne bolesti. Vrlo važno je posumnjati na bolest te uputiti bolesnika na dodatne pretrage. Najučinkovitija dijagnostička metoda probira danas je ultrazvuk srca kojim se mogu indirektno izmjeriti tlakovi u plućnoj cirkulaciji te procijeniti funkcija desnog srca. Završna dijagnostička metoda kojom se dokazuje plućna hipertenzija je kateterizacija desnog srca. U liječenju plućne hipertenzije koriste se danas brojni lijekovi: antagonisti kalcijevih kanala, prostaglandini, antagonisti endotelinskih receptora i inhibitori fosfodiesteraze. U bolesnika u kojih medikamentozna terapija nije učinkovita ostaje kao zadnji korak liječenja transplantacija pluća.

Ključne riječi: plućna hipertenzija; tlak, liječenje; idiopatska.

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DIFFUSE PARENCHYMAL LUNG DISEASE

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Summary

Diffuse parenchymal lung disease (DPLD) is a group of diseases that have the common characteristic of involving the pulmonary parenchyma diffusely. The pulmonary parenchyma consists of all structures distal to the terminal bronchiole. DPLD are numerous, there are some 150 different entities that qualify for the group. Most of these diseases are diagnosed according to common algorithm, but the etiology, prognosis and therapy are quite different. Lung transplantation is the ultimate therapeutic option for some of these diseases, most frequently it is the idiopathic pulmonary fibrosis (IPF).

Keywords: diffuse parenchymal lung disease; lung transplantation; idiopathic pulmonary fibrosis.

INTRODUCTION

Diffuse parenchymal lung disease (DPLD) comprises a number of clinical disorders [1] that affect the alveoli, alveolar septa, respiratory bronchioli, blood vessels, lymph vessels, i.e. the pulmonary parenchyma. These disorders are caused by numerous known agents, may be idiopathic, granulomatous or rare (*Table 1*).

The known causes are diverse inorganic agents leading to pneumoconioses (asbestos, silica, etc.), organic, causing hypersensitivity pneumonitis (farmer's lung, bird fancier's lung, etc.), drugs, irradiation, toxic gases and fumes, bacteria, fungi, viruses, protozoa, and parasitic infections or infestations. When treating a patient with DPLD the clinician must carry out a detailed occupational and environmental history [2] because organic dusts are an increasing cause of occupational exposure and respiratory disease, pneumoconiosis still accounts for a significant proportion of ILDs, new sources of exposure and newly identified agents are emerging and finally more knowledge regarding the toxicological effects of nanoparticles is funda-

mental. The exposure to illicit drugs and personal habits should be considered. The results of recognition of the offending agent result in its avoidance and prevention. For a DPLD to be considered idiopathic all the possible etiologic factors, including environmental or occupational, have to be excluded.

Table 1. Classification of Diffuse Parenchymal Lung Disease (DPLD)

DPLD OF KNOWN CAUSE
• Anorganic particles -pneumoconiosis • Organic particles –hypersensitivity pneumonitis • Gases. • Fumes • Drugs. • Irradiation • Microbes
IDIOPATHIC INTERSTITIAL PNEUMONIAS
• Idiopathic pulmonary fibrosis (IPF), • Non-specific interstitial pneumonia (NSIP), • Organizing pneumonia (OP), • Acute interstitial pneumonia (AIP), • Desquamative interstitial pneumonia (DIP) • Respiratory bronchiolitis-interstitial lung disease (RB-ILD), • Lymphoid interstitial pneumonia (LIP) • Pleuropulmonary fibroelastosis (PPFE)
GRANULOMATOSIS
• Sarcoidosis • Granulomatosis with polyangitis (Wegener's granulomatosis,) • Churg-Strauss Syndrome • Necrotizing sarcoid granuloma • Lymphomatoid granulomatosis • Bronchocentric granulomatosis • Langerhans cell histiocytosis (LCH) • Erdheim-Chester disease
RARE DPLD
• Alveolar proteinosis • Alveolar mikrolithiasis • Amyloidosis • Eosinophilic pneumonia • Lymphangioleiomyomatos(LAM) • Idiopathic pulmonary hemosiderosis • Pulmonary manifestation of connective tissue diseases

Idiopathic interstitial pneumonias according to new classification [3] (Table 2) comprise of several entities, among them there is a new entity called pleuropulmonary fibroelastosis. Also, of great help to the practicing physicians is the inclusion of a category of unclassifiable group of DPLD. The everyday life experience resulted in this change, because almost 30% of these diseases even after the most complete and thorough examination, stay unclassifiable.

The third group consists of diseases that histologically show the pattern of *granuloma*, like sarcoidosis.

And finally, the fourth group consists of diverse, mostly *rare diseases* among them diffuse connective tissue diseases (systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, ankylosing spondylitis, mixed connective tissue), lymphangioleiomyomatosis, alveolar proteinosis and alveolar microlithiasis. Some of these diseases are benign and self-limiting; others are chronic, progressive, irreversible, and fatal. The lung manifestation may be the one manifestation of a systemic process. Or it may be the only organ affected. All DPLDs, however, have certain common clinical, imaging, and physiologic features that should be recognized.

Table 2. Revised ATS/ERS classification of idiopathic interstitial pneumonias (IIPs)³

MAJOR IIPs
• Idiopathic pulmonary fibrosis
• Idiopathic non-specific interstitial pneumonia
• Respiratory bronchiolitis–interstitial lung disease
• Desquamative interstitial pneumonia
• Cryptogenic organizing pneumonia
• Acute interstitial pneumonia
RARE IIPs
• Idiopathic lymphoid interstitial pneumonia
• Idiopathic pleuroparenchymal fibroelastosis
UNCLASSIFIABLE IIPs*

***Causes of unclassifiable idiopathic interstitial pneumonia include** (1) inadequate clinical, radiologic, or pathologic data and (2) major discordance between clinical, radiologic, and pathologic findings that may occur in the following situations:(a) previous therapy resulting in substantial alteration of radiologic or histologic findings (e.g., biopsy of desquamative interstitial pneumonia after steroid therapy, which shows only residual nonspecific interstitial pneumonia [153]);(b) new entity, or unusual variant of recognized entity, not adequately characterized by the current American Thoracic Society/European Respiratory Society classification(e.g., variant of organizing pneumonia with supervening fibrosis) (79); and(c) multiple high-resolution computed tomography and/or pathologic patterns that may be encountered in patients with idiopathic interstitial pneumonia.

COMMON CLINICAL FEATURES

A thorough occupational, history of recent or past exposure to inorganic or mineral particles, or to organic dusts and animal antigens (pets) should be identified. Drugs and chemicals known to cause ILD, history of pulmonary infections (particularly HIV), immune disorders, and collagen vascular disorders should be inquired. A smoking history, the country of origin and recent travel history are often critical for establishing the diagnosis.

Dyspnea is the most frequent symptom of DPLD. At first, dyspnea is evident on exercise; later it progresses to breathlessness at rest. The duration of progressive dyspnea usually ranged from months to years. Dyspnea is commonly associated with dry cough, particularly on exertion, and fatigue is frequently present. Fever, chills, and weight loss are the main symptoms in interstitial pulmonary infections but may also occur in collagen vascular disorders.

In 10% to 15% of the patients who have DPLD, tachypnea is present. Auscultation of the lungs reveals end-inspiratory bibasilar “Velcro®-like” crackles. Wheezing is present in 20% of the patients with hypersensitivity pneumonitis as well as the squawk, the ominous auscultatory sign, typical for advanced HP. Digital clubbing is common in idiopathic pulmonary fibrosis (IPF) and asbestosis.

The most helpful *diagnostic procedures* are radiographic imaging, chest X-ray and high resolution computed tomography (HRCT), bronchoscopy with bronchoalveolar lavage and transbronchial lung biopsy, lung function tests and finally the open lung biopsy.

Chest roentgenogram is abnormal in more than 90% of the patients who have DPLD.

High-resolution computed tomography (HRCT) [4] is superior to conventional radiography in delineating the presence and extent of parenchymal involvement in DPLD. HRCT is also useful in characterization of the pattern of the disease, narrowing the differential diagnosis, as a guide to the site of biopsy and as an aid in the follow up of the patients.

As a result of inflammation and fibrosis of the alveolar and the vicinal structures, the lung become stiff and have low lung compliance. Lung volumes are reduced, diffusing capacity is impaired, and alveolar-to-arterial oxygen difference is widened either at rest or exercise or both. The large airway function usually remains normal, but the small airway dysfunction is often present. Airway obstruction is prominent in some interstitial lung disorders. The exercise testing in everyday management of DPLD is very useful.

Laboratory and immunologic tests are of limited value in establishing the cause of ILD.

Lung biopsy should be performed in diffuse parenchymal lung disease. One should attempt to obtain a lung biopsy early in the course of the disease, particularly in a young and middle-aged patient. Transbronchial biopsy is useful in the diagnosis of sarcoidosis, alveolar proteinosis, miliary tuberculosis, and *Pneumocystis* pneumonia. The procedure has limitations because the amount of tissue obtained is often insufficient for extensive diagnostic studies. Surgical open lung biopsy is being replaced by video assisted thorascopic (VAT) lung biopsy. The diagnostic accuracy of thorascopic lung biopsy is equivalent to open lung biopsy. Furthermore, peri-operative morbidity, length of hospital stay, and duration of chest pain are significantly lower in patients who undergo thorascopic lung biopsy. Recently, it has been reported that the awake VATS lung biopsy is easily feasible by regional anesthesia and resulted in low morbidity, excellent diagnostic yield, short hospital stay, and low cost [5].

Bronchoalveolar lavage (BAL) [6] has expanded our understanding of the pathogenesis of many interstitial lung diseases. The minimally invasive bronchoalveolar lavage (BAL) procedure is an important diagnostic instrument that can facilitate the diagnosis of various diffuse parenchymal lung diseases (DPLD). BAL fluid white blood cell profiles are analyzed, malignant cells looked for, and in certain circumstances particular stains are performed to detect yet other cell types. Additionally, BAL can play a very important role in the diagnosis of respiratory tract infections. All these analyses are usually readily performed in a moderately equipped cytological laboratory.

In the *differential diagnosis* of diffuse parenchymal lung disorders, sarcoidosis, pulmonary affection of connective tissue diseases, idiopathic pulmonary fibrosis, and organizing pneumonia are the most common and are responsible for about two thirds of all cases [7].

MANAGEMENT

The management of diffuse parenchymal lung disease depends upon the precise diagnosis, and disease extent, activity and severity. Exclusion of exposition, etiologic therapy with antibiotics, antifungal agents or antituberculosis drugs, immunosuppressants (corticosteroids, azathioprine, cyclophosphamide, methotrexate, leflunomide), biologicals and immunomodulators (infliximab, interferons, rituximab, nintedanib), antifibrotics (pirfenidon, thalidomide, penicilamin) are all the agent of choice in specific circumstances. Symptomatic therapy (oxygen, antitussives) is used in advanced cases.

Pulmonary rehabilitation is also important. The aim of pulmonary rehabilitation is not only to improve daily functioning but also to help people with DPLD live full, satisfying lives. To that end, pulmonary rehabilitation programs focus on physical exercise, to improve the endurance, breathing techniques that improve lung efficiency, emotional support and nutritional counseling

Lung transplantation (LTx) offers a survival benefit in carefully selected patients with DPLD. Indications for lung transplant referral among fibrotic lung disease [8] are histologic or radiographic evidence of usual interstitial lung disease (UIP) or nonspecific interstitial pneumonia (NSIP), FVC<60% predicted, diffusion capacity <39% of predicted (UIP) or <35% of predicted (NSIP), drop in FVC by $\geq 10\%$ or diffusion capacity by $\geq 15\%$ over 6-month period, drop in SaO₂ on pulse oxymetry by <88% on 6-minute walk test, high-resolution CT imaging with honeycombing (fibrous score>2), and pulmonary hypertension.

DPLD most frequently subjected to lung transplantation are the diseases [9] with UIP like HRCT/PHD pattern (~ 80 % of all LTx for DPLD) like idiopathic pulmonary fibrosis (IPF), chronic hypersensitivity pneumonitis (HP), connective tissue disease (CTD) and miscellanea („lung fibroses”, unclassifiable DPLD, etc.). Diseases with NSIP like HRCT/PHD pattern (~ 4 % of all LTx), sarcoidosis (~2.5 of all LTx) and rare disease: Langerhans cell histiocytosis (LCH), microlithiasis, lymphohangioleiomyomatosis (LAM), pulmonary alveolar proteinosis (PAP) and others, are less frequent thus less frequently subjected to LTx.

Idiopathic pulmonary fibrosis comprises some 23% of all the indications for lung transplantation, raising its proportion in that respect in recent years.

IDOPATHIC PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is archetypal example of «lung fibrosis». It is the most frequent of all “fibroses”.

IPF is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, limited to the lungs, and associated with the histopathologic and/or radiologic pattern of usual interstitial pneumonia (UIP) [10].

The diagnosis of IPF requires: a. Exclusion of other known causes of interstitial lung disease (ILD) (e.g., domestic and occupational environmental exposures, connective tissue disease, and drug toxicity). b. The presence of a UIP pattern on high-resolution computed tomography (HRCT) in patients not subjected to surgical lung biopsy. c. Specific combinations of HRCT and surgical lung biopsy pattern in

patients subjected to surgical lung biopsy. The major and minor criteria proposed in the 2000 ATS/ERS Consensus Statement have been eliminated.

The prevalence of IPF in Europe is 2-23/100.000, Croatia 8/100.00, and in patients above 75 years 177/100.000. The mean length of survival after diagnosis is 3.2–5 years.

Although the disease is idiopathic, many causes or facilitating circumstances are under investigation. Exposure to an inciting agent (smoke, environmental pollutants, environmental dust, viral infections, gastroesophageal reflux disease, chronic aspiration), genetic mutations (surfactant protein C, gene encoding mucin 5B (MUC5B), mutant telomerase known to be associated with familial idiopathic pulmonary fibrosis and telomere shortening which also occurs with aging and can also be acquired are in the focus of extensive research. This telomere shortening could promote the loss of alveolar epithelial cells, resulting in aberrant epithelial cell repair, and therefore should be considered as another potential contributor to the pathogenesis of idiopathic pulmonary fibrosis. Today the aging lung is in the focus of several investigations, not only regarding the pathogenesis of IPF, but also lung cancer and COPD [11].

Clinical presentation most often consists of slowly progressive breathlessness, tightness of the chest and dry cough, which does not respond to antitussive agents. The symptoms are usually present for more than 6 months before presentation. Physical examination reveals bilateral, bibasilar, end-inspiratory fine crackles on auscultation (“Velcro®-like” crackles) and digital clubbing .

The diagnostic approach includes performance of radiological imaging techniques, the plain radiograph of the chest and particularly the high resolution computed tomography (HRCT), assessment of pulmonary function and laboratory tests, and open lung biopsy in those who do not fulfill the diagnostic criteria.

Pulmonary function tests typically reveal restrictive ventilatory changes (reduced vital capacity [VC], and total lung capacity [TLC]) at some point in the course of the disease. The DLco is reduced and may precede the reduction of lung volumes. The respiratory arterial blood gases may be normal, or they may reveal mild hypoxemia. With cardiopulmonary exercise testing early abnormalities of gas exchange could be detected. Pulmonary hypertension rarely occurs at rest, but it is common during exercise.

The plain radiograph is the screening test to detect the presence of IPF, although it may be normal in about 10% of the patients. *HRCT technique* has changed the diagnostic procedure in these patients as it allows earlier diagnosis and helps to narrow the differential diagnosis. The characteristic HRCT in IPF is the summoned feature of different patterns that together make the usual interstitial pneumonia (UIP) ima-

ge ; the honeycombing, reticulation, traction bronchiectasis, architectural distortion and volume loss with no other specific patterns (granuloma, ground-glass, cysts, etc.), (Figure 1), which are inconsistent with UIP pattern [12].

Transbronchial lung biopsy is not helpful in making the diagnosis of IPF, but it can help to exclude some other entities that are considered in differential diagnosis.

Open lung biopsy, or video assisted thoracoscopic (VAT) lung biopsy are performed in the minority of patients. The usual interstitial pneumonia (UIP) is the pathological abnormality essential to the diagnosis of IPF; it refers to a morphologic entity defined by a combination of [1] patchy interstitial fibrosis with alternating areas of normal lung, [2] temporal heterogeneity of fibrosis characterized by scattered fibroblastic foci in the background of dense acellular collagen, and [3] architectural alteration due to chronic scarring or honeycomb change.

It is evident that some *therapies* are effective in the treatment of IPF. Pirfenidone (Esbrie) [13] and Nintedanib (Ofev) [14] show statistically significant improvement in clinical trials, patients taking either drug experienced significantly reduced declines in forced vital capacity compared with patients who received placebo [15].

Future trials are therefore unlikely to use placebo as a control group for ethical reasons. Future clinical assessment will probably include add-on trials in which a new drug is combined with an intervention with established efficacy; this development is in turn likely to herald the use of combination regimens in clinical practice [16].

The combination of inhalation NAC and pifrenidon showed that the progression-free survival (PFS) in IPF was longer in the NAC plus pifrenidone group than in the pifrenidone group [17].

GER medications due to high prevalence of gastroesophageal reflux (GER) showed that the use was associated with a longer survival time, additionally, patients taking GER medications had a lower fibrosis score on HRCT.

Other recommendations are to quit smoking, oxygen therapy to maintain a saturation of at least 90% at rest, with sleep, and with exertion, and vaccination against influenza and pneumococcal infection should be encouraged in all patients with idiopathic pulmonary fibrosis.

If all the earlier mentioned interventions fail, the patient deteriorates, and if the patient accomplishes the indications for lung transplantation he will be referred to the procedure.

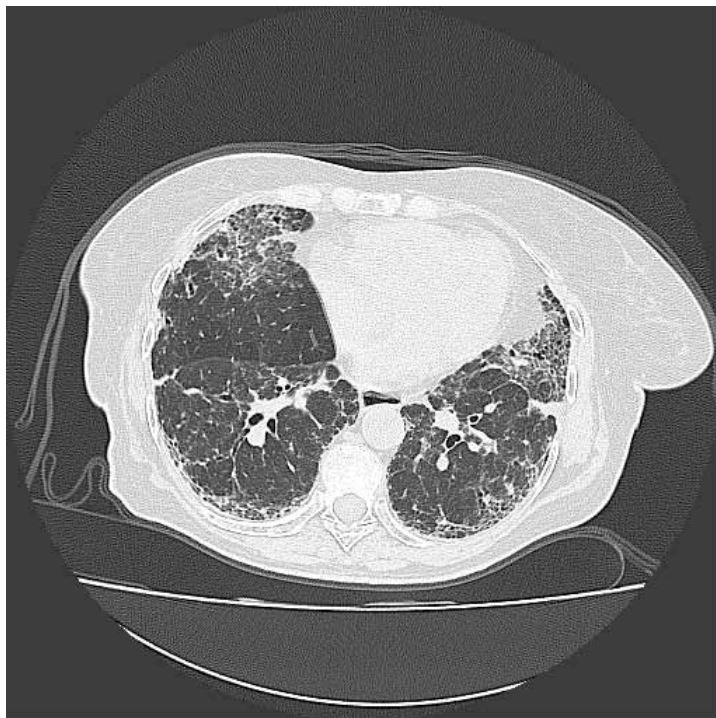


Figure 1. Typical high resolution computed tomography (HRCT) scan in idiopathic pulmonary fibrosis (IPF)

References

- [1] Peroš-Golubičić T, Sharma O. Clinical Atlas of Interstitial Lung Disease, Springer, London 2006. page 10.
- [2] Robalo Cordeiro C, Alfaro TM, Freitas S, Cemlyn-Jones J, and Ferreira A.J. Rare interstitial lung diseases of environmental origin Eur Respir Mon 2011; 54: 301-316.
- [3] William D. Travis, Ulrich Costabel, David M. Hansell, Talmadge E. King, Jr., David A. Lynch, Andrew G. Nicholson, et al An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias Am J Respir Crit Care Med 2013; 188(6) : 733-748.
- [4] Wells AU. High-Resolution Computed Tomography in the Diagnosis of Diffuse Lung Disease: A Clinical Perspective. Semin Respir Crit Care Med. 2003;24:4.

- [5] Pompeo E, Rogliani P, Cristino B, Schillaci O, Novelli G, Saltini C. Awake thoracoscopic biopsy of interstitial lung disease. *Ann Thorac Surg.* 2013;95(2):445-52.
- [6] Peroš-Golubičić T, Smojver-Ježek S. Lung Lavage Cell Profiles in Diffuse Lung Disease, *Coll. Antropol.* 2010; 34(1): 327-335
- [7] Coultas DB, Zumwalt RE, Black WC, Sobonaya RE. The Epidemiology of Interstitial Lung Diseases. *Am J Resp Crit Care Med* 1994; 150:967-72.
- [8] Whitson BA, Hayes D Jr. Indications and outcomes in adult lung transplantation. *J Thorac Dis.* 2014;6(8):1018-23.
- [9] Gottlieb J. Lung transplantation for interstitial lung diseases. See comment in PubMed Commons below *Curr Opin Pulm Med.* 2014;20(5):457-62.
- [10] Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE, Jr., Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Duden RF, Griss BS, Protzko SL, and Schunemann HJ. : An Official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based Guidelines for Diagnosis and Management *Am J Respir Crit Care Med* Vol 183. pp 788-824, 2011.
- [11] Meiners S, Eickelberg O, Königshoff M. Hallmarks of the ageing lung. *Eur Respir J* 2015; 0: 1-21
- [12] Devaraj A. Imaging: how to recognise idiopathic pulmonary fibrosis. *Eur Respir Rev* 2014; 23: 215–219
- [13] King, T.E.Jr.; ...; Peroš-Golubičić, Tatjana; ...; Tazelaar, H.D. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis *N Engl J Med* 2014 ; 370:2083-2092
- [14] Richeldi L, du Bois R, M.D., Raghu R. Efficacy and Safety of Nintedanib in Idiopathic Pulmonary Fibrosis *N Engl J Med* 2014; 370 : 2071-2082
- [15] Loveman E, Copley VR, Colquitt JL, Scott DA, Clegg AJ, Jones J, O'Reilly KM, Singh S, Bausewein C, Wells A. The effectiveness and cost-effectiveness of treatments for idiopathic pulmonary fibrosis: systematic review, network meta-analysis and health economic evaluation. *BMC Pharmacol Toxicol.* 2014;15:63.
- [16] Wuyts WA, Antoniou KM, Borensztajn K, Costabel U, Cottin V, Crestani B, Grutters JC, Maher TM, Poletti V, Richeldi L, Vancheri C, Wells AU. Combination therapy: the future of management for idiopathic pulmonary fibrosis? *Lancet Respir Med.* 2014;2(11):933-42.
- [17] Sakamoto S, Muramatsu Y, Satoh K, Ishida F, Kikuchi N, Sano G, Sugino K, Isobe K, Takai Y, Homma S. Effectiveness of combined therapy with pirfenidone and inhaled N-acetylcysteine for advanced idiopathic pulmonary fibrosis: A case-control study. *Respirology.* 2015 Feb 2. doi: 10.1111/resp.12477. [Epub ahead of print]

Sažetak

Difuzne bolesti plućnog parenhima

Difuzne bolesti plućnog parenhima su grupa bolesti kojima je zajednička osobina da difuzno zahvaćaju plućni parenhim. Plućni se parenhim sastoji od svih struktura koje se prostiru distalno od terminalnih bronhiola. Ove su bolesti brojne, pa tako čak oko 150 različitih entiteta zadovoljava kriterije uključivanja u ovu skupinu bolesti. Dijagnoza većine ovih bolesti postavlja se prema zajedničkom dijagnostičkom algoritmu, ali su etiologija, prognoza i terapija vrlo različite. Transplantacija pluća krajnja je terapijska opcija za neke od ovih bolesti, a najčešće se zahvat se vrši u bolesnika s idiopatskom plućnom fibrozom.

Ključne riječi: difuzne bolesti plućnog parenhima; transplantacija pluća; idiopatska plućna fibroza.

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IMMUNOSUPPRESSIVE THERAPY IN THE LUNG TRANSPLANT RECIPIENT

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Summary

Lung transplantation has become a life-saving procedure for individuals with variety of end-stage respiratory diseases. Optimal immunosuppression remains the key to long-term graft survival. The protocols for immunosuppressive therapy following lung transplantation can be divided into three general categories: induction, maintenance, and treatment of rejection. The goal of induction therapy is to provide intense immunosuppression in the early post-transplantation period, when the risk of allograft rejection is the highest. Induction agents primarily target T lymphocytes, which are considered the effector cells in cell-mediated rejection. Current maintenance therapy typically includes a calcineurin inhibitor, nucleotide blocking agent and corticosteroid. Pulse steroids are generally the first treatment of acute cellular rejection. Therapeutic modalities for treatment of refractory cellular rejection include switch from cyclosporine to tacrolimus, use of lymphocyte depleting agents, azithromycine, and extracorporeal photopheresis. Treatment options for humoral rejections include plasmapheresis and immunoglobulines in combination with rituximab.

Keywords: lung transplantation; immunosuppression; induction therapy; maintenance therapy; rejection.

INTRODUCTION

Lung transplantation has become a life-saving procedure for individuals with variety of end-stage respiratory diseases. The success of transplantation has been closely related to discovery of effective immunosuppressive regimens. In the early 1980s, the introduction of ciclosporin as the mainstay of maintenance immunosuppression was followed by a significant improvement in the survival of lung tran-

splank recipients. Immunosuppressive regimes are employed to reduce the rate of acute and chronic rejection. Adverse effects of immunosuppressive therapy include drug-specific toxicities, opportunistic infections and malignancy. Risk of those events directly correlates with the degree of overall immunosuppression. Therefore, immunosuppressive protocols try to achieve delicate balance between risk of adverse events and rejection. General principle is that the highest concentrations of immunosuppressants are targeted in the early post-transplant period when the risk of acute rejection is the greatest. Immunosuppression is slowly decreased over time (as the risk of acute rejection decreases) to help lower adverse effects. Protocols are consisted of several drugs. Combination therapy targets several steps in T-cell activation, allowing lower doses of each individual drug which provide higher tolerability.

Immunosuppressive regimes are generally defined as induction, maintenance, and treatment of rejection.

Induction therapy

Despite the advances in immunosuppressive therapy, acute allograft rejection is still a significant problem in lung transplantation. More than a third of lung transplant recipients are treated for acute rejection in the first transplant year [1]. The rationale of induction therapy is to use the strongest immunosuppressive drugs at the time when the risk of rejection is highest, which is in the first few weeks following transplantation. Induction regimes consist of agents which primarily target T lymphocytes, which are considered the effector cells in cell mediated rejections. Most frequently used antilymphocytic agents are polyclonal antilymphocyte antibodies, interleukin-2 receptor antagonists and anti-CD52 antibodies (alemtuzumab). Induction therapy also allows later introduction of nephrotoxic immunosuppressive drugs in patients with compromised renal function.

Polyclonal anti-lymphocyte antibodies (ATG)

Polyclonal antibodies are derived from immunization of horses (ATGAM) or rabbits (Thymoglobulin) with human lymphocytes. These preparations contain antibodies directed against many surface T- and B-cell antigens. Antithymocyte globulin depletes circulating lymphocytes through multiple mechanisms, including complement-mediated lysis and opsonization in the spleen and liver [2]. The major acute side effects include: cytokine release syndrome, serum sickness reaction, while some patients experience true anaphylaxis. The glucocorticoids, antihistamines, antipyretics are used for prophylaxis. Leukopenia and thrombocytopenia may require either a reduction in dose or termination of therapy.

Based on the analysis of International Society of Heart and Lung Transplantation (ISHLT) data, ATG - induction significantly reduced the incidence of acute rejection (AR) during the first post-transplantation year, compared to no induction [3]. Also, after 2 years, a trend toward a lower incidence of the bronchiolitis obliterans syndrome (BOS) was documented in the ATG-group (20%) versus the non-induction arm [4]. ATG induction therapy results in significantly lower incidence of acute rejections without an increase in infection complications [5].

Interleukin-2 receptor antagonists (IL2RA)

Interleukin-2 receptor antagonists are monoclonal antibodies that selectively bind to the alpha subunit of the IL-2 receptor (CD25) on activated T cells. By binding this cell surface receptor, these antibodies inhibit T cell proliferation and differentiation without T cell depletion. In contrast to polyclonal antibody preparations, IL-2 receptor antagonists show fewer side effects and are well tolerated.

Induction therapy with IL2RA reduces or delays the incidence of acute rejection, bronchiolitis obliterans syndrome (BOS), and may improve graft and patient survival compared to no induction [4,6].

Alemtuzumab

Alemtuzumab is a humanized rat monoclonal antibody that targets the CD52 antigen expressed on both T and B cells. It leads to depletion of T cells through complement - mediated and direct cellular cytotoxicity [7]. The usual dose of alemtuzumab for induction of immunosuppression is 30 mg i.v. Alemtuzumab can precipitate cytokine storm so patient should be pretreated with steroids. Although alemtuzumab has a half life of 12 days, inflammatory cells need significantly longer time of recovery after alemtuzumab therapy. B cells recover at 12 months, while 50% recovery of T cells is usually reached at 36 months [8]. If alemtuzumab is administered, the maintenance dose of immunosuppressive therapy is tailored in a way that tacrolimus is reduced by 40% of standard target values, while steroids are reduced to 30% of standard dose. Mycophenolate mofetil (MMF) is avoided in the first postoperative year for the risk of severe leukopenia.

Maintenance therapy

Maintenance immunosuppressive therapy is administered to transplant recipients to help prevent acute and chronic allograft rejection. Maintenance therapy generally consists of combined therapy with a calcineurin inhibitor, a nucleotide blocking agent (antimetabolite), and steroids.

Calcineurin-inhibitors (CNI)

Since the induction of cyclosporine in the early 1980s, the calcineurin – inhibitors serve as the backbone of maintenance immunosuppressive therapy in solid organ transplantation. Calcineurin - inhibitors include cyclosporin (CsA) and tacrolimus (TAC).

Cyclosporine (CsA)

Cyclosporine acts by binding to cyclophilin, an intracellular protein. The complex inhibits the activity of calcineurin, which is responsible for the transcription of IL-2 and several other cytokines including TNF-alpha, granulocyte-macrophage colony – stimulating factor and interferon gamma. This prevents activation and proliferation of CD4+ T cells [9].

Drug dosage should be adapted according to the monitoring trough level. Targeted trough levels are 250 – 350 ng/ml in the first post-transplantation year, and should be reduced by 40% in patients who received alemtuzumab induction therapy. Target drug concentrations are typically reduced over time as the risk of acute rejection decreases. The common side effect of cyclosporine, which are related to the concentration of the drug, include nephrotoxicity, hypertension, hyperlipidemia, gingival hyperplasia, hirsutism, and tremor. Nephrotoxicity, the major adverse effect of cyclosporine, results from vasoconstriction of the afferent glomerular arterioles.

Tacrolimus (TAC)

TAC was previously known as FK506. It is a macrolide and is produced by the fungus *Streptomyces tsukubaensis*. TAC binds to the cytoplasmic immunophilin, FK binding protein12, and inactivates calcineurin. Through this pathway it inhibits interleukin – 2 and interferon – gamma production and subsequently T-cell proliferation and activation.

Toxicity profile of tacrolimus is similar to that of cyclosporin A, but it is less nephrotoxic. Recently, tacrolimus has been found to be superior to cyclosporin A in lung transplantation in terms of decreased incidences of acute rejection and chronic rejection [10]. Therefore, the use of tacrolimus has increased steadily, and the drug is now the dominant calcineurin inhibitor.

TAC may be administered as a continuous infusion, or orally. The drug dose should be adjusted as per blood levels. The target levels in the first three postoperative months are 15-18 ng/ml. Therapy is gradually decreased over time, with tacroli-

mus target levels at 8 to 10 ng/mL in a second postoperative year, and 8 ng/ml three years after transplantation. In patients who had received alemtuzumab induction therapy, tacrolimus target levels should be reduced by 40%.

Nucleotide blocking agents

Azathioprine (AZA) and mycophenolate mofetil (MMF) are commonly used nucleotide blocking agents after lung transplantation. Most transplant centers use mycophenolat mofetil as a part of maintenance immunosuppressive therapy, due to its increased selectivity for T- and B- lymphocytes [11].

Mycophenolate mofetil (MMF)

The mechanism of action of MMF is via selective inhibition of T- and B-cell proliferation. Mycophenolate reversibly inhibits de novo synthesis of purines during the S phase, a step that is required for the lymphocyte cell division. T and B lymphocytes use only de novo pathway in purine biosynthesis, whereas other cell lines that use both de novo and salvage pathways are not inhibited [12]. Mycophenolate's principal toxicities occur in the gastrointestinal tract and principally manifest as nausea and diarrhea. Due to potent myelosuppression, careful monitoring of complete blood counts should be performed on regular basis. MMF may be administered either orally or intravenously. The usual dosing schedule is 1000 mg. orally given twice daily. Drug levels can be monitored, although this is not done routinely.

Steroids

Corticosteroids are employed during induction, maintenance and anti-rejection therapy in pulmonary transplantation, emphasizing their crucial role in modulating the host immune response. They suppress prostaglandin synthesis, reduce histamine/bradykinin release, decrease vascular permeability and down-regulate key cytokines by influencing gene transcription [13]. Most common side effects include impaired glucose tolerance, hyperlipidemia, hypertension, hirsutism, M. Cushing, osteoporosis. High-dose steroids are generally administered intraoperatively and postoperatively. Due to the long-term side effects, the corticosteroid dose should be gradual tapering over months. The prednisone dose in the first 3 postoperative month is 0.3 mg/kg body weight, while after first post-transplant year the prednisolone dose is 5 mg once a day.

Treatment of acute and chronic rejection

Rejection therapy refers to immunosuppressive therapy given to reverse an episode of rejection. The intensity and type of rejection therapy depend on the severity of the rejection and type of rejection (whether it is cellular mediated or humoral).

Pulse steroids (500 – 1000 mg/d of methylprednisolone for 3-5 d followed by steroid taper over the ensuing 2-3 weeks) are generally the first treatment for cellular allograft rejection. Despite the many therapeutic options described above, refractory acute and chronic rejection alities in lung transplantation. An internationale retrospective study comparing CsA to tacrolimus revealed a decreased incidence in acute rejection and reversal of refractory acute rejection episodes and improvement of lung function after conversion of CsA to tacrolimus [14]. Therefore, for patients on the cyclosporine based regimen with evidence of rejection, most transplant centers recommend switch maintenance immunosuppressive agent from cyclosporine to tacrolimus.

Azithromycin is a macrolide antibiotic with anti-inflammatory and immunomodulatory properties. Early initiation of azithromycin in lung transplant recipients may prevent the incidence of BOS and prolong BOS free survival. It may improve or stabilize pulmonary function after the onset of BOS, particularly in those with neutrophil and IL-8 predominant BAL [15].

In a case of a refractory cellular rejection, lymphocyte depleting agents (ATG or alemtuzumab) are used. ATG may decrease or stop the decline of lung function in patients presenting with BOS [16].

Other treatment modalities for refractory cellular rejection include extracorporeal photopheresis (ECP). ECP involves leukopheresis followed by incubation of isolated cells with 8-methoxypsoralen and subsequent exposing the cells to ultraviolet light in the presence of 8-methoxypsoralen. The cells are than reinfused to the patient. ECP is thought to induce apoptosis of the mononuclear cells, resulting in immunologic processing of cellular components after re-injection into the circulation that leads to the production of tolerogenic T-lymphocytes [17,18].

Treatment options for humoral rejections include plasmapheresis (5-6 cycles) and immunoglobulines (1-2g/kg over 3-6 days) in combination with rituximab.

Lung transplantation continues to offer patients with end-stage respiratory diseases chance for better quality and lenght of life. A better understanding of the immune response has led to improved monitoring of rejection and the development of better immunosuppressive regimes. In the future, advances in immunosuppression protocols and the use of more specific immune monitor tools are likely to lead to significant improvements in outcomes.

References

- [1] Yusen RD, Christie JD, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: thirtieth adult lung and heart-lung transplant report--2013; focus theme: age. International Society for Heart and Lung Transplantation. *J Heart Lung Transplant*. 2013;32:965-78.
- [2] Valentine VG, Bonvillain RW, Gupta MR, Lombard GA, LaPlace SG, Dhillon GS, Wang G. Infections in lung allograft recipients: ganciclovir era. *J Heart Lung Transplant*. 2008;27(5):528-35.
- [3] Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J, Dobbels F, Rahmel AO, Keck BM, Hertz MI. Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult heart transplant report-2007. *J Heart Lung Transplant*. 2007;26(8):769-81.
- [4] Palmer SM, Miralles AP, Lawrence CM, Gaynor JW, Davis RD, Tapson VF. Rabbit antithymocyte globulin decreases acute rejection after lung transplantation: results of a randomized, prospective study. *Chest*. 1999;116(1):127-33.
- [5] Jaksch P, Wiedemann D, Augustin V, Muraközy G, Scheed A, Kocher AA, Klepetko W. Antithymocyte globulin induction therapy improves survival in lung transplantation for cystic fibrosis. *Transpl Int*. 2013;26(1):34-41.
- [6] Garrity ER Jr, Villanueva J, Bhorade SM, Husain AN, Vigneswaran WT. Low rate of acute lung allograft rejection after the use of daclizumab, an interleukin 2 receptor antibody. *Transplantation*. 2001;71(6):773-7.
- [7] Flynn JM, Byrd JC. Campath-1H monoclonal antibody therapy. *Curr Opin Oncol*. 2000;12(6):574-81. Review.
- [8] Weaver TA, Kirk AD. Alemtuzumab. *Transplantation*. 2007;84(12):1545-7.
- [9] Knoop C, Haverich A, Fischer S. Immunosuppressive therapy after human lung transplantation. *Eur Respir J*. 2004;23(1):159-71. Review.
- [10] Hachem RR, Yusen RD, Chakinala MM, Meyers BF, Lynch JP, Aloush AA, et al. A randomized controlled trial of tacrolimus versus cyclosporine after lung transplantation. *J Heart Lung Transplant*. 2007;26:1012-1018.
- [11] Kobashigawa JA, Miller LW, Russell SD, Ewald GA, Zucker MJ, Goldberg LR, et al. Tacrolimus with mycophenolate mofetil (MMF) or sirolimus vs. cyclosporine with MMF in cardiac transplant patients: 1-year report. *Am J Transplant*. 2006;6:1243-1245.
- [12] Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. *Crit Rev Oncol Hematol*. 2005 Oct;56(1):23-46. Review.
- [13] Adcock I.M., Ito K. Molecular mechanisms of corticosteroid actions. *Monaldi Arch Chest Dis*. 2000;55:256-266.

- [14] Sarahrudi K, Estenne M, Corris P, Niedermayer J, Knoop C, Glanville A, Chaparro C, Verleden G, Gerbase MW, Venuta F, Böttcher H, Aubert JD, Levvey B, Reichenspurner H, Auterith A, Klepetko W. International experience with conversion from cyclosporine to tacrolimus for acute and chronic lung allograft rejection. *J Thorac Cardiovasc Surg.* 2004;127(4):1126-32.
- [15] Verleden GM, Vanaudenaerde BM, Dupont LJ, Van Raemdonck DE. Azithromycin reduces airway neutrophilia and interleukin-8 in patients with bronchiolitis obliterans syndrome. *Am J Respir Crit Care Med.* 2006;174(5):566-70.
- [16] Date H, Lynch JP, Sundaresan S, Patterson GA, Trulock EP. The impact of cytolytic therapy on bronchiolitis obliterans syndrome. *J Heart Lung Transplant.* 1998;17(9):869-75.
- [17] Jaksch P, Scheed A, Keplinger M, Ernst MB, Dani T, Just U, Nahavandi H, Klepetko W, Knobler R. A prospective interventional study on the use of extracorporeal photopheresis in patients with bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant.* 2012;31(9):950-7.
- [18] Jaksch P, Knobler R. ECP and solid organ transplantation. *Transfus Apher Sci.* 2014;50(3):358-62.

Sažetak

Imunosupresivno liječenje nakon transplantacije pluća

Transplantacija pluća je postupak koji spašava život bolesnicima s krajnjim stadijem različitih plućnih bolesti. Optimalna imunosupresija predstavlja ključ dugotrajnog preživljenja presađka. Protokoli imunosupresivnog liječenja nakon transplantacije pluća mogu se podijeliti u tri glavne grupe: indukcijska terapija, terapija održavanja i terapija liječenja reakcija odbacivanja. Cilj indukcijske terapije je osigurati intenzivnu imunosupresiju u ranom posttransplantacijskom periodu, kada je rizik odbacivanja alografta najveći. Indukcijski lijekovi su primarno usmjereni na T limfocite, koji se smatraju glavnim stanicama u stanično posredovanoj reakciji odbacivanja. Trenutno se terapija održavanja tipično sastoji od inhibitora kalcineurina, antimetabolita i kortikosteroida. Pulsne doze kortikosteroida su prvi izbor liječenja akutnog staničnog odbacivanja. Načini liječenja refraktornog staničnog odbacivanja uključuju zamjenu ciklosporina takrolimusom, primjenu monoklonalnih i poliklonalnih antilimfocitnih protutijela, azitromicina i ekstrakorporalne fotofereze. Mogućnosti liječenja humoralnog odbacivanja uključuju plazmaferezu i imunoglobuline u kombinaciji s rituksimabom.

Ključne riječi: transplantacija pluća; imunosupresija; indukcijska terapija; terapija održavanja; odbacivanje.

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THE ROLE OF PULMONARY REHABILITATION BEFORE AND AFTER LUNG TRANSPLANTATION

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Summary

Pulmonary rehabilitation is the key component in treating patients with chronic respiratory diseases. In lung transplantation program it plays an important role as an individualized program for every patient before and after surgery. In these patients reduced ability to exercise is an important predictor of outcome and survival after surgery so feature of pulmonary rehabilitation is immensely important. The intensity of exercise and training regimen depend on the underlying disease. Pretransplant rehabilitation helps optimize and maintain patient's functional status and supplies important interdisciplinary information about the following surgery. It also includes psychosocial support to patients and their caregivers with the goal to reduce stress and improve adjustment while waiting for operation. Posttransplantation rehabilitation starts in the first 24-48 hours after surgery and it is oriented to optimal lung expansion, airway hygiene, efficient breathing, extremity activation and walking. Educational component of the posttransplantation rehabilitation includes teaching recipients about desired health behaviors, risks, adequate medical regimen and healthy diet. Psychosocial support after transplantation involves: helping recipients to recover lost social and family roles, counseling, adapting to new organ and ongoing medical necessities, long-term moderation of their expectations and caring about their psychological wellbeing.

Keywords: pulmonary rehabilitation; lung transplantation; psychosocial support.

Pulmonary rehabilitation has been recognized as a key component in the treatment of patients with chronic respiratory diseases [1]. It is an established procedure in chronic obstructive pulmonary disease (COPD) [2]. Patients with other types of chronic respiratory diseases also have benefit from pulmonary rehabilitation [3].

Despite optimal medical treatment they all have the same problems; suffer from daily symptoms, muscle weakness, exercise intolerance, impaired mood status, poor physical activity and low quality of life. Positive effects of exercise-based pulmonary rehabilitation have been proved in patients with lung cancer (pre-/post-lung resection), chronic respiratory failure due to kyphoscoliosis, pre-/post-lung transplantation, cystic fibrosis, bronchiectasis, severe respiratory failure receiving extracorporeal membrane oxygenation, interstitial lung disease, and pulmonary arterial hypertension. Pulmonary rehabilitation programmes should be adapted to all the patients with chronic respiratory disease other than COPD [4].

Pulmonary rehabilitation provides evidence based assistance with the purpose of the best medical care for patients. New ATS / ERS recommendations (American Thoracic Society / European Respiratory Society Statement) on pulmonary rehabilitation from 2013 aimed that using international scientific expertise, new knowledge and clinical experience of experts in this field.¹

The new ATS/ERS definition of pulmonary rehabilitation is: „Pulmonary rehabilitation is a comprehensive intervention based on a thorough patient assessment followed by patient-tailored therapies which include, but are not limited to, exercise training, education and behaviour change, designed to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence to health-enhancing behaviours” [1].

The objective is long-term improvement through the observance of such healthy behaviours. Changing behaviour is vital for optimization and improvement of health status in patients with chronic respiratory diseases because it reduces the number of interventions. Pulmonary rehabilitation is recognized as the leading strategy to achieve this goal.

Certainly, the exercise is still the basic for rehabilitation, but there is one important role of pulmonary rehabilitation, that is encouraging the ability of patients to engage in collaborative self-management treatment of their disease. This is actually a shift from the traditional didactic model of education and a move towards interactive consultation and involvement of patients in the active control of their disease [2].

Pulmonary rehabilitation program

Pulmonary rehabilitation can be initiated in a stable phase or immediately after the disease exacerbation to reduce symptoms, increase physical activity, promote the independence of patients, increase participation in everyday activities and to improve the quality of life. The indication for pulmonary rehabilitation is not only based on the severity of lung disease, but also of the long-term persistence of symp-

toms, inability to perform daily activities and overall disabilities. The program must be carried out by well-trained interdisciplinary team consisting of: pulmonologist, respiratory therapist, occupational therapist, respiratory nurses, social workers, nutritionists, psychologists and family physicians. The goal of pulmonary rehabilitation is to improve quality of life with emphasis on: learning breathing techniques, respiratory muscles exercise, upper and lower extremities exercise, disease education, psychosocial support, smoking cessation, oxygen therapy, bronchodilator therapy, antibiotics and nutritional support. There is a proven effectiveness of different forms of exercise as part of pulmonary rehabilitation involving interval training, strength training, training of the upper limbs and transcutaneous neuromuscular electrical stimulation [1].

It is well known that patients with chronic respiratory diseases have a significantly higher respiratory work and also greater work of the respiratory muscles compared to healthy person which is particularly visible during physical exercise. Electrophysiological changes that occur during exercise in the respiratory and skeletal muscles are responsible for the lower exercise tolerance. The purpose of inspiratory muscle training (IMT) is to reduce the weakness of the respiratory muscles and improve exercise tolerance. Successful training of respiratory muscles should increase the maximum ventilation during exercise and improve exercise capacity [1].

In pulmonary rehabilitation exercise tolerance is estimated from clinical tests: six minute walking test (6MWT) or cardio pulmonary exercise testing (CPET) at the beginning and end of the program [1]. Nordic walking is feasible, simple and effective in carrying out the training, significantly increases the length of the walking distance (measured from the 6MWT) and has a positive impact on the daily physical activity in patients with chronic lung disease. Nordic walking is a safe physical activity also in end-stage lung disease, including patients waiting for lung transplantation, which leads to improved mobility and quality of life [5].

Pulmonary rehabilitation and lung transplantation

Pulmonary rehabilitation plays an important role in lung transplantation program in terms of individual programs made for each patient before and after the transplant. [6] Before transplantation, pulmonary rehabilitation can help patients in optimizing and maintaining their functional status and can provide the interdisciplinary information about upcoming surgery, postoperative treatment, supervision after surgery and potential complications. In these patients reduced ability to exercise is an important predictor of outcome and survival after surgery, and therefore the role of exercise as a part of pulmonary rehabilitation is very important [7]. Intensi-

ty of exercise and training regimen depends on the underlying disease for which the patient was referred to lung transplantation. Some patients have a very poor effort tolerance and bad constellation of respiratory gases and require low-intensity exercise or interval training. During the exercise, blood pressure, pulse and oxygen saturation are monitored at all times. The aim is to create a really good partnership and communication between patient and pulmonary rehabilitation team thus detecting potential problems and enabling the adjustment of each patient to exercise and other parts of pulmonary rehabilitation program.

The educational component of pulmonary rehabilitation involves teaching patients about risks and benefits of operation, issues relating to care in the postoperative period (controlled cough, techniques of expectoration, wound care ...). It is also very important explain the risks and benefits of immunosuppressive therapy, and plan the necessary visits after transplantation for the purpose of monitoring and control testing.

Some authors studied the effect of interval training in relation to continuous training in candidates for lung transplantation with COPD. Interval training was associated with a lower degree of dyspnea during exercise and less unwanted breaks, but achieved similar improvements in exercise capacity compared with continuous training [7].

Low effort intolerance and functional disability often persists after the lung transplantation, despite the renewal of lung function parameters (normal or near normal) and better constellation of respiratory gases. Skeletal muscle dysfunction plays an important role in such functional disability [9-11].

Muscle weakness that is present preoperatively may worsen during the first weeks after transplantation [11] and may be present up to 3 years post-transplant 12-15 and the maximum capacity of exercise can be reduced to 40-60% of the estimated up to 2 years after transplantation [16-17]. Immunosuppressive drugs may worsen muscle function [17].

Rehabilitation begins in the first 24-48 hours after surgery and is focused on the optimal expansion of the lungs and secretion clearance of respiratory tract as well as the effective breathing, and starts the activation of upper and lower extremities (range of motion, strength and basic transmission for walking). The necessary precautions have been taken to intensive aerobic exercise, especially involving the upper limbs, because it takes 4-6 weeks for the healing of postoperative incision. As the muscular strength and endurance gradually improves, patients may ultimately bear a greater intensity than they were able to achieve preoperatively, because now have normal function of the lungs are no longer limited ventilation [1]. Many studies have shown the importance of exercise in patients before transplantation treatment.

Patients who had implemented exercise before transplantation have significantly better prognosis. [18] It is also recognized that the implementation of pulmonary rehabilitation directly affects the postoperative period in terms of better exercise capacity, muscle function and bone density [1].

Psychosocial aspects of pulmonary rehabilitation

Lung transplantation leads to improved quantity of life but the recipient's quality of life often depends on many pre- and posttransplant factors. Pretransplant rehabilitation includes preparing patients for a lung transplant in psychological sense as well. It is not just a conversation or a psychological evaluation but a complex person-centered process, including fine techniques of guided individual and family counseling, psychoeducation, support and if necessary – psychotherapy. Lung transplant candidates appear to have lower quality of life than candidates for other types of transplantations [20,21] – probably because of higher illness burden. Anxiety and panic disorders are common in patients with lung disease, perhaps because of the shared mechanisms underlying dyspnea, hyperventilation, and symptoms and triggers of anxiety [22].

Beginning with the initiation of transplant evaluation, candidates experience a range of psychosocial challenges that require professional support in pretransplant period, perioperative recovery and long-term adjustment to life with a new organ. These psychosocial factors can either be the cause or the consequence of persons functional capacity, lost social roles and relationships, health behaviors, psychological status and overall adjustment [22].

Specific stressors during evaluation period for lung transplantation include uncertainty about eligibility for lung transplantation, fear of surgery in general, accepting potential changes in life plans, financial problems and conflicting feelings about treatment options [23,24].

In this phase, patients are often reluctant to reveal personal fears and/or psychological problems due to the fear that such information will jeopardize their chances of being accepted as a lung transplant candidate. It is crucial that the professionals working with patients understand this phenomenon and interpret it correctly. Patient's level of adjustment to the need for lung transplantation depends on the disease course, severity and health rate decline.

Interventions are made through pretransplant education, addressing ambiguities, conflicting emotions and moderating expectations. Optimism is desirable in this phase but can easily become unrealistic and cause difficulties in life adjustment after transplant. Expectations moderation during the whole transplantation period is

immensely important because it protects candidates and recipients from emotional „highs“ and „lows“ and focuses them on importance of pursuing necessary health behaviors.

After being listed as a candidate patients and their family experience the most stressful period of the transplantation experience. Balancing the notion that they may not survive the waiting period with the desire to make plans for the posttransplant life, causes anxiety to be present almost constantly [22]. Anxiety and tension are also the result of constant worry about when the phonecall will happen while at the same time experiencing monotony and frustration because of their function limitations caused by the progressed disease. This has an accumulating negative effect on quality of life. Interventions aimed at improving psychosocial outcomes among lung transplant candidates have showed favorable results. Interventions should be weekly, 8 to 12 sessions using cognitive-behavioral strategies to improve coping skills, quality of life, mood symptoms and relationships with family members or caregivers [22]. Stress reducing techniques such as relaxation trainings and/or mindfulness based treatments are desirable.

Interventions are also made at enabling interactions with lung recipients that can give precise advise and support. These interactions are moderated when possible and recipients are educated on how to speak with a candidate.

During the perioperative recovery some recipients feel too optimistic and enthusiastic because of the rapid change in lung function so they can even feel „immune“ to any problems that might arise in that period. Others interpret the expected perioperative difficulties as a sign of overall decline in health and tend to be demoralised. Neither of these two reactions are desirable so during rehabilitation we are determined to predict and prepare candidates for these attitudes and educate the caregivers on how to approach them. Interventions are focused on education about the risk of posttransplant complications and the importance of self-monitoring [22].

Adjustment to lung transplant is complex and often needs support even after the first year has passed and majority of recipients has restored their quality of life to levels comparable to the ones before they became ill. Because majority of recipients have less functional impairments after transplantation, health related quality of life improves but new stressors arise. They have to adapt to ongoing medical issues and reliance on caregivers. Recipients have to find previously „lost“ family and social roles. They have to learn how to be „healthy“ again but responsible and alert. Keeping this balance is a challenge for the patient, family and the whole rehabilitation team.

Treating psychological problems, maintaining adherence to medical regimen, counseling, providing written materials, organizing support groups to help main-

tain good interpersonal connections with other recipients are all crucial parts of psychological support during pre- and posttransplantation rehabilitation.

Conclusion

Further research is needed to understand the degree of benefits of pulmonary rehabilitation and to identify the effects of such structured rehabilitation on transplant patients compared to natural healing process. We see that not all transplant patients achieve the expected gains in muscle strength and exercise capacity after rehabilitation [1]. Reason for this is unclear and requires further researches with the renewal of pulmonary rehabilitation programs.

In the future, we see the need to increase the applicability and availability of pulmonary rehabilitation. This would lead to changes in daily behaviour of our patients, as well as optimize and maintain long-term positive effects of rehabilitation. The aim is to improve pulmonary rehabilitation and to adapt that to needs of each patient individually thus offering better health care and improve their life quality.

References

- [1] Spruit MA, Singh SJ, at all. An Official American Thoracic Society/European Respiratory Society Statement: Key Concepts and Advances in Pulmonary Rehabilitation. *Am J Respir Crit Care Med* Vol 188, Iss. 8, pp e13–e64, Oct 15, 2013.
- [2] Singh SJ, ZuWallack RL, at all. Learn from the past and create the future: the 2013 ATS/ERS statement on pulmonary rehabilitation. *ERJ*. 2013;42(5):1169-74.
- [3] Nici L, Donner C, Wouters E, Zuwallack R, Ambrosino N, Bourbeau J, Carone M, Celli B, Engelen M, Fahy B, et al.; ATS/ERS Pulmonary Rehabilitation Writing Committee. American Thoracic Society/European Respiratory Society Statement on Pulmonary Rehabilitation. *Am J Respir Crit Care Med*. 2006;173:1390-413.
- [4] Spruit MA. Pulmonary rehabilitation. *Eur Respir Rev*. 2014;23(131):55-63.
- [5] Breyer MK, Breyer-Kohansal R, Funk GC, et al. Nordic walking improves daily physical activities in COPD: a randomised controlled trial. *Respir Res*. 2010;11:112.
- [6] Rochester CL. Pulmonary rehabilitation for patients who undergo lung volume-reduction surgery or lung transplantation. *Respir Care*. 2008;53:1196-202.
- [7] Gloeckl R, Halle M, Kenn K. Interval versus continuous training in lung transplant candidates: a randomized trial. *J Heart Lung Transplant*. 2012;31:934-41.
- [8] Jastrzebski D, Ochman M, Ziora D, Labus L, Kowalski K, Wyrwol J, Lutognewska W, Maksymiak M, Ksiazek B, Magner A, et al. Pulmonary rehabilitation in patients referred for lung transplantation. *Adv Exp Med Biol*. 2013;755:19-25.

- [9] Evans AB, Al-Himyary AJ, Hrovat MI, Pappagianopoulos P, Wain JC, Ginns LC, Systrom DM. Abnormal skeletal muscle oxidative capacity after lung transplantation ³¹P-MRS. *Am J Respir Crit Care Med*. 1997;155:615-21.
- [10] Wang XN, Williams TJ, McKenna MJ, Li JL, Fraser SF, Side EA, Snell GI, Walters EH, Carey MF. Skeletal muscle oxidative capacity, fibertype, and metabolites after lung transplantation. *Am J Respir Crit Care Med*. 1999;160:57-63.
- [11] Maury G, Langer D, Verleden G, Dupont L, Gosselink R, Decramer M, Troosters T. Skeletal muscle force and functional exercise tolerance before and after lung transplantation: a cohort study. *Am J Transplant*. 2008;8:1275-81.
- [12] Pantoja JG, Andrade FH, Stoki DS, Frost AE, Eschenbacher WL, Reid MB. Respiratory and limb muscle function in lung allograft recipients. *Am J Respir Crit Care Med*. 1999;160:1205-11.
- [13] Van DerWoude BT, Kropmans TJ, Douma KW, Van Der Bij W, Ouwens JP, Koeter GH, Van DerSchans CP. Peripheral muscle force and exercise capacity in lung transplant candidates. *Int J Rehabil Res*. 2002;25:351-5. Pinet C, Scillia P, Cassart M, Lamotte M, Knoop C, Mélot C, Estenne M. Preferential reduction of quadriceps over respiratory muscle strength and bulk after lung transplantation for cystic fibrosis. *Thorax*. 2004;59:783-9.
- [15] Reinsma GD, ten Hacken NH, Grevink RG, van der Bij W, Koeter GH, van Weert E. Limiting factors of exercise performance 1 year after lung transplantation. *J Heart Lung Transplant*. 2006;25:1310-16.
- [16] Williams TJ, Patterson GA, McClean PA, Zamel N, Maurer JR. Maximal exercise testing in single and double lung transplant recipients. *Am Rev Respir Dis* 1992;145:101-105.
- [17] Mathur S, Reid WD, Levy RD. Exercise limitation in recipients of lung transplants. *Phys Ther*. 2004;84:1178-87.
- [18] Wickerson L, Mathur S, Brooks D. Exercise training after lung transplantation: a systematic review. *J Heart Lung Transplant*. 2010;29:497-503.
- [19] Basara Toromanovic L, Jalušić Glunčić T. Psihološka potpora bolesnicima s plućnim bolestima. *Medix*. 2014:214-219.
- [20] Ortega T, Deulofeu R, Salamero P, et al. Health-related quality of life before and after a solid organ transplantation (kidney, liver, and lung) of four Catalonia hospitals. *Transplant Proc*. 2009;41:2265-7.
- [21] Myaskovsky L, Dew MA, Switzer GE, et al. Quality of life and coping strategies among lung transplant candidates and their family caregivers. *Soc Sci Med*. 2005;60:2321-32.
- [22] Rosenberger, EM; Dew, MA; DiMartini AF; DeVito Dabbs, AJ; Yusen, RD. Psychosocial issues facing lung transplant candidates, recipients and family caregivers. *Thorac Surg Clin*. 2012; 22(4):517-29.

- [23] Dew, MA.; DiMartini, AF.; Kormos, RL. Stress of organ transplantation. In: Fink, G., editor. Encyclopedia of stress. 2nd ed. Oxford, UK: Academic Press (Elsevier), 2007;35-44.
- [24] Barbour KA, Blumenthal JA, Palmer SM. Psychosocial issues in the assessment and management of patients undergoing lung transplantation. Chest. 2006;129:1367-74.

Sažetak

Uloga plućne rehabilitacije prije i nakon transplantacije

Plućna rehabilitacija ključna je komponenta u liječenju osoba s kroničnim bolestima dišnog sustava, a u programu transplantacije pluća igra važnu ulogu u smislu individualnog programa za svakog bolesnika prije i nakon transplantacije. Kod ovih je pacijenata nemogućnost vježbanja snažan predskazatelj ishoda operacije i preživljenja, stoga je baš ovo obilježje plućne rehabilitacije od iznimne važnosti. Intenzitet i režim vježbanja određen je karakteristikama bolesti od koje je pacijent bolovao prije transplantacije. Pretransplantacijska plućna rehabilitacija bolesnicima pomaže u optimiziranju i održavanju funkcionalnog statusa prije operacije uz pružanje interdisciplinarnih informacija važnih za predstojeću operaciju i oporavak. Također uključuje psihosocijalnu potporu za pacijenta i članove obitelji radi umanjivanja stresa i povećanja prilagodbe u periodu čekanja transplantacije. Posttransplantacijska rehabilitacija započinje u prvih 24-48 sati nakon operacije i usmjerena je na optimalno širenje pluća i toaletu dišnih putova, učinkovito disanje, aktivaciju ekstremiteta i hod. Edukacijska komponenta posttransplantacijske rehabilitacije uključuje poučavanje o željenom zdravstvenom ponašanju, rizicima, adekvatnom režimu uzimanja lijekova i zdravoj prehrani. Psihosocijalna potpora odnosi se na pomoć u ponovnom preuzimanju izgubljenih socijalnih i obiteljskih životnih uloga, savjetovanje, prilagodbu na novi organ i daljnje medicinske potrebe, dugoročno moderiranje očekivanja te brigu o psihičkoj dobrobiti pacijenta.

Ključne riječi: plućna rehabilitacija; transplantacija pluća; psihosocijalna potpora.

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CYSTIC FIBROSIS

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Summary

Cystic fibrosis is the most common autosomal recessive genetic disease that limits lifespan in white populations. Hundreds of mutations have been discovered, with a huge range of clinical expressions. Identification of subjects with cystic fibrosis and carriers of the mutation enables genetic counseling and disease prevention. Early diagnosis, nowadays often through neonatal screening, allows early multidisciplinary approach with a positive impact on longevity and an increasing number of adult patients. As more patients live longer, the epidemiological indicators change and the number of complications on different organs increase.

Standardization of care in cystic fibrosis centers with continuous multidisciplinary and longitudinal follow-up from pediatric age to adulthood contributes largely to better control of the disease and improving the quality of life.

Keywords: cystic fibrosis; pathogenesis; disease presentation; diagnosis, treatment.

Cystic fibrosis (CF) is a hereditary disorder with autosomal recessive inheritance affecting many organs, primarily the respiratory system, the digestive tract and the reproductive system [1]. The first disease description dates seventy years ago, when patients died mostly in infancy due to severe malabsorption and diarrhea [2]. Life-expectancy of patients born nowadays is 50 years of longer [3,4]. Patients' outcome is determined by the course of lung disease that is the main cause of a shortened life-span.

The disease incidence is approximately 1 in every 3,200 live births in northern and western Europe and USA, 1 in 15,000 African-American births and 1 in 32,000 Asian-American births [5]. Cystic fibrosis is caused by mutations in a single gene colloquially called the CF gene that encodes the *cystic fibrosis transmembrane conductance regulator* (CFTR) protein [6]. Abnormal chloride conductance in apical membra-

nes of epithelial cells lining the airways, pancreatic ducts, sweat ducts, intestines, biliary tree, and vas deferens results in impaired ion composition of secretions that becomes dehydrated and viscous; therefore the alternative name for the disease – *mucoviscidosis*. The consequence of liquid depletion in secretions is mucus obstruction leading to fibrotic and cystic deformations and finally tissue destruction [4]. It is worth mentioning that CFTR has other regulatory functions besides chloride transport. By now around 2000 mutations of the CFTR-gene have been described, but the functional importance of many is not known. The mutation F508del is found in approximately 70% of CF patients in northwestern Europe and USA.

Within the last decades the life span of CF patients has been notably prolonged, thanks to the development of new therapeutical approaches and interventions with impact on the disease course. Some important interventions are: disease detection through neonatal screening [7], aggressive nutritional support [8], and new treatments for lung inflammation, infection and mucocilliary clearance [9,10,11]. Further progress came with exciting new treatments aiming to treat causes rather than just symptoms, i.e. mutation-specific therapies allowing read-through of premature stop codons, CFTR potentiators and CFTR correctors [12,13]. Nowadays lung transplantation is a treatment option for end-stage lung disease [14]. Specialized CF centers with a multidisciplinary team have a crucial, central role in developing and implementing new therapies based on understanding the underlying pathophysiology of the disease [15].

Despite of well known diagnostic algorithms for classic and non-classic CF [16], CF is not always a straightforward diagnosis. There is a small subset of patients, often adults, who have atypical clinical manifestations [17,18]. Classic CF presentation includes pancreatic insufficiency, malnutrition, chronic lung disease and in postpubertal males obstructive azoospermia. The diagnosis is usually suspected within the first years of life and easily confirmed. On the contrary, patients with atypical presentations are often pancreatic sufficient and have mild clinical symptoms. The diagnosis is delayed to adolescence or adulthood, sometimes with the burden of equivocal results of diagnostic tests.

Special challenge present patients that might have CF, but they do not meet classic diagnostic criteria for CF, i.e. those having intermediary or normal concentrations of chloride in the sweat, those carrying only one mutation or those with a monosymptomatic phenotype. These patients are referred to as having a CFTR-related disease. It is important to differentiate them from CF patients, for psychological and practical reasons, because they have much better prospects [19,20].

The clinical syndrome we recognize as CF has considerable variability, and at least some of this variability seems to be associated with specific gene mutations.

However, individuals with the same genotype can differ considerably, i.e. regarding the severity of lung disease. This can be explained by the influence of other intrinsic factors like gene modifiers and extrinsic factors such as life-style, environmental influences, treatment choices etc. [21]. Therefore one should be extremely careful when predicting the disease severity and patient's prognosis merely on the genotype.

With the prolonged life expectancy of CF patients, especially those born in this millennium, there are societies with a bigger proportion of adult patients than children. In Canada the median age of survival has increased from 37.3 years in 2002 to 47.7 years in 2012. [4]. The proportion of adult patients in Croatia is constantly increasing in the past two decades as well; they are 1/5 of all registered patients [22].

Patophysiology

Most studies regarding how CFTR dysfunction leads to the phenotypic disease were performed on epithelial cells of CF airway samples. Bulk of evidence suggests that the main features are diminished chloride efflux to the lumen and excess sodium and water reabsorption, leading to dehydration of airway surface materials. Airway surface liquid depletion is thought to impair mucociliary clearance. Thus, the viscous mucus accumulates obstructing the airways and phlegm harbors pathogenic bacteria. The clinical consequence is persistent cough and bronchitis, often accompanied with broncho-obstruction – the earliest clinical signs of impaired lung function in CF.

With time, mucus on the epithelium forms plaques with anaerobic niches that can bind opportunistic bacterial pathogens. Characteristically, *Pseudomonas aeruginosa* over time adjusts to this media, synthesizes an alginate coat and forms biofilms that are very difficult to clear with standard antibiotic treatment. The consequence of this is a vicious circle of phlegm retention, infection and chronic neutrophil inflammation that perpetuates itself [5,9,23].

Many patients develop a pronounced inflammatory reaction already in early age, prior to infections. This is believed to be a direct effect of CFTR dysfunction leading to dysregulated host inflammatory response. In any case, a strong and constant neutrophilic airway inflammation enhanced with chronic infection and other inflammatory stimuli is a milestone of CF airway disease [5,9].

Impaired electrolyte conductance contributes to an impaired bioelectric potential difference (PD) across the epithelial membrane. The PD can be measured *in vivo* in the nasal epithelium and *in vitro* in rectal biopsies. Assessing basal PD and changes in response to various stimuli is used as a diagnostic tool in doubtful cases and in research laboratories to test the effectiveness of new drugs for CF [5,9].

The pathophysiology of changes within the pancreas are similar to those in the airways: dysfunctional CFTR gene leads to lack of chloride secretion in pancreatic ducts and as a consequence reduced chloride/bicarbonate exchange, resulting in a smaller volume of pancreatic juices that is rich in proteins and poor in bicarbonates. These alterations are responsible for obstruction and autodigestion of the pancreas that starts early in embryonic life, causing exocrine pancreatic insufficiency at birth or within the first months or years of life [24].

Impaired ion conductance exists in bile ducts and in *vas deferens* as well; viscous secretions obstruct the canals and lead to reactive inflammation and finally fibrotic degeneration [25].

In contrast to epithelial cells, the functioning CFTR protein in sweat glands enables reabsorption of salt and water from the sweat. Therefore the sweat concentration of chloride and sodium is increased in CF and this is the main laboratory test to diagnose the disease. Only a small proportion of patients (1- 2%) with some rare mutations have normal sweat electrolyte concentrations [26].

Clinical features

Lung disease is present in virtually all CF patients and is the determining factor of survival. Some patients experience early respiratory symptoms in infancy with nonproductive, exhausting cough. Symptoms mimic bronchiolitis with obstructive dyspnea and wheezing. However, the lung disease may evolve gradually, in months and even years, presenting as chronic broncho-obstruction, bronchitis, recurrent pneumonias and hyperinflation of the lung parenchyma. As the disease progresses, peribronchal fibrosis, atelectasis and bronchiectasis develop. Attributes of chronic lung disease are chronic respiratory insufficiency and *cor pulmonale* [8,23].

In addition to chronic lung disease patients experience pulmonary exacerbations, sometimes pneumothorax in the advanced disease. In adolescents and adults (rarely in children) one should consider *allergic bronchopulmonary aspergillosis* in refractory exacerbations.

Initially, the pathogens causing lung infection are common microorganisms for the age of the child (viruses, *Haemophilus* and *Pneumococcus* strains). Later, usual isolates are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia*. *Pseudomonas* infections are difficult to eradicate due to its' capacity to transform and form biofilms [26].

Chronic pancreatic insufficiency is present in 85% of the CF population. Lack of exocrine pancreatic secretion leads to maldigestion and secondary malabsorption, steatorrhea and increased intestinal nitrogen loss [24]. Malnutrition, specifically

long-lasting energy deficiency, has a negative impact on the respiratory system, enhancing the number of lung infections and degree of inflammation that favor structural changes in the airways [24,26].

Endocrine function of Langerhans islets is usually maintained until adolescence and adulthood. Finally about one third of patients develop *cystic fibrosis related diabetes* (CFRD), a unique form of diabetes that has characteristics of type I and type II diabetes [27].

Neonates with CF can present with transient *cholestatic jaundice* caused by obstruction of intrahepatic bile ducts. Older patients are at risk for *focal biliary cirrhosis*, but clinically apparent liver disease occurs in only a small portion of patients and usually presents by 15 years of age. Liver failure is the second leading cause of death in CF [26].

Neonates can also present with *meconium ileus*. Older children and adults can develop *distal obstructive syndrome* (DIOS), a syndrome of partial or complete postneonatal distal small bowel obstruction caused by stool plugs, also known as meconium ileus equivalent [5,9].

As a consequence of the chronic inflammation, hypomotility, recurrent infections and frequent antibiotic use CF patients can develop *small bowel bacterial overgrowth syndrome*, one of the factors contributing to malabsorption and malnutrition [28].

Excessive loss of sodium and chloride through sweating sometimes causes *hyponatremic dehydration* and *hypochloremic metabolic alkalosis*. This metabolic imbalance can be the presenting symptom in early infancy [29].

Growth and thriving largely depends on adequate substitution of pancreatic enzymes and hypercaloric nutrition. Often, puberty is delayed for one or two years due to impaired growth and maturation [29].

Most male patients (98%) are azoospermic and infertile due to vas deferens obstruction, with normal other reproductive functions. Women are fertile, although they may experience difficulties in getting pregnant due to dehydrated cervical secretions [30].

Diagnosis

CF is primarily a clinical diagnosis. It should be suspected in any child who demonstrates typical clinical features of CF or who was born to a family with a known history of CF or has a positive newborn screening. Suspicion is followed by laboratory testing to prove CFTR dysfunction. This includes either measuring sweat chloride concentration, or genetic testing, or measurement of the nasal PD [31]. The sweat test is the long-standing standard and mostly used to confirm CF. Mutation

identification is not obligatory for the diagnosis in patients with clinical symptoms and elevated sweat chlorides. However, knowing the mutations is relevant for family genetic counseling and important because some mutations can be corrected with the new therapies.

Neonatal screening has been implemented in many countries. This allows disease detection in the pre-symptomatic phase. Early implementation of various therapeutic procedures should provide better disease control; ensure optimal nutritional status and altogether better prognosis [7].

Treatment options

CF treatment consists of life-lasting measures to control symptoms and disease progression, as there is still no causative therapy [9]. However, alternative drug treatment that affects intercellular function, like trafficking, expression or function of CFTR has emerged. One of these mutation specific treatments is registered for use in 2012.y: a CFTR potentiator *ivacafor* (Kalydeco) for patients with G551D mutation. In progress and with promising results are studies with a corrector compound (VX809, *lumakaftor*) and a drug that induces read-through of premature stop codons in class I mutations (PTC124-*ataluren*). Some more substances targeting different ways of correcting the CFTR function or biochemical aberrations of CF are under investigation [32].

While waiting for therapies that treat the basic defect, the milestone of CF therapy remains *symptomatic treatment*. It is mostly focused on respiratory and gastrointestinal symptoms with nutritional support.

Respiratory treatment is focused on managing infection, inflammation and improving airway clearance, and, for end stage lung disease, lung transplantation (9).

Infections. Aggressive antibiotic therapy was one of the main contributors to longer survival of CF patients. Patients move from phases of rather stable lung disease with occasional exacerbations to the state of chronic infection. In each phase antibiotic treatment plays an important role. Antibiotics can be given intravenously, orally and in inhalations. Nowadays inhaled antibiotics (tobramycin, colistin, aztreonam) are the treatment choice for both early *Pseudeomonas* eradication and control of chronic colonization [26,33,34].

Inflammation. Neutrophil-dominated inflammation in the airways is a hallmark of CF. Although the mechanism leading to pronounced inflammation is not well understood, inflammation contributes to lung damage and disease severity. *Oral corticosteroids* yield improvement in lung function and reduce pulmonary exacerbations, but their use is highly limited due to unacceptable adverse event profile.

Unfortunately, *inhaled corticosteroids* have no proven effect on the course of CF lung disease and are not recommended unless marked bronchial hyper-reactivity is present.

High dose *oral ibuprofen* was shown to be effective in slowing the decline in FEV₁, but it is not an accepted treatment in all centers [5,35].

Macrolide antibiotics have received attention in CF because of their remarkable effect in patients with diffuse panbronchiolitis. Azithromycin is nowadays recognized and advised as an immunomodulatory agent for CF, but the exact mechanism of action remains unclear.

Mucolytic therapy. Airway surface liquid depletion and viscid airway secretions are also a hallmark of CF. Mucolytics such as *N-acetylcysteine* have little effect on lung disease in these patients. On the contrary, inhaled *donorase-alfa* (recombinant human DNase) effectively reduces sputum viscosity and number of exacerbations, and improves lung function in patients with mild to modest lung disease [36].

Short-term *hypertonic saline solution* improves lung function in CF patients, but long-term effects have to be yet proven. Inhaled *powdered mannitol* (Bronchitol, Ardiol) is an alternative and acceptable mucolytic [36].

Lung transplantation. This is the final therapeutic option for end-stage lung disease. In the past decades the procedure itself and post-transplant care have improved substantially [37]. The best timing and selection of candidates for transplantation are still a matter of dispute. It is important to recognize that lung transplantation is not a cure for CF patients, but represents a transfer to a new health-state in which all other manifestations of CF persist and may worsen.

Airway clearance techniques. Chest physical treatment is an inevitable part of CF care. There are many techniques: simple percussion, different methods aiming to provoke coughing, high frequency chest wall oscillatory drainage, autogenic drainage etc. The choice depends on technical availability, competence of the staff, age and preferences of the patient.

Active **immunization** is an important issue. Patients should follow the national program. No consensus has been reached about extra immunization so far, but there are suggestions that vaccination against pneumococcus, chickenpox, the flu and hepatitis A are beneficial [38].

Gastrointestinal system and nutrition. With longer survival of CF patients the numbers of gastrointestinal complications increase. Therefore collaboration with other specialists like gastroenterologists, endocrinologists, dietitians etc. is an imperative.

Nutritional therapy is based on three main components: pancreatic enzyme replacement, vitamin supplementation and quantitatively and qualitatively balanced diet ensuring at least 20% more calories than the RDA values in order to meet raised

energy demands caused by the hypermetabolic state [39]. Patients with CF and poor nutritional status are more prone to lung infections and earlier decline in FEV₁, so gastroenterological therapy must be included in everyday care.

Conclusion

When CF is suspected in the presence of typical clinical features it must be confirmed by laboratory testing. The awareness of doctors to recognize the symptoms, especially pediatricians who see patients at an early age is crucial for detecting the disease, therefore competence should be worked on.

Neonatal screening enables detecting the disease in asymptomatic individuals. However, it raises some controversies and new challenges regarding treatment and prevention of complications. Main determinants of CF outcome is early detection and good control of pulmonary disease, that can be achieved through multidisciplinary treatment including a pulmonologists, gastroenterologists, dietitians and other specialties. With better care and longer lifespan of many patients we are witnessing a dramatic increase in the proportion of adult patients, in some countries surpassing the pediatric population. This brings new challenges, especially for doctors in adult medicine who face some new and less known CF presentations emerging during the life course.

It is now well established that best care for CF patients is provided in specialized CF centers that ensure multidisciplinary and longitudinal follow-ups of patients from infancy to adulthood, taking in consideration special needs and difficulties in translation from pediatric to adult CF units. Based on European experiences and published recommendations for standards of care, it is imperative to reorganize medical care for CF patients in Croatia, where already one fifth patients are adults [22].

References

- [1] Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005; 352:1992-2001.
- [2] Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease: a clinical and pathological study. *Am J Dis Child* 1938; 56:344-399.
- [3] Dodge JA, Lewis PA, Stanton M, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947-2003. *Eur Respir J* 2007;29: 522-526.
- [4] Canadian Cystic Fibrosis Patient Data Registry Report 2007. Canadian Cystic Fibrosis Foundation: 2007 Toronto, Canada.
- [5] O'Sullivan B, Freedman SD. Cystic fibrosis. *Lancet* 2009;373:1891-904.

- [6] Harris A. Cystic fibrosis gene. *Br Med Bull* 1992;48:738-53. (Harris A, Argent AE. The cystic fibrosis gene and its product CFTR. *Semin Cell Biol* 1993;4:37-44.
- [7] Farrell PM, Kosorok MR, Rock MJ, Laxova A, Zeng L, Lai HC, et al. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *Pediatrics* 2001;107:1-13.
- [8] Kalnins D, Wilschanski M. Maintenance of nutritional status in patients with cystic fibrosis: new and emerging therapies. *Drug Design, Development and Therapy* 2012;6:151-161.
- [9] Ratjen FA. Cystic fibrosis: pathogenesis and future treatment strategies. *Respir Care* 2009;54:595-605.
- [10] Anderson P. Emerging therapies in cystic fibrosis. *Ther Adv Respir Dis* 2010;4:177-85.
- [11] Ashlock MA, Beall RJ, Hamblett NM, Konstan MW, Penland CM, Ramsey BW, et al. A pipeline of therapies for cystic fibrosis. *Semin Respir Crit Care Med* 2009;30:611-626.
- [12] Barrett PM, Alagely A, Topol EJ. Cystic fibrosis in an era of genomically guided therapy *Human Molecular Genetics*, 2012;2:66-71.
- [13] van Goor F, Yu H, Burton B, Hoffman BJ. Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J Cyst Fibros* 2014;13:29-36.
- [14] Adler FR, Aurora P, Barker DH, Barr ML, et al. Lung transplantation for cystic fibrosis. *Proc Am Thorac Soc* 2009;6:619-33.
- [15] Mahadeva R, Webb K, Westerbeek RC, Carroll NR, Dodd ME, Bilton D, et al. Clinical outcome in relation to care in centres specialising in cystic fibrosis: cross sectional study. *BMJ* 1998;316:1771-1775.
- [16] Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: A consensus statement. *J Pediatr* 1998;132:589-95.
- [17] Castellani C, Cuppens H, Macek JrM, Cassiman JJ, Kerem E, Durie P, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros* 2008;7:79-196.
- [18] Zielenski J. Genotype and phenotype in cystic fibrosis. *Respiration* 2000; 117-33.
- [19] De Boeck K, Wilschanski M, Castellani C, Taylo, C, Cuppens H, Dodge J, et al. Cystic fibrosis: terminology and diagnostic algorithms. *Thorax* 2006;61:627-63.
- [20] Goubau C, Wilschanski M, Skalicka V, Lebecque P, Southern KW, Sermet I, et al. Phenotypic characterisation of patients with intermediate sweat chloride values: towards validation of the European diagnostic algorithm for cystic fibrosis. *Thorax* 2009;64:683-691.

- [21] Slieker MG, Sanders EA, Rijkers GT at al. Disease modifying genes in cystic fibrosis. *J Cyst Fibros* 2005;4:7-13.
- [22] T-Drinković D, Tješić-D D, Kelečić J, Votava-Raić A. Data from the Croatian CF Registry. *J Cyst Fibros* 2008;7: S115.
- [23] Koch C, Hoby N. Pathogenesis of cystic fibrosis. *Lancet* 1993;341:1065-69
- [24] Whitcomb DC. Genetic aspects of pancreatitis. *Annu Rev Med* 2010; 61:413-2
- [25] Moyer K, Balistreri W. Hepatobiliary disease in patients with cystic fibrosis. *Curr Opin Gastroenterol* 2009;25:272-8.
- [26] Gibson RL, Burns JL, Ramsey BW. State of the Art: Pathophysiology and Management of Pulmonary Infections in Cystic Fibrosis. *Am J Respir Crit Care Med* 2003;168:918-51.
- [27] Moran A, Brunzell C, Cohen RC, Katz M, et al.: the CFRD Guidelines Committee. Clinical Care Guidelines for Cystic Fibrosis-Related Diabetes. *Diabetes Care* 2010;33: 2697-2708.
- [28] Bures J, Cyrany J, Kohoutova D, Forstel M, et al. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol* 2010 28;16:2978-990.
- [29] Kovács L, Hlavatá K, Jankó V. Pseudo-Bartterov syndróm u dojčafa s falošne negativným výsledkom skríningu na cystickú fibrózu. *Pediatr prax* 2012;13:266-267.
- [30] Ahmad A, Ahmed A, Patrizio P. Cystic fibrosis and fertility. *Curr Opin Obstet Gynecol.* 2013;22:59-63.
- [31] Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: A consensus statement. *J Pediatr* 1998;132:589-95.
- [32] Sears EH, Gertman EJ, Casserly BP. Treatment options for cystic fibrosis: state of the art and future perspectives. *Rev Recent Clin Trials* 2011;6:94-107.
- [33] Moss RB. Administration of aerosolized antibiotics in cystic fibrosis patients. *Chest* 2001;120:107-113.
- [34] Giugno H, Castanos C, Lubatti A, Pinheiro JL, Hernandez C, Gonzalez Pena H. Early antibiotic treatment for eradication od initial infection by *Pseudomonas aeruginosa* in patient with cystic fibrosis. *Arch Argent Pediatr* 2010;108:141-7.
- [35] Nichols DP, Konstan MW, Chmiel JF. Anti-inflammatory therapies for cystic fibrosis-related lung disease. *Clin Rev Allergy Immunol* 2008;35:135-53.
- [36] Wallis C. Mucolytic therapy in cystic fibrosis. *J R Soc Med* 2001;94:17-24.
- [37] Kotsimbos T, Williams TJ, Anderson GP. Update on lung transplantation: programmes, patients and prospects. *Eur Respir Rev* 2012;21:271-305.
- [38] Tješić-Drinković Do, Tješić-Drinković Du, Votava R i sur. Procijepljenost djece s cističnom fibrozom. *Pediatr Croat* 2004;48:157-63.
- [39] Sinaasappel M, Stern M, Littlewood J. Nutrition in patients with CF: a European consensus. *J Cystic Fibros* 2002; 2:51-75.

Sažetak

Cistična fibroza

Cistična fibroza je najčešća nasljedna, autosomalno recesivna bolest koja utječe na dužinu života u bijeloj rasi. Danas je prepoznato više stotina mutacija uz veliku varijaciju ekspresije bolesti. Prepoznavanje bolesnika i nosilaca mutacije pruža mogućnost genskog savjetovanja i prevencije bolesti. Rano dijagnosticiranje, sve češće pomoću neonatolog probira, omogućuje rani multidisciplinarni terapijski pristup uz značajno produljenje životnog vijeka i sve većeg broja odraslih bolesnika. Dužinom životnog vijeka mijenjaju se epidemiološki pokazatelji bolesti te se uočava sve više komplikacija bolesti na drugim organskim sistemima.

Osnivanje specijaliziranih centara za liječenje cistične fibroze s multidisciplinarnom longitudinalnom skrbi za bolesnike od pedijatrijske do odrasle dobi predstavlja daljnji doprinos kontroli bolesti i kvalitete života bolesnika.

Ključne riječi: cistična fibroza; patogeneza; klinička slika; dijagnostika; liječenje.

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Symposium

TUMORS OF THE GASTROINTESTINAL
TRACT: COLORECTAL CANCER

Guest Editors:

Antonio Juretić

PREFACE

Colorectal cancer (CRC) appears at the molecular level and in terms of treatment effectiveness and survival, respectively, to be a heterogeneous disease. A majority of CRCs are sporadic and believed to be influenced by diet, lifestyle, environmental factors, and acquired somatic mutations. To emphasize this complexity regarding the CRC incidence, pathogenesis and complexity in diagnosis and treatments, the 1st scientific meeting titled *Tumors of the Gastrointestinal Tract: Colorectal Cancer* was organized on December 21st, 2014 by the Croatian Academy of Science and Arts, Department of Medical Sciences, and by the School of Medicine of the University of Zagreb, Department of Clinical Oncology. The Organizing Committee comprised the following members of the Department of Clinical Oncology: Prof. Lidija Bekeć-Orešković, MD, PhD, Prof. Nikola Đaković, MD PhD, Prof. Antonio Juretić, MD, PhD, Academy President Prof. Zvonko Kusić, MD, PhD, and Prof. Fedor Šantek, MD, PhD.

The aim of this meeting was to recapitulate the data which indicate that CRC is a heterogeneous group of tumors. The topics presented at this meeting were the following: CRC incidence, importance of optimal nutrition, molecular pathogenesis, novel diagnostic procedures and algorithms, pathohistological recommendations and algorithms, surgical approaches, systemic therapy, radiation therapy, drug pharmacogenomics and patient pharmacogenetics, therapy side-effects, and the importance of a multidisciplinary team in making therapeutic decisions and in taking care of patients.

Regretfully, not all papers and all presentations are included in this issue, but most of them are. We hope that the papers presented will provide the readers with sufficient information for better understanding of colorectal cancer incidence, pathogenesis, diagnosis and treatment options in a clinical setting.

On behalf of the Organizing Committee,
Antonio Juretić

COLORECTAL CANCER: AN INTRODUCTION

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Summary

This introductory type of article provides a brief overview of the current clinical status of sporadic colorectal cancer (CRC). CRC ranks as one of the most prevalent and incident cancers and is also between the deadliest ones worldwide, along with lung and breast cancers. A majority of CRCs are sporadic, with age being the most important risk factor. CRC appears to be a complex, heterogeneous disease that involves multiple signaling pathways and tumors that appear histologically identical may have different prognoses and different responses to treatment. CRC develops through gradual accumulation of genetic and epigenetic changes, resulting in the transformation of normal colonic mucosa into invasive cancer. Basically, the treatment for colorectal cancer varies by tumor location, stage at diagnosis and patient's general condition. Any available data about the genes and/or molecular pathways that regulate activities such as cell growth, death or apoptosis, DNA repair, malignant alteration, etc. should also be taken into consideration. Recent polychemotherapy protocols along with the use of inhibitors of the vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) pathways have enhanced the therapeutic responses and potentially also the patient prognoses. The treatment of CRC is expected to become more and more routinely based on identified molecular CRC subtypes and on validated prognostic and predictive biomarkers relatively soon (within several years), which should provide to patients better therapeutic outcomes with less side-effects.

Keywords: colorectal cancer; biomarkers; chemotherapy; targeted therapy; radiotherapy; personalized medicine.

Colorectal cancer (CRC) ranks as one of the most common cancer types diagnosed worldwide and also as one of leading causes of cancer death [1-3]. Clinical experience, supported by various pathohistological, chromosomal, and molecular biology data and analysis, indicate that CRC is a heterogeneous disease, so patients with clinicopathologically similar tumors and with the same disease stage might differ strikingly in terms of treatment response and survival, respectively [4-9].

The aim of this short overview is to recapitulate the data which indicate that CRC is a heterogeneous group of tumors and to present the topics presented by the lecturers and discussed at this meeting. The topics were the following: CRC incidence, importance of optimal nutrition, molecular pathogenesis, novel diagnostic procedures and algorithms, pathohistological recommendations and algorithms, surgical approaches, systemic therapy, radiation therapy, drug pharmacogenomics and patient pharmacogenetics, therapy side-effects, and the importance of a multi-disciplinary team in making therapeutic decisions and in taking care of patients.

Our understanding of colorectal cancer as a disease is in fact continuously evolving. There are a number of known genes controlling growth factor pathways that may drive the development and progression of colorectal cancer. The risk of occurrence and occurrence of colorectal cancer depends on the genetic characteristics of the individual (heritage and epigenetic changes), their diet, intestinal flora, and lifestyle. Colorectal cancer is hereditary in less than 5% of patients. In most patients with colorectal cancer (approximately 95%), the impact of heritage is not so high, so we refer to it as sporadic colorectal cancer. The lifestyle and nutritional recommendations, particularly for sporadic CRC prevention, include increasing dietary fiber intake, reducing red and processed meat consumption and alcoholic drinks, as well as regular physical exercise [8-11].

Two to three major genetic pathways are implicated in colorectal cancer. Most of sporadic CRC (at least two thirds) derive from malignant altered adenoma. In addition to genetic mutations leading to the activation of several protooncogenes and to the inactivation of several tumor suppressor genes such as APC (adenomatous polyposis colis), TP53 gene and LOH (loss of heterozygosity), these tumors also demonstrate chromosome instability. This is a so-called phenotype or pathway of chromosome instability (CIN) and microsatellite stability (MSS) [8,9].

A subset of about 15% of CRC display a distinct type of genomic instability, referred to as microsatellite instability (MSI) pathway. Instability in two or more of several distinct marker loci (MLH1, MSH2, MSH6, PMS2) is defined as MSI-high (MSI-H) tumor, whereas those with one unstable marker are designated MSI low (MSI-L). Tumors with no positive marker at any of the investigated loci are classified as microsatellite-stable (MSS). The MSI subtype is caused by a defective DNA mismatch repair (MMR) system. The technical determination of the MSI status is based either on PCR analysis or immunohistochemistry (IHC). In about one third of cases, the MSI-H phenotype results from a germline mutation in one of the MMR genes. More commonly, MSI-H is sporadic and due to somatic or epigenetic events, for example due to MLH1 gene promoter methylation [8,9,12].

The epigenetic inactivation of the MLH1 gene in CRC is generally associated with an extensive hypermethylation of many other promoter regions, also known as the CpG island methylation phenotype or pathway (CIMP). The CIMP subtype includes a broader spectrum of tumors than the MSI subtype. Technological advances allowing for genome-wide DNA methylation profiling have revealed recently, that aberrant DNA methylation is not restricted to promoters, but occurs genome-wide, including intragenic and intergenic regions. Promoter methylation is often linked to the silencing of genes, whereas intragenic methylation is positively correlated with gene expression [8,9].

All these subtypes may require different clinical management and treatment strategies. For example, in patients with stage II disease mismatch repair deficiency (dMMR) is a predictive biomarker for lack of significant benefit from adjuvant single-agent fluoropyrimidine chemotherapy in this population [4,6].

Another CRC classification system, based on the cancer genome atlas (TCGA) analysis, considers the rate of somatic mutations that can vary considerably among tumors. About 16% of tumors were found to be hypermutated (defined as >12 mutations per 10⁶ bases) with somatic events in either DNA mismatch repair genes—manifesting also as the MSI-H phenotype — or in a related DNA repair gene (such as POLE) not entirely. TGFBR2 and BRAF were frequent targets of mutation, too, found in about 50% of hypermutated, but less than 5% of non-hypermutated tumors. In contrast, the group of non-hypermutated tumors is very well associated with CIN+ tumors. The most frequently mutated genes among them were APC, TP53, KRAS, PIK3CA, FBXW7, and NRAS, indicating that hypermutated and non-hypermutated tumors progress through different sequences of genetic events and pathways [8,9,13].

Moreover, recent unsupervised approach using gene modules resulted in the identification and/or proposition of five distinct molecularly defined CRC subtypes, which adds a new layer of complexity to CRC heterogeneity and opens new opportunities for understanding the disease [8,14].

All this emerging data confirm that CRC, despite similar histology across cases, is a heterogeneous group of tumors that can be subdivided by their molecular features and treated differently; e.g., microsatellite unstable (MSI) versus microsatellite stable (MSS), RAS mutated versus wildtype, BRAF mutated versus wildtype, and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) mutated versus wildtype [4,8,9,13,14].

A majority of newly diagnosed patients present with stage I – III disease that is confined to the bowel and regional lymph nodes, whereas about a third is diagnosed with metastatic disease. Early stage CRC might be curable with surgery alone or in combination with systemic adjuvant chemotherapy and radiotherapy (for rectal

cancer). Radiotherapy can be applied as neoadjuvant or as adjuvant. Nevertheless, a considerable proportion of these patients experience disease recurrence after surgical resection (and adjuvant therapy) or develop metastatic disease, indicating again that supplemental parameters and biomarkers for risk estimation in clinical practice beyond the TNM classification and pathological risk factors are needed. In developed countries, there is a trend of improvement in CRC mortality, probably due to an earlier diagnosis through screening, more precise diagnostic equipment and algorithms, and better treatment modalities [4-7,15].

The backbone of chemotherapy in CRC is 5-fluorouracil (5-FU), a pyrimidine analog that disrupts DNA and RNA synthesis. When 5-FU is administered intravenously, it is given with leucovorin, a folate analog that stabilizes thymidylate synthetase and enhances the cytotoxicity of 5-FU. Oral capecitabine is a prodrug, that is enzymatically converted to 5-FU in the body and can be used instead of 5-FU. 5-FU is active as a single agent, but its efficacy and patients' survival are improved in a metastatic setting when combined with oxaliplatin, a platinum derivative and alkylating agent, or with irinotecan, a topoisomerase I inhibitor that affects DNA repair. In selected patients, a feasible treatment option may be a combination of all three agents (FOLFOXIRI). There is evidence for improved response rate and survival with the latter chemotherapy protocol, but at the cost of increased toxicity (side-effects), including neurotoxicity and neutropenia. Various studies have investigated whether molecular differences between patients can predict response to standard chemotherapy drugs to facilitate a more personalized approach to chemotherapy. Analyses regarding the genes or their products that may be targeted by such cytostatics or may be involved in the metabolism of these cytostatics or in repairing the damage to (tumor) DNA molecule caused by these agents are not in routine use. In a disease metastatic setting, including the above-mentioned cytotoxic drugs, both the vascular endothelial growth factor (VEGF)-A-targeted antibody bevacizumab and the epidermal growth factor receptor (EGFR)-targeted antibodies cetuximab and panitumumab have clear efficacy when combined with particular cytotoxic chemotherapeutic regimens, the latter group in KRAS and NRAS-wildtype disease only. For bevacizumab, we have no predictive parameter or marker. Another biologic agent that targets the VEGF pathway is aflibercept, a recombinant protein with VEGF receptors 1 and 2 that targets VEGF-A, VEGF-B, and placental growth factor (PlGF). The US Food and Drug Administration (FDA) also approved the novel agent regorafenib for chemotherapy-refractory mCRC in 2013. Regorafenib is an oral tyrosine kinase inhibitor that has multiple targets, including VEGF receptors 1, 2, and 3 [4-7,16-19].

In conclusion, treatment and outcomes for patients with mCRC have been steadily improving with the introduction of newer polychemotherapy protocols along

with the use of inhibitors of the vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) pathways. Molecular heterogeneity of CRC is believed to be one of the main factors responsible for the considerable variability in patients' response to treatment. Whenever possible, treatment should be individualized according to patient's pharmacogenetic characteristics and the finding with respect to the predictive genetic alterations in autologous tumor cells. Such individualized approach (personalized cancer medicine) may result in more rational, higher-quality and more effective treatment with less adverse reactions [4-6, 8, 9, 20].

References

- [1] Globocan 2012. Estimated cancer incidence, mortality and prevalence world-wide in 2012. International Agency for Research on Cancer (IARC). Available from: <http://globocan.iarc.fr>.
- [2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015;65:5-29. doi: 10.3322/caac.21254.
- [3] Registar za rak. Hrvatski zavod za javno zdravstvo. Available from: <http://www.hzjz.hr/rak/novo.htm>.
- [4] NCCN clinical practice guidelines in oncology: Colon cancer, version 2.2015. Available from: <http://www.nccn.org>.
- [5] NCCN clinical practice guidelines in oncology: Rectal cancer, version 2.2015. Available from: <http://www.nccn.org>.
- [6] Van Cutsem E, Cervantes A, Nordlinger B, Arnold D; ESMO Guidelines Working Group. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2014;25 Suppl 3:iii1-9. doi: 10.1093/annonc/mdu260.
- [7] van de Velde CJ, Boelens PG, Borrás JM, Coebergh JW, Cervantes A, Blomqvist L, Beets-Tan RG, van den Broek CB, Brown G, Van Cutsem E, Espin E, Haustermans K, Glimelius B, Iversen LH, van Krieken JH, Marijnen CA, Henning G, Gore-Booth J, Meldolesi E, Mroczkowski P, Nagtegaal I, Naredi P, Ortiz H, Pahlman L, Quirke P, Rödel C, Roth A, Rutten H, Schmoll HJ, Smith JJ, Tanis PJ, Taylor C, Wibe A, Wiggers T, Gambacorta MA, Aristei C, Valentini V. EURECCA colorectal: multidisciplinary management: European consensus conference colon and rectum. *Eur J Cancer*. 2014;50:1.e1-1.e34. doi: 10.1016/j.ejca.2013.06.048.
- [8] Dienstmann R, Salazar R, Tabernero J. The evolution of our molecular understanding of colorectal cancer: what we are doing now, what the future holds, and how tumor profiling is just the beginning. *Am Soc Clin Oncol Educ Book*. 2014;91-9. doi: 10.14694/EdBook_AM.2014.34.91.

- [9] Akkad J, Bochum S, Martens UM. Personalized treatment for colorectal cancer: novel developments and putative therapeutic strategies. *Langenbecks Arch Surg.* 2015;400:129-43. doi: 10.1007/s00423-015-1276-0.
- [10] Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: Pathologic aspects. *J Gastrointest Oncol.* 2012;3:153-73. doi: 10.3978/j.issn.2078-6891.2012.030.
- [11] Stadler ZK, Schrader KA, Vijai J, Robson ME, Offit K. Cancer genomics and inherited risk. *J Clin Oncol.* 2014;32:687-98. doi: 10.1200/JCO.2013.49.7271.
- [12] Kloor M, Staffa L, Ahadova A, von Knebel Doeberitz M. Clinical significance of microsatellite instability in colorectal cancer. *Langenbecks Arch Surg.* 2014;399:23-31. doi: 10.1007/s00423-013-1112-3.
- [13] Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature.* 2012 Jul 18;487(7407):330-7. doi:10.1038/nature11252.
- [14] Budinska E, Popovici V, Tejpar S, D'Ario G, Lapique N, Sikora KO, Di Narzo AF, Yan P, Hodgson JG, Weinrich S, Bosman F, Roth A, Delorenzi M. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. *J Pathol.* 2013;231:63-76. doi: 10.1002/path.4212.
- [15] Binefa G, Rodríguez-Moranta F, Teule A, Medina-Hayas M. Colorectal cancer: from prevention to personalized medicine. *World J Gastroenterol.* 2014;20:6786-808. doi: 10.3748/wjg.v20.i22.6786.
- [16] Ciombor KK, Wu C, Goldberg RM. Recent therapeutic advances in the treatment of colorectal cancer. *Annu Rev Med.* 2015;66:83-95. doi: 10.1146/annurev-med-051513-102539.
- [17] Kirstein MM, Lange A, Prenzler A, Manns MP, Kubicka S, Vogel A. Targeted therapies in metastatic colorectal cancer: a systematic review and assessment of currently available data. *Oncologist.* 2014;19:1156-68. doi: 10.1634/theoncologist.2014-0032.
- [18] Stahel R, Bogaerts J, Ciardiello F, de Ruyscher D, Dubsky P, Ducreux M, Finn S, Laurent-Puig P, Peters S, Piccart M, Smit E, Sotiriou C, Tejpar S, Van Cutsem E, Tabernero J. Optimising translational oncology in clinical practice: Strategies to accelerate progress in drug development. *Cancer Treat Rev.* 2015;41:129-135. doi: 10.1016/j.ctrv.2014.12.004.
- [19] Gustavsson B, Carlsson G, Machover D, Petrelli N, Roth A, Schmoll HJ, Tveit KM, Gibson F. A Review of the evolution of systemic chemotherapy in the management of colorectal cancer. *Clin Colorectal Cancer.* 2015;14:1-10. doi: 10.1016/j.clcc.2014.11.002.
- [20] Kalia M. Personalized oncology: recent advances and future challenges. *Metabolism.* 2013 Jan;62 Suppl 1:S11-4. doi: 10.1016/j.metabol.2012.08.016.

Sažetak

Kolorektalni rak – uvodnik

U prikazanom uvodnom radu dan je kratak pregled kliničkih aktualnosti u sporadičnom kolorektalnom raku (KR). KR je u svijetu, zajedno s rakom pluća i rakom dojke, jedan od najčešćih tipova raka kako po incidenciji tako i u prevalenciji. Nažalost, među zloćudnim tumorima je jedan i od češćih uzroka smrti. Većina KR je sporadična, s dobi kao jednim od najvažnijih faktora rizika. Dijagnoza kolorektalnog raka bazira se na patohistološkim karakteristikama, ali rezultati molekularnih istraživanja ukazuju da se radi o skupini heterogenih tumora, koji se razlikuju u patogenezi, molekularnim aberacijama, prognozi i odgovoru na primijenjeno liječenje. KR se razvija kroz postepenu akumulaciju genskih i epigenetskih promjena, s posljedicom transformacije normalni stanica mukoze u invazivni rak. Strategija liječenja raka kolorektuma se prvenstveno temelji na smještaju tumora, procjeni uznapredovalosti i proširenosti tumora te općem stanju bolesnika. Ipak, sve češće kada je moguće, u obzir se uzimaju geni i/ili molekularni putevi koji reguliraju aktivnosti kao npr. rast i diobu stanica, njihovu smrt ili apoptozu, popravak molekula DNA, i druge slične stanične aktivnosti. Razmjerno noviji polikemoterapijski protokoli kao i uporaba inhibitora vaskularnog endotelijalnog faktora rasta i receptora za epidermalni faktor rasta poboljšali su liječenje, a moguće i prognozu bolesnika. Za očekivati je da će se unutar nekoliko slijedećih godina liječenje bolesnika s kolorektalnim rakom sve više i više temeljiti na nalazu molekularnih subtipova raka i prema validiranim prognostičkim i prediktivnim parametrima, jer bi takav pristup trebao osigurati bolju terapijsku učinkovitost i manje nuspojava.

Ključne riječi: kolorektalni rak; biomarkeri; kemoterapija; ciljana terapija; radioterapija; personalizirana medicina.

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EPIDEMIOLOGY OF COLORECTAL CANCER IN CROATIA AND WORLDWIDE

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Summary

Colorectal cancer (CRC) is the third most common cancer in men worldwide (746,000; 10%) and second most common in women (614,000 cases; 9.2%). Incidence and mortality are on the rise in many developing countries, closing the gap to the western, industrialized populations. In Croatia it is the second malignant cause of death in both males and females, while it ranks second in female incidence and third in male incidence. Trends in CRC in Croatia still display an increase in the standardized rates of incidence and mortality, however, the data for five-year survival for Croatia from recently published international studies indicate an improvement. The epidemiological data clearly point to CRC as one of the most important health issues of Croatian society. Its increasing incidence and mortality trends need to be tackled with a multidisciplinary approach, ensuring the availability of new treatment options and drugs when necessary, but also insisting on the secondary prevention (population-based screening) and primary prevention (changes in nutritional habits and levels of physical activity with educational activities targeted towards wider audience).

Keywords: colorectal cancer; incidence; mortality; prevalence; Croatia.

The International Agency for Research on Cancer (IARC) estimates that over 14 million people throughout the world develop cancer every year, while 8,2 million die of cancer annually; that number will increase to 19,3 million incident cases and 11,4 million deaths by year 2025 [1]. Colorectal cancer (CRC) is an important health burden throughout the world; according to GLOBOCAN 2012 [2] 1.36 million new cases of CRC in the world occur each year, 447,000 of which in Europe. It makes it the third most common cancer in men worldwide (746,000; 10%) and second most

common in women (614,000 cases; 9.2%). The highest incidence rates of CRC are attributable to Australia and New Zealand; the highest mortality rates are observed in Central and Eastern Europe. The incidence of CRC is increasing in many developing countries with rates in the countries of Eastern Europe that recently underwent a major economic transition already reaching or exceeding those of the industrial western countries [3]. Reports from Eastern Asian countries also indicate a rapid rise in CRC incidence, closing the gap to Western populations [4,5].

The most recent data for Croatia (incidence for 2012 (6); mortality for 2013 [7]) indicate that, in males, colorectal cancer is ranked third in incidence (1.700 new cases; crude incidence rate 82.8/100,000) and second in cancer causes of death (1.182 deaths; crude mortality rate 57.6/100,000). In females, colorectal cancer is ranked second in incidence (1.261 new cases; crude incidence rate 57.3/100,000) and second in cancer related mortality (855 deaths; crude mortality rate 38.8/100,000). The distribution of stages for CRC registered in the Croatian Cancer Registry in 2012 indicates that it was diagnosed at the localised stage in 20% of cases, with regional spreading or regional metastases in 38% of the cases, in 25% there were distant metastases at time of diagnosis, while for 17% of the cases the information on stage at diagnosis was missing.

According to the GLOBOCAN 2012 estimates [2], in males in Europe, Croatia is ranked 8th in incidence (age-standardized rate (ASR(W)) 44.2/100,000) and 3rd in mortality (ASR(W) 26.7/100,000); higher mortality rates are observed only in Hungary and Slovakia. For women, Croatia is ranked 16th in incidence (24.7/100,000) and 2nd in mortality (ASR(W) 13.0/100,000), with only Hungary having higher mortality rates. The estimated 5-year prevalence is 293.3/100,000 in males and 204.8/100,000 in females [1]. Also, the cumulative risk (age 0-74) of incident colorectal cancer for Croatia was 5.25% in males and 2.86% for females, as shown in figures 1 and 2. The discrepancy between the incidence and mortality rank of Croatia in Europe strongly indicates the need for improvement of prevention and treatment.

As for epidemiological situation in Croatia, a recent publication [8] described increasing trends in incidence and mortality of CRC in men in the period 1988-2008, with estimated annual percent changes (EAPC) of +2.9% for incidence and +2.1% for mortality. In women, the increase in incidence rates was not statistically significant, while mortality rates showed an increase for the period from 1994-2008, with EAPC of +1.1%. Given the high incidence and increasing mortality, Croatian Society of Coloproctology and Croatian Society of Oncology launched an incentive for quality control and referral improvements for colorectal cancer patients, recognizing the current lack of availability of some treatment modalities at different hospitals [9].

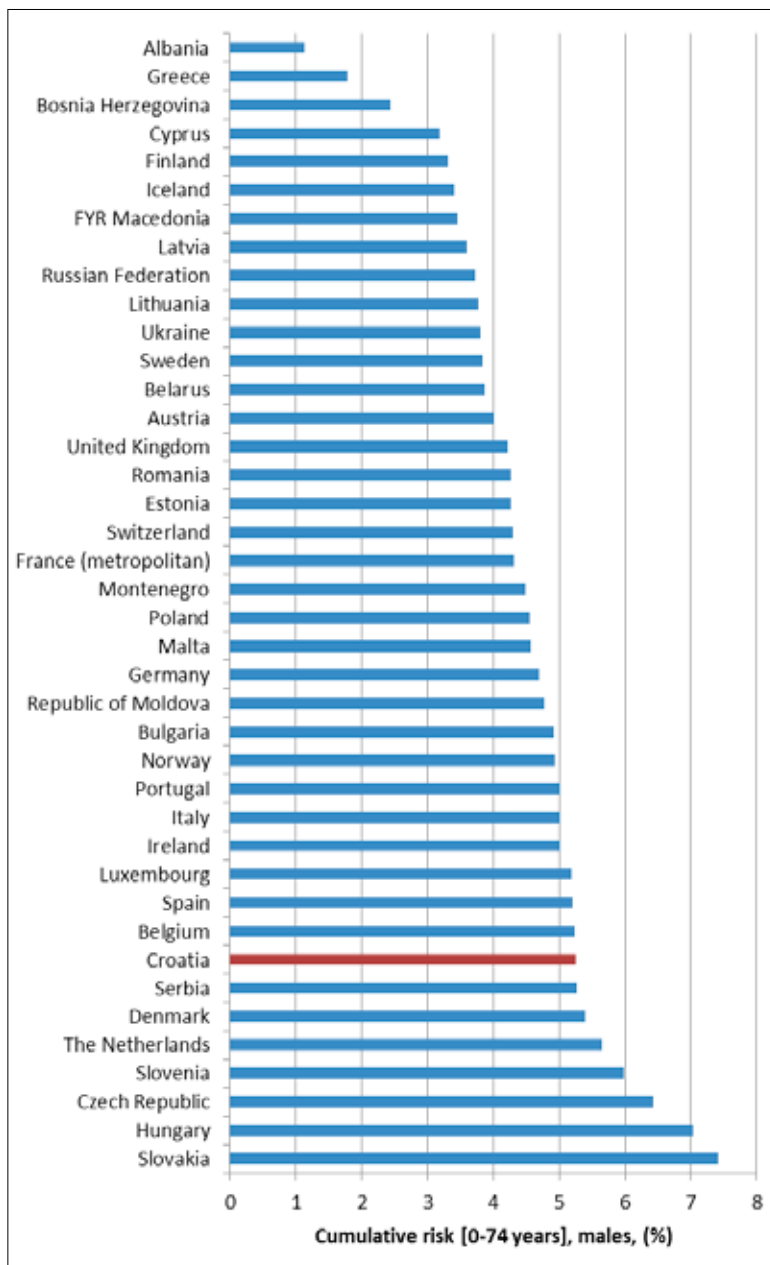


Figure 1. Cumulative risk (0-74 years) of incident colorectal cancer in Europe, males. Source: GLOBOCAN 2012 (2)

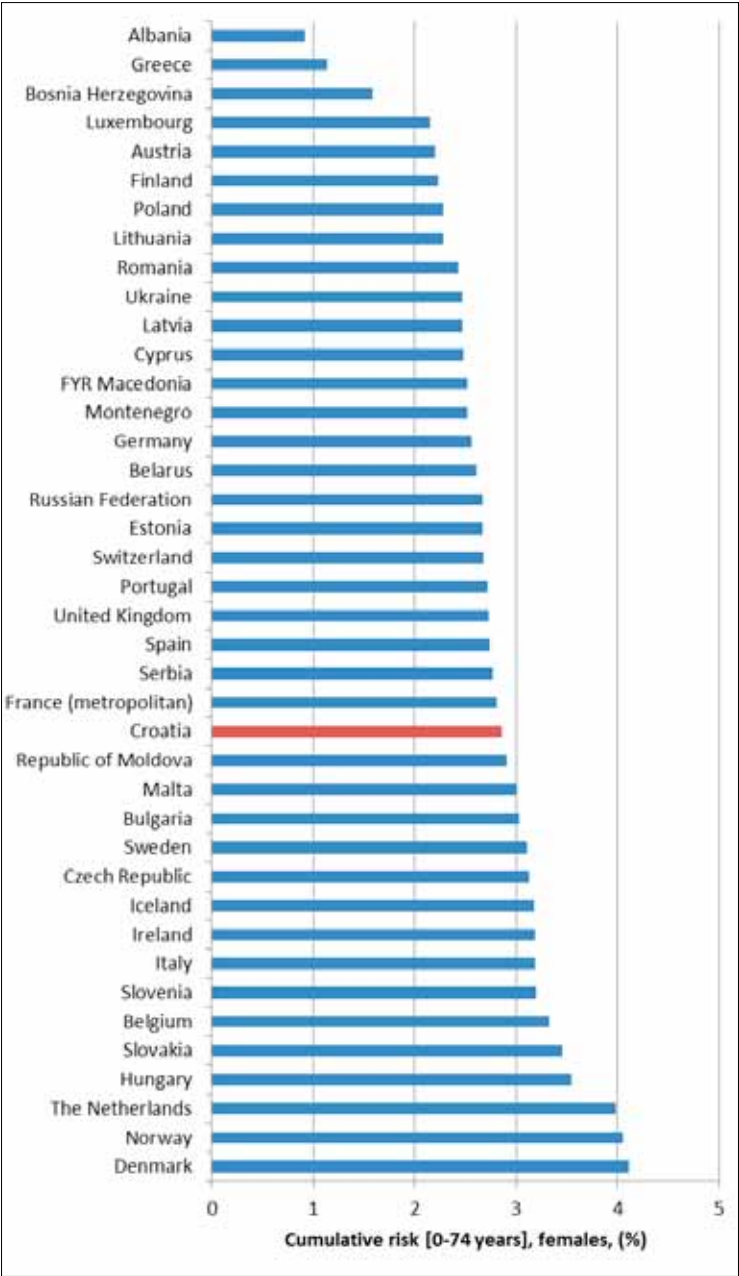


Figure 2. Cumulative risk (0-74 years) of incident colorectal cancer in Europe, females. Source: GLOBOCAN 2012 (2)

The EUROCARE-5 study has provided data on cancer survival in European countries, and for the first time in the EUROCARE history data from Croatia were included. The study indicated that the 5-year relative survival of Croatian cancer patients (diagnosed from 2000-2007 and followed-up until the end of 2008) was 49.6% for colon cancer, and 48.5% for rectal cancer (both sexes combined) [10]. This was lower than survival in western European countries (Austria 61.2%/61.1%), comparable to similar European countries (Slovakia 51.4%/44.7%) and higher than survival in Eastern European countries such as Bulgaria (45.2%/38.4%).

The recently published CONCORD-2 study evaluated cancer survival for over 25 million cancer patients from 279 cancer registries in 67 countries [11]. The data for Croatia (19,846 eligible patients for colon cancer and 12,240 for rectal cancer) indicate that the net five-year survival has improved from 50.1% in the 1998-1999 period to 52.0% in 2005-2009 period, while the improvement in rectal cancer was from 44.6% in the 1998-1999 period to 48.2% in the 2005-2009 period.

Results of colorectal cancer screening in Croatia indicate that, in the first cycle, a total of 1.404.300 individuals were invited to screening. In total, 288.935 (21%) persons returned the test packages with a correctly placed stool specimen on FOBT cards. Over 10,000 colonoscopies were performed and 576 colon cancers were discovered [12].

In conclusion, the epidemiological data indicate that CRC is one of the most important health issues of Croatian society. Its rising incidence and mortality trends need to be tackled with a multidisciplinary approach, ensuring the availability of new treatment options and drugs, but also insisting on the secondary prevention (population-based screening) and primary prevention (changes in nutritional habits and levels of physical activity, as well as appropriate educational activities).

References

- [1] Stewart BW, Wild CP, eds. *World Cancer Report 2014*. Lyon, France: International Agency for Research on Cancer, 2014.
- [2] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1. 0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. Visited: <http://globocan.iarc.fr>. 12.1.2014.
- [3] Center MM, Jemal A, Ward E. International trends in colorectal cancer incidence rates. *Cancer epidemiology, biomarkers & prevention* : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2009;18(6):1688-94.

- [4] Sung JJ, Lau JY, Goh KL, Leung WK, Asia Pacific Working Group on Colorectal C. Increasing incidence of colorectal cancer in Asia: implications for screening. *The Lancet Oncology*. 2005;6(11):871-6.
- [5] Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: environmental factors, molecular pathways, and opportunities for prevention. *World journal of gastroenterology : WJG*. 2014;20(20):6055-72.
- [6] Incidencija raka u Hrvatskoj. Bilten br. 37. Zagreb: Hrvatski zavod za javno zdravstvo. 2014.
- [7] Poljicanin T, Benjak T, eds. Hrvatski zdravstveno-statistički ljetopis za 2006. godinu. Zagreb: Hrvatski zavod za javno zdravstvo. 2007.
- [8] Kirac I, Sekerija M, Simunovic I, Zgaga L, Velimir Vrdoljak D, Kovacevic D, et al. Incidence and mortality trends of gastric and colorectal cancers in Croatia, 1988-2008. *Croat Med J*. 2012;53(2):124-34.
- [9] Vrdoljak E, Plestina S, Dintinjana RD, Tomas I, Sobat H, Separovic R, et al. [Clinical recommendations for diagnosis, treatment and monitoring of patients with colorectal cancer]. *Liječ Vjesn*. 2011;133(11-12):366-9.
- [10] Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EURO CARE-5--a population-based study. *The Lancet Oncology*. 2014;15(1):35-47.
- [11] Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, et al. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet*. 2015;385(9972):977-1010.
- [12] Antoljak N, Sekerija M. Epidemiology and screening of colorectal cancer. *Libri Oncol*. 2013;41(1-3):3-8.

Sažetak

Epidemiologija kolorektalnog raka u Hrvatskoj i svijetu

Kolorektalni karcinom diljem svijeta zauzima treće mjesto među najčešćim zloćudnim bolestima muškaraca (746.000; 10%) i drugo mjesto učestalosti kod žena (614.000; 9,2%). U zemljama u razvoju incidencija i mortalitet su u porastu, što pridonosi smanjenju raskoraka prema zapadnim, industrijaliziranim populacijama. U Hrvatskoj je kolorektalni karcinom drugi uzrok smrtnosti od zloćudnih bolesti i kod muškaraca i žena. Kod žena se po incidenciji svrstava na drugo mjesto, a kod muškaraca na treće. Trendovi kod kolorektalnog karcinoma u Hrvatskoj još uvijek imaju sklonost porasta u standardiziranim stopama incidencije i mortaliteta, međutim podaci o petogodišnjem preživljenju iz recentno objavljenih međunarodnih studija ukazuju na poboljšanja. Epidemiološki podaci jasno ukazuju kako je kolorektalni karcinom jedna od najvažnijih javnozdravstvenih stavki u Hrvatskoj. Njegova rastuća incidencija i mortalitet upozoravaju na važnost uloge multidisciplinarnog pristupa, osiguranje dostupnosti novih modaliteta liječenja kada je potrebno, kao i inzistiranje na sekundarnoj prevenciji (probir na populacijskoj razini) i primarnoj prevenciji (promjene prehrambenih navika i razine tjelesne aktivnosti, uz edukaciju šire javnosti o problemu kolorektalnog karcinoma).

Ključne riječi: kolorektalni rak; incidencija; mortalitet; prevalencija; Hrvatska.

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CLINICAL NUTRITION AND COLORECTAL CANCER

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Summary

Cachexia-anorexia syndrome, characterized by anorexia and loss of adipose tissue and skeletal muscle mass, is common feature in majority of patients with cancer, especially in cancer localized in gastrointestinal tract. Malnutrition is recognized as a negative prognostic factor in patients with colorectal cancer. Malnutrition in cancer patients reduces response to specific on-cological therapy, slows down recovery after surgical treatment, leads to impairment of quality of life and decreases survival. It is considered that development of cancer cachexia is a multifactorial process, however inflammation plays a major role. Therefore, targeting inflammation may represent an effective strategy to prevent/treat cachexia. Treatment should be patient-tailored and based on a multimodal approach.

Keywords: colorectal cancer; cancer anorexia-cachexia syndrome; nutritional support; eicosapentanoic acid; megestrol – acetate.

INTRODUCTION

Colorectal cancer is the third most common cancer, with 1.4 million new cases diagnosed in 2012 [1]. Among patients with colorectal cancer, cancer anorexia-cachexia syndrome has a high prevalence and large impact on morbidity and mortality, as well as on patients quality of life [2]. In addition, it has been showed that undernutrition in patients with colorectal cancer represents economic burden to health care system, with average cost per patient about 1050 EUR [3].

According to recent definition, cachexia is defined as a multifactorial syndrome characterized by severe body weight, muscle and in lesser extent fat loss and increased protein catabolism, due to underlying disease[s]. Recent findings have revealed that biochemical, metabolic and molecular disturbances that lead to cachexia, are present even without significant body weight loss [4,5]. Goals of nutritional inter-

ventions are reduction of the prevalence and severity of cancer cachexia which would allow initiation and completion of active anticancer therapies (chemotherapy and or radiotherapy); and to improve quality of life [6,7].

PATHOGENESIS OF CANCER CACHEXIA IN COLORECTAL CANCER

There are several factors in patients with colorectal cancer that can lead to cancer anorexia-cachexia syndrome. Cancer growth and dissemination, as well as cancer treatments (surgery, chemotherapy, and radiation therapy), interfere with taste, ingestion, swallowing and digestion of food; which may predispose patients to malnutrition. Except reduced food intake, abnormal metabolism is an important factor in the pathophysiology of cancer-cachexia, characterised by a negative protein and energy balance.

It is considered that metabolic changes in cancer cachexia are mediated with pro-inflammatory cytokines [8,9]. Immune and tumor cells produce cytokines, which support the cancer growth and lead to psychobehavioural symptoms (fatigue, depression, cognitive changes), drug resistance and toxicity, pain, anorexia and cachexia, cancer recurrence and progression [10]. Mediators connected with development of cachexia are interleukin-1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor-TNF- α , and interferon-g (IFN-g); which are secreted from patients' mononuclear cells. Tumor cells also produce important factors: lipid mobilizing factor (LMF), that has an important role in stimulation of triglyceride hydrolysis in fat tissue; and proteolysis inducing factor (PIF) that activates NFkB and STAT3 which stimulate synthesis of IL-6 and IL-8, and promote the ATP-ubiquitin proteolytic pathway. This represents the most important factor in muscle mass degradation; it stimulates synthesis of acute phase proteins (especially C-reactive protein) and reduces synthesis of other proteins in liver. As previously mentioned, metabolic disturbances in cachexia cause loss of up to 80% of total body storage of fat tissue and especially muscle tissue.

In cancer cachexia syndrome gluconeogenesis from amino acids, lactate and glycerol, is frequently increased. Cory's cycle is activated, production and recycling of glucose is increased, and impaired glucose tolerance and insulin resistance are present [11,12,13].

There are also some important abnormalities in lipid metabolism. Lipoprotein lipase activity is reduced, lipogenesis decreased; while lipolysis, glycerol and fatty acids turnover, as well as lipid oxidation are increased [14,15].

Protein metabolism alterations in cancer cachexia syndrome are also frequently present and include previously mentioned synthesis of acute phase proteins and muscle wasting [2]. Except ATP-ubiquitin dependent pathway, three proteolytic sy-

stems have been described: the caspases, the lysosome, the Ca^{2+} -dependent pathway. It is considered that all these systems have an important role in muscle depletion [16].

Imbalance between anti-inflammatory cytokines and pro-inflammatory cytokines leads to increased resting energy expenditure, an important factor in cancer cachexia pathogenesis [17].

SCREENING AND ASSESSMENT OF MALNUTRITION

A simple and quick screening method for assessment of metabolic risk, recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN), is NRS-2002 and can be used in all oncological patients [18]. In addition, Fearon et al. have recommended a model for evaluation of nutritional status of oncological patients. This group of authors proposed a definition of cachexia: cachexia is generally defined as being involuntary weight loss of $>5\%$ from historical weight, a body mass index (BMI) $<20\text{ kg/m}^2$ with any degree of weight loss $>2\%$ or a skeletal muscle index consistent with sarcopenia with any degree of weight loss $>2\%$. Furthermore, it was emphasized that cachexia represents a spectrum: patients can progress from pre-cachectic phase to cachexia syndrome and finally to refractory cachexia, the point at which the disease is no longer responsive to treatment or when treatment benefits are outweighed by burden and risk [19]. Nutritional assessment should include clinical and nutritional history, stage of disease (predictor of risk of malnutrition), physical assessment, functional assessment (eg. hand-grip test, spirometry), anthropometric measurements (body weight, body mass index), laboratory tests (C-reactive protein, CBC, serum albumin, creatinine, electrolytes, etc.), body composition and energy expenditure [16].

TREATMENT OF CANCER CACHEXIA

Currently, international guidelines on nutrition support in patients with cancer have been established, such as the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines and the American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines [20-22]. Croatian national guidelines for treatment of patients with cancer cachexia are available since 2007 [23]. Several years ago Muscaritoli and his collaborators have developed a new approach to cancer cachexia, called "parallel pathway" [16]. This pathway represents a multidisciplinary approach to cancer patients through a collaboration between physician, dietician, psychologist and nurse. The goal of "parallel pathway", showed in *figure 1*, is prevention and correction of malnutrition, and prevention or delay the onset of cancer

cachexia. The current approach in treatment of cancer cachexia depends on the stage of cachexia. Nutritional counseling is the first step. Enteral nutrition via tube can be applied in severe cachectic patients with a functional gastrointestinal tract, but who are not capable of oral food intake. However, in most patients, sip feeding is applicable. Parenteral nutrition is an optional therapeutic modality and can be used alone or in combination with enteral nutrition.

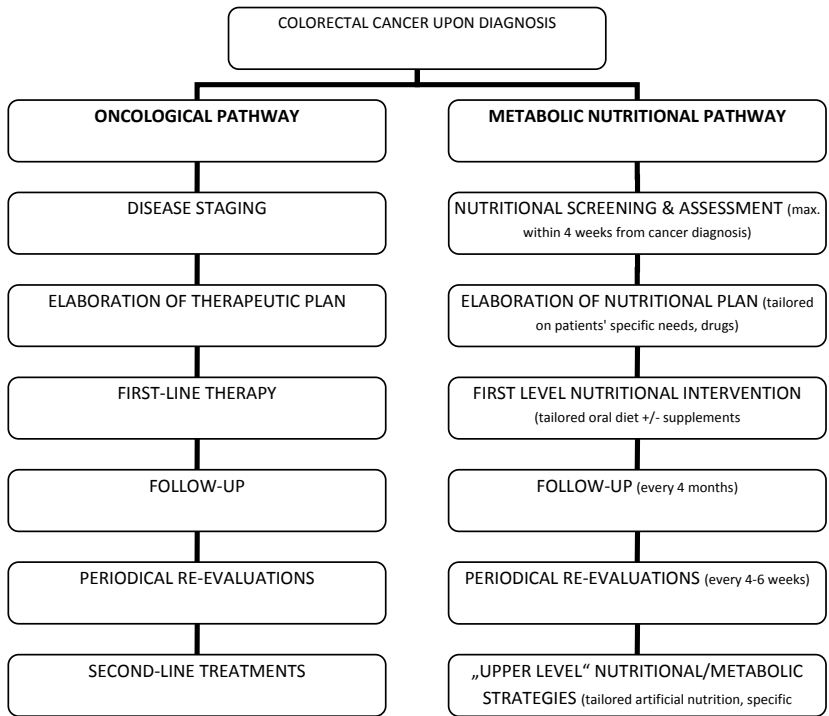


Figure 1. Adapted from *The “parallel pathway”: a novel nutritional and metabolic approach to cancer patients* by Maurizio Muscaritoli, et al. **Left side:** The oncological pathway during cancer patient’s diagnosis and treatment is displayed. **Right side:** The nutritional and metabolic intervention according to the relative oncological step

Nutritional counseling has an important role in cancer patients management. Results of Ravasco et al. prospective, randomized, controlled trial have shown that nutritional counseling in patients with colorectal cancer treated by radiotherapy is the most effective method to improve nutritional status, nutritional intake, and

quality of life in this clinical setting [24]. Recently published randomized controlled trial evaluated the effect of dietary advice dedicated to increase intake in older patients at risk for malnutrition during chemotherapy, versus usual care, on one-year mortality. Results have shown that early dietary counseling was efficient in increasing intake but had no beneficial effect on mortality or secondary outcomes. This lack of effect can be explained by cancer cachexia antianabolism [25]. Evaluation of effectiveness of nutritional support in patients with colorectal cancer receiving chemotherapy demonstrated that simultaneous individualized dietary counseling and nutritional support are effective in improving nutritional status thereby reducing chemotherapy-induced morbidity [26].

The routine use of parenteral nutrition for patients with colorectal cancer is not recommended. However, results from a prospective, randomized clinical trial have shown that early supplementation of parenteral nutrition is capable of improving quality of life, chemotherapy-related toxicity and body composition in patients with advanced colorectal carcinoma undergoing palliative treatment [27].

Patients with colorectal cancer often undergo elective or urgent surgery. Traditionally, postoperative management of these patients involved avoidance of oral intake of fluids or nutrients. However, new studies indicate that early oral feeding after elective colorectal surgery is not only well tolerated by patients but also has positive effects on postoperative outcomes [28]. Furthermore, it has been shown that early enteral nutrition is associated with less anastomotic leakage in patients undergoing extensive rectal surgery [29].

Except nutritional counseling and nutritional support, physical activity plays an important role in management of patients with cancer cachexia. Proposed mechanisms which can lead to improvement are: increase in insulin sensitivity, protein synthesis rate and anti-oxidative enzyme activity; and a suppression of the inflammatory response and an enhancement of immune function [30,31].

AGENTS STUDIED IN CANCER CACHEXIA

There are several nutrients, such as eicosapentaenoic acid (EPA), an omega-3 polyunsaturated acid, and drugs such as appetite stimulants, antioxidants and antiinflammatories, that have been proposed as potential treatment options for cancer cachexia syndrome. Bearing in mind complex nature of cancer cachexia in colorectal cancer, there is no one specific, globally effective or accepted treatment for this condition.

It has been shown that EPA inhibits cancer growth during tumorigenesis and early stages of development through a variety of mechanisms. Proposed mechanisms are apoptosis, cell signaling and gene expression. The anti-tumor properties of

EPA continue with disease progression, improving the efficacy of chemotherapy by protecting non-target tissues and improving its effect on tumor tissue. EPA supplementation may be responsible for stabilization in dose-limiting toxicity [32,33].

Another widely used agent, in treatment of cancer cachexia, is megestrol acetate (MA) that increases appetite and augmentation of body mass. However, during usage of MA, possible adverse effects, such as thromboembolic incidents, uterine bleeding hypertension, hyperglycemia, peripheral edema and adrenal suppression may occur. Progestational drugs, cannabinoids, and cyproheptadine are used in the clinic as appetite stimulans in the therapy of cancer-induced anorexia and cachexia syndrome. These drugs have been shown to be partially effective in reversing or maintaining the symptom of body weight loss in patients with chronic illness [34, 35]. There are several other agents evaluated in studies, such as thalidomide, branched chained amino acids, corticosteroids, anabolic steroids, hydralazine sulfate and ghrelin. Probably combination therapies that target multiple pathways, such as eicosapentaenoic acid administered in combination with exercise, appetite stimulants, antioxidants or anti-inflammatories, have potential in the treatment of this complex syndrome and require further development [35].

CURRENT CROATIAN NATIONAL GUIDELINES

In 2007, Croatian guidelines were developed for the use of eicosapentaenoic acid and megestrol acetate in cancer cachexia syndrome. Based on relevant literature, guidelines suggest simultaneous use of EPA at a standard dosage of 2.2 g/day and MA at a dosage of 400 mg/day for 8 weeks in menagement of patiens with cancer cachexia.

References

- [1] <http://www.wcrf.org/int/data-specific-cancers>
- [2] Muscaritoli M, Anker SD, Argilés J, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr. 2010 Apr;29(2):154-9. doi: 10.1016/j.clnu.2009.12.004. Epub 2010 Jan 8.
- [3] Benković V, Kolčić I, Ivičević Uhernik A, et al. The economic burden of disease-related undernutrition in selected chronic diseases. Clin Nutr. 2014 Aug;33(4):689-93. doi: 10.1016/j.clnu.2013.09.006. Epub 2013 Sep 18.
- [4] Bossola M, Muscaritoli M, Costelli P, et al. Increased muscle proteasome activity correlates with disease severity in gastric cancer patients. Ann Surg. 2003 Mar;237(3):384-9.

- [5] Bossola M, Muscaritoli M, Costelli P, et al. Increased muscle ubiquitin mRNA levels in gastric cancer patients. *Am J Physiol Regul Integr Comp Physiol*. 2001 May;280(5):R1518-23.
- [6] Dobrila-Dintinjana R, Guina T, Krznarić Ž, et al. Effects of Nutritional Support in Patients with Colorectal Cancer during Chemotherapy. *Coll Antropol*. 2008;32: 737-740,
- [7] Markman B, Rodríguez-Freixinos V, Tabernero J. Biomarkers in colorectal cancer. *Clin Transl Oncol*. 2010;12:261-70.
- [8] Ravasco P, Monteiro-Grillo I, Camilo M. How relevant are cytokines in colorectal cancer wasting? *Cancer J*. 2007;13:392-8.
- [9] Shibata M, Nezu T, Kanou H, et al. Decreased production of interleukin-12 and type 2 immune responses are marked in cachectic patients with colorectal and gastric cancer. *J Clin Gastroenterol*. 2002;34:416-20.
- [10] Seruga B, Zhang H, Bernstein LJ, Tannock IF. Cytokines and their relationship to the symptoms and outcome of cancer. *Nat Rev Cancer*. 2008 Nov;8(11):887-99. doi: 10.1038/nrc2507. Epub 2008 Oct 10.
- [11] De Blaauw I, Deutz NE, Von Meyenfeldt MF. Metabolic changes in cancer cachexia--first of two parts. *Clin Nutr*. 1997 Aug;16(4):169-76.
- [12] Rossi Fanelli F, Cangiano C, Muscaritoli M, et al. Tumor-induced changes in host metabolism: a possible marker of neoplastic disease. *Nutrition*. 1995 Sep-Oct;11(5 Suppl):595-600.
- [13] Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells. Isolation, sequence, and activity of the promoter for type II hexokinase. *J Biol Chem*. 1995 Jul 14;270(28):16918-25.
- [14] Costelli P, Tessitore L, Batetta B, et al. Alterations of lipid and cholesterol metabolism in cachectic tumor-bearing rats are prevented by insulin. *J Nutr*. 1999;129:700-706.
- [15] Muscaritoli M, Cangiano C, Cascino A, et al. Plasma clearance of exogenous lipids in patients with malignant disease. *Nutrition*. 1990; 6:147-151.
- [16] Muscaritoli M, Molino A, Gioia G, et al. The "parallel pathway": a novel nutritional and metabolic approach to cancer patients. *Intern Emerg Med*. 2011 Apr;6(2):105-12. doi: 10.1007/s11739-010-0426-1. Epub 2010 Jul 2.
- [17] Argiles JM, Lopez-Soriano FJ. Catabolic proinflammatory cytokines. *Curr Opin Clin Nutr Metab Care*. 1998;1:245-251.
- [18] Kondrup J, Allison M, Elia M, et al. ESPEN guidelines for nutritional screening 2002. *Clin Nutr* 2003; 22: 415-21.
- [19] Fearon KCH, Strasser F, Anker SD, et al. Definition and classification of cancer cachexia: an international consensus. *Lancet Oncol*. 2011;12(5):489-495. doi: 10.1016/S1470-2045(10)70218-7.

- [20] Arends J, Bodoky G, Bozzetti F, et al. ESPEN Guidelines on Enteral Nutrition: Non-surgical oncology. *Clin Nutr.* 2006 Apr;25(2):245-59. Epub 2006 May 12.
- [21] Bozzetti F, Arends J, Lundholm K, et al. ESPEN Guidelines on Parenteral Nutrition: non-surgical oncology. *Clin Nutr.* 2009 Aug;28(4):445-54. doi: 10.1016/j.clnu.2009.04.011. Epub 2009 May 23.
- [22] Huhmann MB, August DA. Review of American Society for Parenteral and Enteral Nutrition (ASPEN) Clinical Guidelines for Nutrition Support in Cancer Patients: nutrition screening and assessment. *Nutr Clin Pract.* 2008 Apr-May;23(2):182-8. doi: 10.1177/0884533608314530.
- [23] Krznaric Z, Juretic A, Samija M, et al. Hrvatske smjernice za primjenu eikoza-pentaenske kiseline i megestrolacetata u sindromu tumorske kaheksije [article in Croatian]. *Lijec Vjesn* 2007; 129: 381-6.
- [24] Ravasco P, Monteiro-Grillo I, Vidal PM, et al. Dietary counseling improves patients outcomes: a prospective, randomized, controlled trial in colorectal cancer patients undergoing radiotherapy. *J Clin Oncol.* 2005;23:1431-1438.
- [25] Bourdel-Marchasson I, Blanc-Bisson C, Doussau A, et al. Nutritional Advice in Older Patients at Risk of Malnutrition during Treatment for Chemotherapy: A Two-Year Randomized Controlled Trial *PLoS One.* 2014; 9(9): e108687. Published online 2014 Sep 29. doi: 10.1371/journal.pone.0108687PMCID: PMC4181649
- [26] Dobrila-Dintinjana R, Trivanovic D, Zelić M. Nutritional support in patients with colorectal cancer during chemotherapy: does it work? *Hepatogastroenterology.* 2013 May;60(123):475-80.
- [27] Hasenberg T, Essenbreis M, Herold A, et al. Early supplementation of parenteral nutrition is capable of improving quality of life, chemotherapy-related toxicity and body composition in patients with advanced colorectal carcinoma undergoing palliative treatment: results from a prospective, randomized clinical trial. *Colorectal Dis.* 2010 Oct;12(10 Online):e190-9. doi: 10.1111/j.1463-1318.2009.02111.x.
- [28] Dag A, Colak T, Turkmenoglu O, et al. A randomized controlled trial evaluating early versus traditional oral feeding after colorectal surgery. *Clinics (Sao Paulo).* 2011 Dec; 66(12): 2001–2005. doi: 10.1590/S1807-59322011001200001
- [29] Boelens PG, Heesakkers FF, Luyer MD. Reduction of postoperative ileus by early enteral nutrition in patients undergoing major rectal surgery: prospective, randomized, controlled trial. *Ann Surg* 2014 Apr;259(4):649-55.
- [30] Radbruch L, Elsner F, Trottenberg P, et al. Clinical practice guidelines on cancer cachexia in advanced cancer patients. Aachen, Department of Palliative Medicine/European Palliative Care Research Collaborative. 2010.
- [31] Ardies CM. Exercise, cachexia, and cancer therapy: a molecular rationale. *Nutr Cancer.* 2001;42(2):143–157. doi: 10.1207/S15327914NC422_1.

- [32] Baracos VE, Mazurak VC, Ma DWL. n-3 Polyunsaturated fatty acids throughout the cancer trajectory: influence on disease incidence, progression, response to therapy and cancer-associated cachexia. *Nutr Res Rev.* 2004;17(2):177-192. doi: 10.1079/NRR200488.
- [33] Murphy RA, Mourtzakis M, Chu QSC, et al. Supplementation with fish oil increases first-line chemotherapy efficacy in patients with advanced nonsmall cell lung cancer. *Cancer.* 2011;117(16):3774-3780. doi: 10.1002/cncr.25933.
- [34] Inui A. Cancer anorexia-cachexia syndrome: current issues in research and management. *Inui ACA Cancer J Clin.* 2002 Mar-Apr; 52(2):72-91.
- [35] Suzuki H, Asakawa A, Amitani H, et al. Cancer cachexia—pathophysiology and management. *J Gastroenterol.* 2013 May; 48(5): 574-594.

Sažetak

Klinička prehrana i kolorektalni karcinom

Kaheksija-anoreksija sindrom je karakteriziran anoreksijom i gubitkom masnog tkiva i skeletne mišićne mase. Sindrom je prisutan u većine onkoloških bolesnika, osobito u bolesnika s tumorima probavnog sustava. Odavno je prepoznato kako malnutricija predstavlja negativni prognostički faktor kod bolesnika s kolorektalnim karcinomom. Malnutricija u onkoloških bolesnika dovodi do slabijeg odgovora na specifičnu onkološku terapiju, sporijeg oporavka nakon kirurških zahvata, do smanjenja kvalitete života, ali i sveukupnog preživljenja. Istraživanja su pokazala kako je tumorska kaheksija multifaktorijalan proces. Međutim, smatra se kako glavnu ulogu u patogenezi tumorske kaheksije ima upala. Stoga je upravo djelovanje na upalni proces potencijalni terapijski cilj u liječenju, odnosno prevenciji kaheksije. Liječenje tumorske kaheksije trebalo bi biti individualizirano i temeljeno na multimodalnom pristupu.

Ključne riječi: kolorektalni karcinom; sindrom tumorske kaheksije-anoreksije; nutritivna potpora; eikosapentanoična kiselina; megestrol-acetat.

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MOLECULAR STRATIFICATION OF COLORECTAL CANCER

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Summary

Colorectal cancer (CRC) is one of the most common cancers and one of the leading causes of cancer death in the Western world. The disease arises from the accumulation of mutations in oncogenes, tumor suppressor genes and mismatch repair genes during progression from normal colon epithelium to adenoma and metastatic carcinoma. Improved understanding of genetic events that underline tumor development and progression may contribute to new strategies for prevention, diagnosis as well as for therapy. The results of Human genome project showed that each person has its unique genetic "signature" as a consequence of a large number of inherited genetic variations. There are also sporadic molecular changes specific for each tumor type. This mini review will give some information about the most frequent molecular genetics changes in colorectal cancer and its molecular stratification due to its heredity, germline pharmacogenetic markers, somatic mutations and microsatellite instability.

Keywords: colorectal cancer; biomarkers; molecular stratification.

Colorectal cancer (CRC) is one of the most common cancers and represents the third most diagnosed cancer in both men and women. Despite advances in surgery, chemotherapy and screening it is still one of the leading causes of cancer related death in the Western world [1].

Of all human cancers, the molecular genetic alterations in colorectal cancer are best understood. The disease arises from the accumulation of mutations in oncogenes, tumor suppressor genes and mismatch repair (MMR) genes during progression from normal colon epithelium to adenoma and metastatic carcinoma (Figure 1) [2].

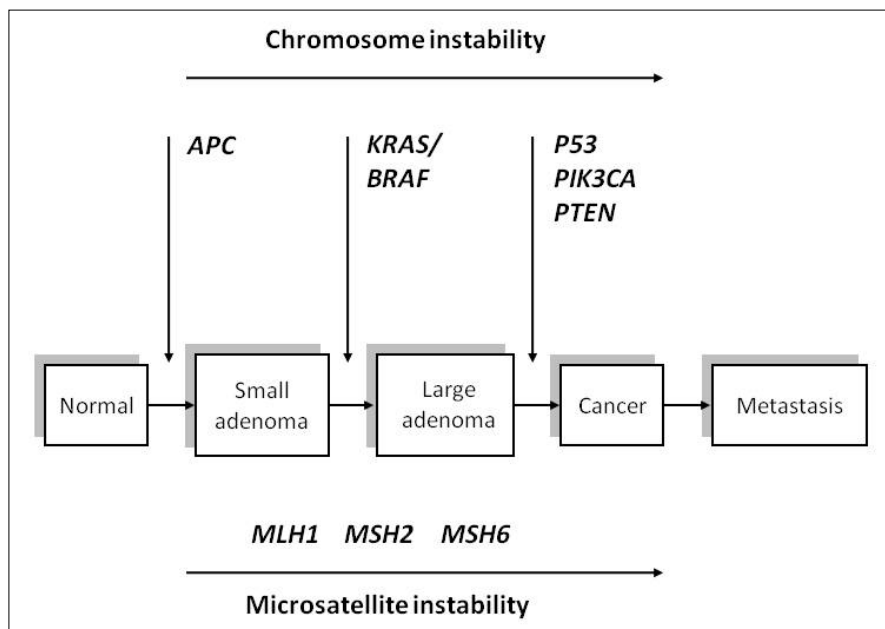


Figure 1. Multistep model of colorectal cancer tumorigenesis.

The majority of colon cancers (80%) arise in sporadic form and only 20% of the patients have a family history of CRC. However, only 5-6% of CRC arise from a germline mutation in high penetrant genes, adenomatous polyposis coli (*APC*) gene (1%) and MMR genes (5%) while the remaining CRCs have a positive family history but cannot be categorized to any hereditary CRC syndrome. These familial CRC are caused by single nucleotide polymorphisms (SNP) in less penetrant genes such as cytokines. Many susceptibility loci have been identified, however, their value in CRC risk prediction remains low [3,4].

The development of colorectal cancer is a multistep process caused by progressive accumulation of genetic and epigenetic changes that cause activation of oncogenes and/or inactivation of tumor suppressor genes. The earliest trigger is the mutation of the *APC* (adenomatous polyposis coli) gene. Mutations in oncogenes (*KRAS*) and other tumor suppressor genes (*p53*, *DPC4*) drive tumor towards malignant transformation and metastasis. Alongside with gene mutations, deregulated expression of oncogenes and/or tumor suppressor genes can also occur as a consequence of epigenetic modifications of their promoters [3,5-7].

Improved understanding of genetic events that underline tumor development, progression and metastasis may contribute to new strategies for prevention, screening, diagnosis as well as for therapy. Survival of patients with metastatic colorectal cancer has improved over the past several decades, due to the development of new combinations of standard chemotherapy such as 5-fluorouracil (5-FU), irinotecan, and oxaliplatin, as well as to the introduction of new targeted therapies. Among the available targeted therapies are monoclonal antibodies that target the epidermal growth factor receptor (EGFR) which have clearly demonstrated efficacy in the treatment of metastatic CRC [8,9].

Individual patient response to therapy could be very diverse, even if their disease seems similar when evaluating clinicopathological parameters. Many drugs commonly used in clinical practice show interindividual variations in efficacy, dose requirements as well as the presence of side effects. There is increasing evidence that treatment response is based on the genetic “signature” of the individuals as well as on the molecular changes in the tumor itself. Pharmacogenetics is the field of personalized medicine that examines the impact of genetic variations on the individual response to drug treatment. These variations are often due to germ line mutations in genes that encode for drug metabolizing enzymes, transporters, cellular targets and signaling pathway. For example, patients with variant DPYD*2A in dihydropyrimidine dehydrogenase (DPD) gene are at high risk to develop severe 5-FU toxicity as well as patients with a variant UGT1A1*28 in uridine diphosphat-glucuronosyl transferase 1A1 (UGT1A1) gene are at a higher risk of developing neutropenia and diarrhea when treated with irinotecan for CRC. Knowledge about the influence of these variations on drug response can be used to identify, through pretreatment genetic screening, the patients who will be responders to a specific drug and those at greater risk to develop an adverse drug reaction [10-13].

An important distinction between pharmacogenetics in oncology and other therapeutics fields is that somatic mutations, frequently acquired in cancer tissues, also contribute to the variations in treatment outcome, sensitivity or resistance to cancer therapy [13,14].

The epidermal growth factor receptor (EGFR) signaling pathway plays an important role in normal cells as well as in tumorigenesis. EGFR also represents an important target for cancer treatment because its activation stimulates key processes involved in tumor growth and progression, including proliferation, angiogenesis, invasion, metastasis, and drug sensitivity (Figure 2) [8,15,16].

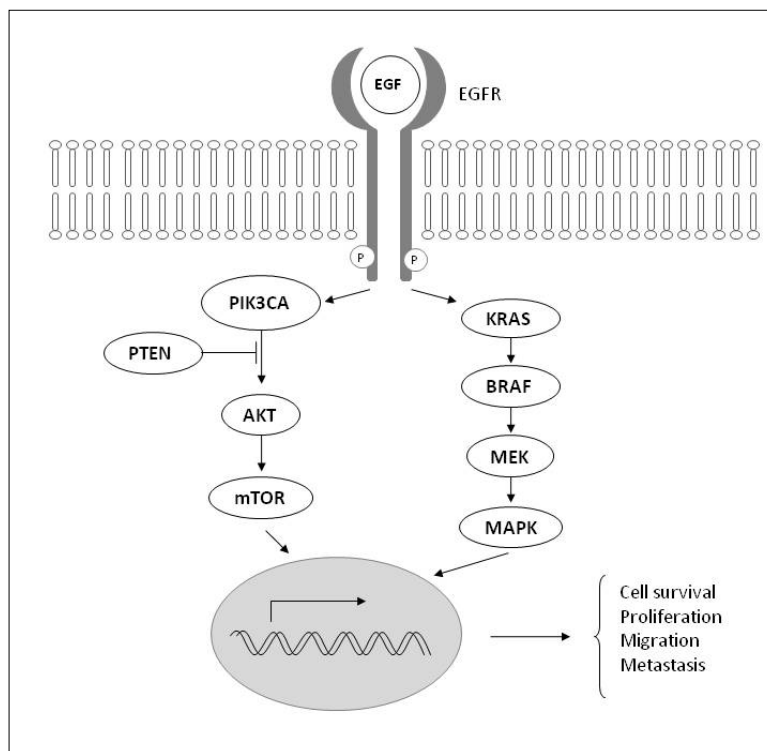


Figure 2. Epidermal growth factor receptor (EGFR) signaling pathway.

Novel therapeutic agents, anti-EGFR monoclonal antibodies, have been shown to be effective therapies for patients with metastatic CRC, but only in a small proportion of patients [15]. Alterations within the EGFR signaling cascade, such as gene mutations in its downstream regulators, have been shown to contribute to colorectal carcinogenesis, but are also predictive biomarkers of anti-EGFR therapy. Genetic analyses showed that the presence of somatic mutations in the KRAS, found in 40-60% of CRC, can predict lack of response and resistance to anti-EGFR antibodies in patients with metastatic CRC [16].

KRAS mutations, most commonly codon 12/13 missense mutations, lead to constitutive activation of the KRAS protein. Because KRAS is the downstream effector of EGFR, activating mutations in the KRAS gene lead to an independent activation of the downstream signal transduction. This unregulated downstream signaling will not be blocked by antibodies that target the EGFR receptor and KRAS mutation status is a strong predictive marker of resistance to EGFR targeted therapy [17]. Un-

fortunately at least 60% of patients with wild type KRAS gene will still not respond to anti-EGFR therapies, suggesting the need for additional biomarkers to help distinguished responders from no responders [17,18]

The BRAF gene encodes a serine-threonine protein kinase that is downstream of KRAS in the MAPK signaling pathway and mutations in these two genes are mutually exclusive. BRAF mutations occur in 5-22% of all colorectal cancers, and in 40-52% of CRC with microsatellite instability [19,20]. Analysis for mutations in both genes could identify as many as 40% of patients who have no chance of responding to this class of drugs. Retrospective studies suggest that concomitant detection of KRAS and BRAF mutations, combined with detection of mutations in two other genes (PIK3CA and PTEN), can identify up to 70% of patients who are unlikely to respond to anti-EGFR therapies [18].

Lynch syndrome (LS) is a dominantly inherited cancer syndrome in which predisposition to colorectal, endometrial, gastric and other cancers occurs due to the mutations in MMR genes. Mismatch repair proteins form a complex that detects and corrects replication errors. A deregulated MMR system due to the mutations in MMR genes leads to accelerated accumulation of somatic mutations, often resulting in carcinogenesis. The most frequently mutated MMR genes are MSH2 and MLH1, in 70-90% of Lynch syndrome, with the remaining 10-30% distributed equally in MSH6 and PMS2 [21].

Microsatellite instability (MSI) is the molecular marker of MMR genes mutations and DNA mismatch repair deficiency. Since its initial description in hereditary colorectal cancer in 1993 and its association with Lynch syndrome, the most common inherited cancer predisposition syndrome, new results suggests that MSI status may also be of prognostic and predictive biomarker in the management of sporadic CRC. To examine the presence of MSI in a CRC tumor the National Cancer Institute (NCI) workshop (1997) recommended to analyze a panel of five microsatellites including two mononucleotide (BAT25, BAT26) and three dinucleotide markers (D2S123, D5S346 and D17S250), (Figure 3), [22,23]. Depending on the number of microsatellite markers displaying additional alleles, MSI can be rated as MSI-high (MSI-H, >2 out of 5 markers), MSI-low (MSI-L, 1 out of 5), or microsatellite stable (MSS, 0 out of 5). MSI-H status could be finding in the most cases of LS colorectal tumors but also in 15% of sporadic CRC [24]. Most of these tumors also exhibit loss of MLH1 protein expression as a consequence of the epigenetic silencing of MLH1 gene promoter. Recent findings demonstrated that inactivation of the MMR system with MSI-H status and MLH1 protein negative tumors in hereditary as well as in sporadic CRC tumors can result in resistance to 5-FU treatment and could be used as valuable pharmacogenetic marker [25].

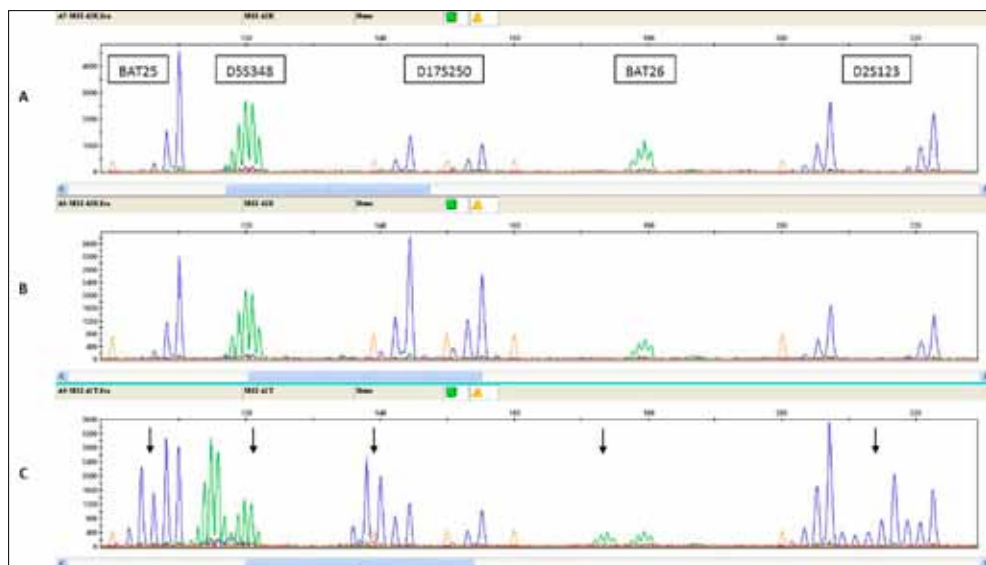


Figure 3. Analysis of microsatellite instability (MSI-high tumor). A. Blood; B. Normal tissue adjacent to tumor; C. Tumor tissue.

Clinicians have long known that each patient and their disease are unique. Recent advances in molecular medicine have enabled a more detailed understanding of the impact of genetics in disease development and progression. Each individual cancer as well as colorectal cancer has a set of molecular alterations that can be defined as the genetic “signature” of that particular tumor. Recent genomic analyses of different human tumors confirmed that individual tumors exhibit heterogeneous patterns of somatic mutations, gene amplifications and deletions, epigenetic profiles, and gene expression profiles. Modern molecular platforms give the ability to characterize tumors at multiple levels and to define biological subtypes of cancer. Cancers could be divided into subtypes that are more or less aggressive. Identification of a more aggressive subtype may influence the type of their treatment. Molecular stratification and sub classification of tumors will add a step to traditional clinical practice and better diagnosis and therapy of different types of cancer as well as colorectal cancer [26,27].

As more biomarkers will be identified and validated predicting testing will be used more extensively in clinical decision making. Optimization of tools to predict the risk for developing cancer, to diagnose a disease at an early stage, to give a prognosis, and predict treatment response is of great importance to the patients as well as to the health professionals.

References

- [1] Kinzler, KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell*. 1996; 87: 159-70.
- [2] Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med*. 2004; 10: 789-99. doi:10.1038/nm1087
- [3] Migliore L, Migheli F, Spisni R, Coppede F. Genetics, cytogenetics and epigenetics of colorectal cancer. *J Biomed Biotech*. 2011; Article ID 792362. doi:10.1155/2011/792362
- [4] Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. *Gastroenterology*. 2010; 138: 2044-58. doi: 10.1053/j.gastro.2010.01.054.
- [5] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990; 61: 759-767.
- [6] Berg M, Soreide K. Genetic and epigenetic traits as biomarkers in colorectal cancer. *Int J Mol Sci*. 2011; 12: 9426-39. doi:10.3390/ijms12129426
- [7] Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. *Gut*. 2011; 60: 116-29. doi: 10.1136/gut.2009.206250.
- [8] Tol J, Punt CJA. Monoclonal antibodies in the treatment of metastatic colorectal cancer: a review. *Clin Therapeut*. 2010; 32: 437-53. doi: 10.1016/j.clinthera.2010.03.012.
- [9] Wang HL, Lopategui J, Amin MB, Patterson SD. KRAS mutation testing in human cancers: the pathologist's role in the era of personalized medicine. *Adv Anat Pathol*. 2010; 17: 23-32. doi: 10.1097/PAP.0b013e3181c6962f.
- [10] Roses AD. Pharmacogenetics and the practice of medicine. *Nature*. 2000; 405: 857-65.
- [11] Yalcin S. The increasing role of pharmacogenetics in the treatment of gastrointestinal cancers. *Gastrointest Cancer Res*. 2009; 3: 197-203. doi:10.1038/35015728
- [12] Yong WP, Innocenti F, Ratain MJ. The role of pharmacogenetics in cancer therapeutics. *Br J Clin Pharmacol*. 2006; 62:35-46. doi: 10.1111/j.1365-2125.2006.02591.x
- [13] Esplin ED, Snyder MP. Genomic era diagnosis and management of hereditary and sporadic colon cancer. *World J Clin Oncol*. 2014; 5:1036-47. doi: 10.5306/wjco.v5.i5.1036
- [14] De Palma M, Hanahan D. The biology of personalized cancer medicine: facing individual complexities underlying hallmark capabilities. *Mol Oncol*. 2012; 6:111-27. doi: 10.1016/j.molonc.2012.01.011
- [15] Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *JNCI*. 2009; 101: 1308-12. doi: 10.1093/jnci/djp280.

- [16] Heinemann V, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. *Cancer Treatment Rev.* 2009; 35: 262-71. doi: 10.1016/j.ctrv.2008.11.005.
- [17] Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au H-J, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med.* 2008; 359: 1757-65. DOI: 10.1056/NEJMoa0804385
- [18] Pappou EP, Ahuja N. The role of oncogenes in gastrointestinal cancer. *Gastrointest Cancer Res: GCR.* 2010; Suppl 1: S2-S15.
- [19] Garnett MJ, Marais R. Guilty as charged: B-RAF is a human oncogene. *Cancer Cell.* 2004; 6: 313-9. doi: 10.1016/j.ccr.2004.09.022
- [20] Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. RAF/RAS oncogenes and mismatch-repair status. *Nature.* 2002; 418: article 934. doi: 10.1038/418934a
- [21] Cohen SA, Leininger A. The genetic basis of Lynch syndrome and its implications for clinical practice and risk management. *Appl Clin Genet.* 2014; 7:147-58. doi: 10.2147/TACG.S51483.
- [22] Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshelman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 1998; 58:5248-57.
- [23] Umar A, Boland CR, Terdiman JP, Syngal S, de Chapelle A, Rushoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004; 96:261-8. doi: 10.1093/jnci/djh034
- [24] Heinimann K. Toward a molecular classification of colorectal cancer: the role of microsatellite instability status. *Frontiers Oncol.* 2013; 3:272. doi:10.3389/fonc.2013.00272
- [25] Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, Balaguer F, Sempere L, Xicola RM, Bujanda L, Reñé JM, Clofent J, Bessa X, Morillas JD, Nicolás-Pérez D, Pons E, Payá A, Alenda C; Gastrointestinal Oncology Group of the Spanish Gastroenterological Association. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. *Eur J Cancer.* 2009; 2:365-73. Doi:10.1016/j.ejca.2008.07.016

- [26] Tan P. Divide and conquer: progress in the molecular stratification of cancer. *Yonsei Med J.* 2009; 50:464-473. doi: 10.3349/ymj.2009.50.4.464
- [27] Ogino S, Fuchs CS, Giovannucci E. How many molecular subtypes. Implications of the unique tumor principle in personalized medicine. *Expert Rev Mol Diagn.* 2012; 12:621-628. doi: 10.1586/erm.12.46.

Sažetak

Molekularna stratifikacija kolorektalnog raka

Kolorektalni karcinom je jedan od najčešćih zloćudnih tumora i jedan od vodećih uzroka smrti od raka u zemljama razvijenog svijeta. Oboljenje nastaje kroz duži niz godina tijekom kojih kao posljedica mutacija u onkogenima, tumor supresorskim genima i genima za popravak DNA iz normalne sluznice kolona preko adenoma nastaje zloćudni invazivni karcinom. Dobro poznavanje molekularne podloge nastanka i napredovanja kolorektalnog karcinoma doprinosi boljoj prevenciji, ranoj dijagnostici i uspješnijoj terapiji ovog zloćudnog tumora. Rezultati istraživanja u području molekularne patologije raka ukazuju na to da svaki tumor posjeduje svoj vlastiti genetski otisak koji je rezultat individualnih, za taj tumor specifičnih, molekularnih promjena ali i genetičkih karakteristika oboljele osobe. U ovom kratkom preglednom radu opisane su najčešće molekularno genetičke promjene u kolorektalnom karcinomu te molekularna stratifikacija ovog zloćudnog tumora s obzirom na nasljednost, farmakogenetske biljege, stečene mutacije i mikrosatelitnu nestabilnost.

Ključne riječi: kolorektalni rak; biomarkeri; molekularna stratifikacija.

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PROTOCOL FOR THE PRIMARY COLON AND RECTUM CARCINOMA SPECIMEN EXAMINATION

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Summary

The College of American Pathologists offered protocol to assist pathologists in providing clinically useful and relevant information when reporting results of colon cancer surgical specimen examination. These recommendations are accepted by Croatian Society of Pathology and forensic medicine, were published and recommended for every-day pathologists work. These protocols are an educational tool to assist pathologists in the useful reporting of relevant information and applies to all primary carcinomas of the colon and rectum. Currently, the seventh edition TNM staging system for carcinoma of the colon and rectum of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) is recommended.

Keywords: colon carcinoma; prognostic factors; predictive factors.

Surgical procedures for diagnosis colon carcinoma are: excisional biopsy (polypectomy), local transanal resection, colectomy (total, partial or segmental) as well as rectal resection. Pathologists should after correct grossing of specimens report several facts which have influence on staging and prognosis of patients with colorectal carcinoma such as: anatomic site, histological type (WHO), histological grade, TNM status, margins, lympho-vascular and perineural invasion, perforation, microsatellite instability, RAS status as well as treatment effect.

Anatomic Sites

The protocol applies to all carcinomas arising in the colon and rectum. The colon is divided in right, transverse and left colon. The right colon is subdivided into the cecum and the ascending colon. The left colon is subdivided into the descending

and sigmoid colon [1,2]. When measuring below with a rigid sigmoidoscope, rectum extends 16 cm from the anal verge. Tumors located at the border between 2 subsites of the colon (eg, cecum and ascending colon) are registered as tumors of the subsite that is more involved. If two subsites are involved to the same extent, the tumor is classified as an "overlapping" lesion. A tumor is classified as rectal if its inferior margin lies less than 16 cm from the anal verge or if any part of the tumor is located at least partly within the supply of the superior rectal artery. A tumor is classified as rectosigmoid when differentiation between rectum and sigmoid is not possible [3].

Histologic Types

For consistency in reporting, the histologic classification proposed by the World Health Organization (WHO) is recommended and follows [4]:

- Adenocarcinoma
- Mucinous (colloid) adenocarcinoma (greater than 50% mucinous)
- Signet-ring cell carcinoma (greater than 50% signet-ring cells)
- Squamous cell carcinoma
- Adenosquamous carcinoma
- Medullary carcinoma
- Small cell carcinoma (high-grade neuroendocrine carcinoma)
- Undifferentiated carcinoma
- Other (specify)

By convention, signet-ring cell carcinomas, small cell carcinomas, and undifferentiated carcinomas are high grade. The only histologic types of colorectal carcinoma that have been shown to have adverse prognostic significance independent of stage are signet-ring cell carcinoma and small cell carcinoma (high-grade neuroendocrine carcinoma), [5,6]. Medullary carcinoma is a distinctive histologic type strongly associated with high levels of microsatellite instability (MSI-H), indicative of defects in normal DNA repair gene function. Medullary carcinoma may occur either sporadically or in association with hereditary nonpolyposis colon cancer (HNP-CC), [7,8]. This tumor type is characterized by solid growth in nested, organoid, or trabecular patterns, with no immunohistochemical evidence of neuroendocrine differentiation. Medullary carcinomas are also characterized by numerous tumor infiltrating lymphocytes. The term "carcinoma, NOS" (not otherwise specified) is not part of the WHO classification.

Histologic Grade

A number of grading systems for colorectal cancer have been suggested, but a single widely accepted and uniformly used standard for grading is lacking. Most systems stratify tumors into 3 or 4 grades as grade 1 (well differentiated, grade 2 (moderately differentiated, grade 3 (poorly differentiated) and grade 4 (undifferentiated). Despite a significant degree of interobserver variability, histologic grade has repeatedly been shown by multivariate analysis to be a stage-independent prognostic factor [9,10]. Specifically, it has been demonstrated that high tumor grade is an adverse prognostic factor. It is known that in most studies documenting the prognostic power of tumor grade, 2-tiered grade stratification were used as follows: *a) low grade*: well differentiated and moderately differentiated (histologically greater than or equal to 50% gland formation), *b) high grade*: poorly differentiated and undifferentiated (less than 50% gland formation). Therefore, in light of its proven prognostic value, relative simplicity, and reproducibility, a 2-tiered grading system for colorectal carcinoma (ie, low grade and high grade) is recommended.

Lympho-Vascular and Perineural Invasion

Venous invasion has been demonstrated by multivariate analysis to be an independent adverse prognostic factor [11]. Invasion of extramural veins, in particular, has been shown to be an independent indicator of unfavorable outcome and increased risk of occurrence of hepatic metastasis. The significance of intramural venous invasion is less clear, because data specific to this issue are lacking. In several studies, both lymphatic invasion and perineural invasion have been shown by multivariate analysis to be independent indicators of poor prognosis and for that reason the presence or absence of tumor invasion of small, thin-walled vessels should be reported in all cases [12].

Perforation

Tumor perforation is an uncommon complication of colorectal cancer, but one that is associated with a poor outcome, including high in-hospital mortality and morbidity. Perforation of the uninvolved colon proximal to an obstructing tumor is also associated with high mortality because of generalized peritonitis and sepsis. Reported perforation rates range from 2.6% to 9%. Perforation is more likely to occur in older patients [13].

Mesorectal margin

The quality of the surgical technique is a key factor in the success of surgical treatment for rectal cancer, both in the prevention of local recurrence and in long-term survival. Numerous studies have demonstrated that total mesorectal excision (TME) improves local recurrence rates and the corresponding survival by as much as 20%. This surgical technique entails precise sharp dissection within the areolar plane outside (lateral to) the visceral mesorectal fascia to remove the rectum. This plane encases the rectum, its mesentery, and all regional nodes and constitutes Waldeyer fascia. High-quality TME surgery reduces local recurrence from 20% to 30%, to 8% to 10% or less, and increases 5-year survival from 48% to 68%. Adjuvant therapy in the presence of a high-quality TME may further reduce local recurrence (from 8% to 2.6%), [14].

Pathologic evaluation of the resection specimen has been shown to be a sensitive means of assessing the quality of rectal surgery. It is superior to indirect measures of surgical quality assessment, such as perioperative mortality, rates of complication, number of local recurrences, and 5-year survival. It has been shown that macroscopic pathologic assessment of the completeness of the mesorectum of the specimen, scored as complete, partially complete, or incomplete, accurately predicts both local recurrence and distant metastasis. Microscopic parameters, such as the status of the circumferential resection margin, the distance between the tumor and nearest circumferential margin (ie. "surgical clearance"), and the distance between the tumor and the closest distal margin, are all important predictors of local recurrence and may be affected by surgical technique. There is strong evidence that the status of the circumferential resection margin is a powerful predictor of local recurrence but is inconsistently evaluated and underreported.

The nonperitonealized surface of the fresh specimen is examined circumferentially, and the completeness of the mesorectum is scored as described as incomplete, nearly complete and complete. The entire specimen is scored according to the worst area. These stages are as follows:

Incomplete

- Little bulk to the mesorectum
- Defects in the mesorectum down to the muscularis propria
- After transverse sectioning, the circumferential margin appears very irregular

Nearly Complete

- Moderate bulk to the mesorectum
- Irregularity of the mesorectal surface with defects greater than 5 mm, but none extending to the muscularis propria
- No areas of visibility of the muscularis propria except at the insertion site of the levator ani muscles

Complete

- Intact bulky mesorectum with a smooth surface
- Only minor irregularities of the mesorectal surface
- No surface defects greater than 5 mm in depth
- No coning toward the distal margin of the specimen
- After transverse sectioning, the circumferential margin appears smooth

Histopathologic Features Suggestive of MSI

Identification of MSI-H colorectal tumors is important, as mismatch repair deficiency may serve as a prognostic marker of patient outcome, a predictive marker of response to chemotherapy, and as a screening tool for HNPCC (Lynch Syndrome). Revised Bethesda guidelines for HNPCC detection recommend testing colorectal tumors for MSI under the following circumstances [15]:

1. Colorectal cancer diagnosed in a patient who is younger than 50 years
2. Presence of synchronous, metachronous, or other HNPCC-associated tumors (endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, small bowel, and brain tumors and sebaceous adenomas and keratoacanthomas), regardless of age
3. Colorectal cancer with MSI-H histology† in a patient who is younger than 60 years
4. Colorectal cancer in 1 or more first-degree relatives with an HNPCC-related tumor, with 1 of the cancers being diagnosed in a person younger than 50 years
5. Colorectal cancer diagnosed in 2 or more first- or second-degree relatives with HNPCC-related tumors, regardless of age

MSI-H histologic features are defined as presence of tumor-infiltrating lymphocytes (only moderate- and high-density intratumoral lymphocytes (approximately 3 or more per high-power field using hematoxylin-eosin-stained sections should be considered significant), Crohn-like lymphocytic reaction (lymphoid aggregated

or follicles are the tumor edge, not associated with preexisting lymph node), mucinous/signet-ring cell differentiation, or medullary growth pattern.[16] Other pathologic features associated with MSI-H status in colorectal carcinomas include right-sided location, high-grade histology, and lack of dirty necrosis [17].

Margins

It may be helpful to mark the margin(s) closest to the tumor with ink following close examination of the serosal surface for puckering and other signs of tumor involvement. Margins marked by ink should be designated in the macroscopic description of the surgical pathology report. The serosal surface (visceral peritoneum) does not constitute a surgical margin.

In addition to addressing the proximal and distal margins, the circumferential (radial) margin must be assessed for any segment either or incompletely encased by peritoneum. The circumferential (radial) margin represents the adventitial soft tissue margin closest to the deepest penetration of tumor and is created surgically by blunt or sharp dissection of the retroperitoneal or subperitoneal aspect, respectively. Multivariate analysis has suggested that tumor involvement of the circumferential (radial) margin is the most critical factor in predicting local recurrence in rectal cancer [18]. The circumferential (radial) margin is considered negative if the tumor is more than 1 mm from the inked nonperitonealized surface but should be recorded as positive if tumor is located 1 mm or less from the nonperitonealized surface because local recurrence rates are similar with clearances of 0 to 1 mm.

The mesenteric resection margin is the only relevant circumferential margin in segments completely encased by peritoneum (eg, transverse colon). Involvement of this margin should be reported even if tumor does not penetrate the serosal surface. Sections to evaluate the proximal and distal resection margins can be obtained either by longitudinal sections perpendicular to the margin or by en face sections parallel to the margin. The distance from the tumor edge to the closest resection margin(s) may also be important, particularly for low anterior resections. For these cases, a distal resection margin of 2 cm is considered adequate; for T1 and T2 tumors, 1 cm may be sufficient distal clearance. Anastomotic recurrences are rare when the distance to the closest transverse margin is 5 cm or greater. In cases of carcinoma arising in a background of inflammatory bowel disease, proximal and distal resection margins should be evaluated for dysplasia and active inflammation [19].

Treatment Effect

Neoadjuvant chemoradiation therapy in rectal cancer is associated with significant tumor response and downstaging [20]. Because eradication of the tumor, as detected by pathologic examination of the resected specimen, is associated with a significantly better prognosis, specimens from patients receiving neoadjuvant chemoradiation should be thoroughly sectioned, with careful examination of the tumor site. Minimal residual disease has been shown to have a better prognosis than gross residual disease. Although several grading systems for tumor response have been advocated, a 3-point tumor regression grade has been shown to provide good inter-observer reproducibility compared with 5-grade schemas, and to provide similar prognostic significance. Tumor regression should be assessed only in the primary tumor; lymph node metastases should not be included in the assessment. Acellular pools of mucin in specimens from patient receiving neoadjuvant therapy are considered to represent completely eradicated tumor and are not used to assign pT stage or counted as positive lymph nodes [21].

Tumor Deposits (Discontinuous Extramural Extension)

Irregular discrete tumor deposits in pericolic or perirectal fat away from the leading edge of the tumor and showing no evidence of residual lymph node tissue, but within the lymphatic drainage of the primary carcinoma, are considered peritumoral deposits or satellite nodules and are not counted as lymph nodes replaced by tumor. Most examples are due to lymphovascular or, more rarely, perineural invasion. Because these tumor deposits are associated with reduced disease-free and overall survival their number should be recorded in the surgical pathology report [22,23]. If tumor deposits are observed in lesions that would otherwise be classified as pT1 (tumor confined to submucosa) or pT2 (tumor confined to muscularis propria), then the primary tumor classification is not changed, but the nodule is recorded in a separate N category as N1c.

TNM and Anatomic Stage/Prognostic Groupings

Surgical resection remains the most effective therapy for colorectal carcinoma, and the best estimation of prognosis is derived from the pathologic findings on the resection specimen. The anatomic extent of disease is by far the most important prognostic factor in colorectal cancer. The protocol recommends the TNM staging system of the AJCC and the UICC but does not preclude the use of other staging systems.

By AJCC/UICC convention, the designation “T” refers to a primary tumor that has not been previously treated. The symbol “p” refers to the pathologic classification of the TNM, as opposed to the clinical classification, and is based on gross and microscopic examination. pT entails a resection of the primary tumor or biopsy adequate to evaluate the highest pT category, pN entails removal or biopsy of nodes adequate to validate lymph node metastasis, and pM implies microscopic examination of distant lesions. Clinical classification (cTNM) is usually carried out by the referring physician before treatment during initial evaluation of the patient or when pathologic classification is not possible [24].

TNM Descriptors

T Category

For colorectal carcinomas, “carcinoma in situ” (pTis) as a staging term includes cancer cells confined within the glandular basement membrane (intraepithelial carcinoma, synonymous with high-grade dysplasia) or invasive into the mucosal lamina propria, up to but not through the muscularis mucosae (intramucosal carcinoma). Tumor extension through the muscularis mucosae into the submucosa is classified as T1. When tumor invades muscular layer is characterised as T2, while infiltrating subserosa as T3. Direct invasion of other organs or structures includes invasion of other segments of colorectum by way of the serosa or mesocolon (eg, invasion of the sigmoid colon by carcinoma of the cecum) is classified as pT4. In such a case, both an adjacent organ and the visceral peritoneum are penetrated by tumor. For rectal tumors, invasion of the external sphincter is classified as T3, whereas invasion of the levator ani muscle(s) is classified as T4. Tumor in veins or lymphatics does not affect the pT classification. pT4 tumors can be divided in pT4a (serosal involvement by tumor cells), and pT4b (direct invasion of adjacent organs). It has been demonstrated by multivariate analysis that pT4a and pT4b tumors have a negative impact on prognosis [25].

N Category

The accuracy and predictive value of stage II assignment are directly proportional to the thoroughness of the surgical technique in removing all regional nodes and the pathologic examination of the resection specimen in identifying and harvesting all regional lymph nodes for microscopic assessment. It has been suggested that 12 lymph nodes be considered the minimal acceptable harvest from a careful specimen dissection. Increasingly, however, evidence indicates that this bar should be raised, as the greater the number of nodes examined, the greater the likeliho-

od that metastasis will be found, suggesting that no minimum number of nodes accurately or reliably stages all patients [26]. Routine assessment of regional lymph nodes is limited to conventional pathologic techniques (gross assessment and histologic examination), and data are currently insufficient to recommend special measures to detect micrometastasis or ITCs. Thus, neither multiple levels of paraffin blocks nor the use of special/ancillary techniques such as immunohistochemistry are recommended for routine examination of regional lymph nodes [24].

Regional lymph nodes. The number of lymph nodes recovered from resection specimen is dependent on several factors. Surgical technique, surgery volume, and patient factors (eg, age and anatomic variation) alter the actual number of nodes in a resection specimen, but the diligence and skill of the pathologist in identifying and harvesting lymph nodes in the resection specimen also are major factors. Lymph nodes may be more difficult to identify in specimens from patients who are obese or elderly, or after neoadjuvant therapy. Because it has been shown that nodal metastasis in colorectal cancer is often found in small lymph nodes (<5 mm in diameter), diligent search for lymph nodes is required on gross examination of resection specimens. If fewer than 12 lymph nodes are found, reexamining the specimen for additional lymph nodes, with or without visual enhancement techniques, should be considered [9]. The pathology report should clearly state the total number of lymph nodes examined and the total number involved by metastases [24].

Nonregional Lymph Nodes. For microscopic examination of lymph nodes in large resection specimens, lymph nodes must be designated as regional versus non-regional, according to the anatomic location of the tumor. Metastasis to nonregional lymph nodes is classified as distant metastasis and designated as M1.

Lymph Nodes Replaced by Tumor. A tumor nodule in the pericolic/perirectal fat without histologic evidence of residual lymph node tissue is classified as a tumor deposit (peritumoral deposit or satellite nodule) and is not considered a positive lymph node. Such tumor deposits may represent discontinuous spread, lymph-vascular spread with extravascular extension, or totally replaced lymph nodes. In the absence of unequivocal lymph node metastases, tumor deposits are recorded as N1c [24].

Micrometastasis and Isolated Tumor Cells. A micrometastasis is defined as tumor measuring greater than 0.2 mm but less than or equal to 2.0 mm in greatest dimension. Micrometastases are classified as N1(mic) or M1(mic) in lymph nodes or at distant sites, respectively. Isolated tumor cells (ITCs) are defined as single tumor cells or small clusters of tumor cells measuring 0.2 mm or less, usually found by special techniques such as immunohistochemical staining, and are classified as N0.

Because the biologic significance of ITCs (either a single focus in a single node, multiple foci within a single node, or micrometastatic involvement of multiple nodes) remains unproven, N0 is considered justified. The number of lymph nodes involved by micrometastases or ITCs should be clearly stated.

TNM Anatomic Stage/Prognostic Groups

Pathologic staging is usually performed after surgical resection of the primary tumor. Pathologic staging depends on pathologic documentation of the anatomic extent of disease, whether or not the primary tumor has been completely removed. If a biopsied tumor is not resected for any reason (eg, when technically unfeasible), and if the highest T and N categories or the M1 category of the tumor can be confirmed microscopically, the criteria for pathologic classification and staging have been satisfied without total removal of the primary cancer [24].

TNM Stage Groupings

Stage 0	Tis	N0	M0*
Stage I	T1	N0	M0
	T2	N0	M0
Stage IIA	T3	N0	M0
Stage IIB	T4a	N0	M0
Stage IIC	T4b	N0	M0
Stage IIIA	T1–T2	N1	M0
	T1	N2a	M0
	T3–T4a	N1	M0
Stage IIIB	T2–T3	N2a	M0
	T1–T2	N2b	M0
	T4a	N2a	M0
	T3–T4a	N2b	M0
Stage IIIC	T4b	N1–N2	M0
	Any T	Any N	M1a
Stage IVB	Any T	Any N	M1b

Molecular Studies

Detection of defects in mismatch repair in colorectal carcinomas is important for detection of Lynch syndrome (a subset of HNPCC accounting for approximately 2% of all colorectal carcinomas), and examination of the tissue for defective DNA mismatch repair is recommended if any of the criteria in the revised Bethesda gui-

delines are met such as primarily on at-risk populations, such as colorectal cancer patients younger than 50 years or patients with a strong family history of HNPCC-associated tumors (eg, colorectal, endometrial, gastric, or upper urinary tract urothelial carcinoma). In addition, emerging data suggest that MSI-H in sporadic colon cancers is associated with better outcome and may serve as a predictor of response to 5-FU-based chemotherapy, although these latter indications for testing are not clearly established and have not been accepted as standard of care [27].

Patients with an MSI-H phenotype may have a germline mutation in one of several DNA mismatch repair (MMR) genes (eg, *MLH1*, *MSH2*, *MSH6*, or *PMS2*) and after appropriate genetic counseling may want to consider having such testing. Best method for testing is for that reason to use at least 5 microsatellite markers, generally mononucleotide or dinucleotide repeat markers (recomendation is to use a 5-marker panel consisting of 3 dinucleotide and 3 mononucleotide repeats for MSI testing. Many laboratories now use a commercially available kit for MSI testing that uses 5 mono-nucleotide markers. The pathologists should help identify areas of the tumor for DNA isolation that have at least this minimum content of tumors cells. MSI testing is frequently done in conjunction with immunohistochemical (IHC) testing for DNA MMR protein expression (ie, *MLH1*, *MSH2*, *MSH6*, *PMS* expression). If the results of DNA MMR IHC and MSI testing are discordant (eg, MSI-H phenotype with normal IHC or abnormal IHC with MSS phenotype), then the laboratory should make sure that the same sample was used for MSI and IHC testing and that there was no sample mix-up. Ideally, the results of DNA MMR IHC and MSI testing should be incorporated into the surgical pathology report for the colorectal cancer case and an interpretation of the clinical significance of these findings provided. If DNA MMR IHC has not been performed, this testing should be recommended for any cases that show an MSI-H phenotype because this information will help identify the gene that is most likely to have a germ-line mutation (eg, a patient whose tumor shows loss of *MSH2* and *MSH6* expression, but retention of *MLH1* and *PMS2* expression, is likely to have an *MSH2* germline mutation).

Analysis for somatic mutations in the V600E hot spot in *BRAF* may be indicated for tumors that show MSI-H, as this mutation has been found in sporadic MSI-H tumors but not in HNPCC-associated cancers. Use of *BRAF* mutational analysis as a step before germline genetic testing in patients with MSI-H tumors may be a cost-effective means of identifying patients with sporadic tumors for whom further testing is not indicated [28].

The presence of the ras gene (*KRAS* and *NRAS*) mutation has been shown to be associated with lack of clinical response to therapies targeted at the epidermal

growth factor receptor (EGFR). While clinical guidelines for RAS mutational analysis are evolving, current provisional recommendations from the American Society for Clinical Oncology are that all patients with stage IV colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for *KRAS* and *NRAS* mutations [24,29].

References

- [1] Edge SB, Byrd DR, Carducci MA, Compton CC, editors. AJCC Cancer Staging Manual. 7. New York, NY: Springer; 2009.
- [2] Greene FL, Compton CC, Fritz AG, Shah J, Winchester DP, editors. AJCC Cancer Staging Atlas. New York, NY: Springer; 2006.
- [3] Fielding LP, Arsenault PA, Chapuis PH, et al. Clinicopathological staging for colorectal cancer: an international documentation system (IDS) and an international comprehensive anatomical terminology (ICAT) J Gastroenterol Hepatol. 1991;6(4):325-44.
- [4] Hamilton SR, Vogelstein B, Kudo S, et al. Carcinoma of the colon and rectum. In: Hamilton SR, Aaltonen LA, editors. Pathology and Genetics of Tumours of the Digestive System. Lyon, France: IARC Press; 2000. pp. 103-143. World Health Organization Classification of Tumours.
- [5] Kang H, O'Connell JB, Maggard MA, Sack J, Ko CY. A 10-year outcomes evaluation of mucinous and signet-ring cell carcinoma of the colon and rectum. Dis Colon Rectum. 2005;48(6):1161-8.
- [6] Bernick PE, Klimstra DS, Shia J, et al. Neuroendocrine carcinomas of the colon and rectum. Dis Colon Rectum. 2004;47(2):163-9.
- [7] Wick MR, Vitsky JL, Ritter JH, Swanson PE, Mills SE. Sporadic medullary carcinoma of the colon: a clinicopathologic comparison with nonhereditary poorly differentiated enteric-type adenocarcinoma and neuroendocrine colorectal carcinoma. Am J Clin Pathol. 2005;123(1):56-65.
- [8] Jass JR. Pathology of hereditary nonpolyposis colorectal cancer. Ann NY Acad Sci. 2000;910:62-73. discussion 73-64.
- [9] Jass JR, Atkin WS, Cuzick J, et al. The grading of rectal cancer: historical perspectives and a multivariate analysis of 447 cases. Histopathology. 1986;10(5):437-59.
- [10] Aust DE. WHO classification 2010 for the lower gastrointestinal tract: what is new? Pathology. 2011;32:326-31. doi: 10.1007/s00292-011-1505-4.
- [11] Fujita S, Shimoda T, Yoshimura K, Akasu T, Moriya Y. Prospective evaluation of prognostic factors in patients with colorectal cancer undergoing curative resection. J Surg Oncol. 2003;84(3):127-31.

- [12] Huh JW, Kim HR, Kim YJ. Lymphovascular or perineural invasion may predict lymph node metastasis in patients with T1 and T2 colorectal cancer. *J Gastrointest Surg.* 2010 ;14:1074-80. doi: 10.1007/s11605-010-1206-y.
- [13] Anwar MA, D'Souza F, Coulter R, Memon B, Khan IM, Memon MA. Outcome of acutely perforated colorectal cancers: experience of a single district general hospital. *Surg Oncol.* 2006;15(2):91–6.
- [14] Kapiteijn E, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer [summary for patients in *Med J Aust.* 2002;177(10):563–564; PMID: 12429007] *N Engl J Med.* 2001;345(9):638-46.
- [15] Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004;96(4):261-8.
- [16] Greenson JK, Bonner JD, Ben-Yzhak O, et al. Phenotype of microsatellite unstable colorectal carcinomas. *Am J Surg Pathol.* 2003;27(5):563-70.
- [17] Kim JH¹, Kang GH¹. Molecular and prognostic heterogeneity of microsatellite-unstable colorectal cancer. *World J Gastroenterol.* 2014 Apr 21;20(15):4230-43. doi: 10.3748/wjg.v20.i15.4230.
- [18] Birbeck KF, Macklin CP, Tiffin NJ, et al. Rates of circumferential resection margin involvement vary between surgeons and predict outcomes in rectal cancer surgery. *Ann Surg.* 2002;235(4):449-57.
- [19] Ferko A, Orhalmi J, Dusek T, Chobola M, Hovorkova E, Nikolov DH. Higher risk of incomplete mesorectal excision and positive circumferential margin in low rectal cancer regardless of surgical technique. *Wideochir Inne Tech Malo Inwazyjne.* 2014;9:569-77. doi: 10.5114/wiitm.2014.45733. Epub 2014 Oct 7.
- [20] Gavioli M, Luppi G, Losi L, et al. Incidence and clinical impact of sterilized disease and minimal residual disease after preoperative radiochemotherapy for rectal cancer. *Dis Colon Rectum.* 2005;48(10):1851-7.
- [21] Ryan R, Gibbons D, Hyland JMP, et al. Pathological response following long-course neoadjuvant chemoradiotherapy for locally advanced rectal cancer. *Histopathology.* 2005;47(2):141-6.
- [22] Lo DS, Pollett A, Siu LL, et al. Prognostic significance of mesenteric tumor nodules in patients with stage III colorectal cancer. *Cancer.* 2008;112(1):50-4.
- [23] Puppa G, Maisonneuve P, Sonzogni A, et al. Pathological assessment of pericolic tumor deposits in advanced colonic carcinoma: relevance to prognosis and tumor staging. *Mod Pathol.* 2007;20(8):843-55.
- [24] Washington MK, Berlin J, Branton P, Burgart LJ, Carter DK, Fitzgibbons PL, et al. Protocol for the primary colon and rectum carcinoma specimen examination. *Arch Pathol Lab Med.* 2009; 133: 1539-51.

- [25] Gunderson LL, Jessup MJ, Sargent DJ, Greene FL, Stewart A. TN categorization for rectal and colon cancers based on national survival outcome data. *J Clin Oncol.* 2008;26(15S):4020.
- [26] Cserni G, Vinh-Hung V, Burzykowski T. Is there a minimum number of lymph nodes that should be histologically assessed for a reliable nodal staging of T3N0M0 colorectal carcinomas? *J Surg Oncol.* 2002;81(2):63-9.
- [27] Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med.* 2003;249:247-57.
- [28] Bessa X, Balleste B, Andreu M, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. *Clin Gastroenterol Hepatol.* 2008;6:206-14.
- [29] Raskov H, Pommergaard H, Burcharth J, Rosenberg J. Colorectal carcinogenesis-update and perspectives. *World J Gastroenterol.* 2014;20:18151-64.

Sažetak

Protokol za patohistološki pregled kirurških uzoraka kolorektalnog karcinoma

Američka udruga patologa izradila je protokol preuzimanja uzoraka i pisanja izvješća za uzorke kolorektalnog karcinoma koji će pružiti dovoljno relevantnih podataka za kliničku uporabu. Ove preporuke su prihvaćene od strane Hrvatske udruge patologa i sudskih medicinaru, te publicirane kao pomoć patolozima u svakodnevnom radu. Navedeni protokoli su edukativni i služe patolozima u pisanju patohistoloških izvješća koja sadržavaju relevantne informacije i primjenjuju se kod svih primarnih kolorektalnih karcinoma. Za procjenu stadija proširenosti kolorektalnog karcinoma trenutačno se koristi sedmo izdanje TNM sustava koje je temeljeno na preporuci Američkog zajedničkog komiteta za rak (AJCC) i Internacionalne udruge protiv raka (UICC).

Ključne riječi: karcinom debelog crijeva; prognostički čimbenici; prediktivni čimbenici.

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ENDOSCOPIC INNOVATIONS IN DIAGNOSIS AND MANAGEMENT OF COLORECTAL CANCER

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Summary

Colonoscopy is the gold standard in diagnosis of colorectal cancer that in most instances arises from precursor lesion, adenomatous polyp. However, white light forward viewing colonoscopy is not a perfect method, up to a quarter of adenomas are being missed during standard procedures. Therefore, new techniques and technologies are being developed in order to increase adenoma detection rate, either through better resolution and magnification of the image (high-definition, high-magnification endoscopes) or by augmenting the overview of colonic mucosa (Full Spectrum Endoscopy colonoscope, Third-Eye Retroscope). Besides adenoma detection, new technologies allow better tissue characterisation and in vivo discrimination between non-neoplastic and neoplastic lesions (conventional chromoendoscopy, virtual chromoendoscopy, confocal laser endomicroscopy, endocytoscopy). In addition to diagnostic procedures, therapeutic techniques are also evolving. Formerly, all of the flat or depressed colorectal lesions, encountered during colonoscopy, were referred to surgery. Today, endoscopic mucosal resection is becoming a routine method for the treatment of early gastrointestinal mucosal lesions of less than 2 cm in diameter. For larger lesions, endoscopic submucosal dissection, a state-of-the-art technique, is indicated, but currently carried out only in tertiary centres.

Endoscopic innovations are leading into new era of colorectal cancer diagnosis and management, hopefully resulting in decrease of incidence, morbidity and mortality.

Keywords: colorectal cancer; diagnosis; disease management; colonoscopy; innovations.

INTRODUCTION

Colorectal cancer (CRC) is second most common cancer among Croatian men and women and second leading cause of death from cancer [1,2]. It is also among five most common cancers worldwide and although it is more common in richer

countries, incidence of the disease is rising in some developing countries [2,3]. As opposed to other common cancers such as lung, breast and prostate, where the focus is early detection, CRC can be prevented since the majority of CRCs arise from precursor lesions, adenomatous polyps [4]. Indeed, studies have confirmed that removal of adenomatous polyps leads to reduction of cancer and subsequent reduction of mortality [5]. Given the above, CRC screening is widely recommended with colonoscopy being the gold standard for CRC diagnosis [6]. However, standard white light forward viewing colonoscopy is not a perfect method, reported adenoma miss rates are up to 27% [7-12]. Therefore, new endoscopic techniques and technologies are being developed in order to reduce adenoma miss rates and the incidence of interval cancer. It is recommended for every colorectal lesion encountered on colonoscopy to be removed and examined by a pathologist, a costly strategy that increases the risk of polypectomy complications [6,13]. Regarding the latter, new technologies are emerging designed to enable in vivo tissue characterization ("optical biopsies") and "leave in situ" approach for hyperplastic polyps or "resect and discard" approach for diminutive adenomatous polyps [14]. Information obtained by "optical biopsies" also impact therapeutic strategy selection since tissue biopsies are not recommended prior to endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD). EMR and ESD are endoscopic techniques for removal of flat pre-cancerous and cancerous lesions, that can, in carefully selected patients, replace surgery [15,16]. In this review we will perustrate advances in endoscopy regarding colon cancer diagnosis and management. Even though some commercially accessible for more than a decade, many technologies are not widely available due to their price and the need for expertise in interventional gastroenterology.

ENDOSCOPIC INNOVATIONS IN COLON CANCER SCREENING

Colonoscopy is the gold standard in CRC screening however, for moderate risk population, it has its limitations. Patients percieve bowel preparation, crucial for adequate examination, upleasant, the procedure carries the small risk of bowel perforation and in case of sedation, cardiopulmonary complications. Accordingly, many adult patients opt for other, less invasive procedures including stool based tests (guiaac-based fecal occult blood test, fecal immunochemical test, stool DNA test) and full or partial structural exams (flexible sigmoidoscopy, double-contrast barium enema, computed tomographic colonography) [6,13].

Currently, a new endoscopic procedure, colon capsule endoscopy (CCE), that may be useful for improving compliance with CRC screening, is being evaluated.

Even though similar to small bowel device, the capsule is bigger (11x32 mm vs. 11x26 mm) with video capture components on both ends of the capsule. After initial activation, the colon capsule captures images for 5 minutes, then transits into sleep mode for 2 hours. Once reactivated, approximately at the level of terminal ileum, the capsule records images for about 10 hours. Data are recorded via an antenna - lead array similar to that used in other capsule endoscopy procedures. Images are then transferred from a recording device to a workstation for formal review and report generation.

CCE has been shown to be a safe procedure when used in average-risk individuals with a very low rate of technical failures (3%) and a high capsule excretion rate of about 90% [17,18].

As in all other forms of screening, any positive findings require conventional colonoscopy for tissue sampling or polypectomy.

Quality of screening colonoscopy is measured by adenoma detection rate and cecal intubation rate. Factors impacting procedure quality, besides bowel preparation and colonoscopist's proficiency, include patient's compliance conditioned by abdominal discomfort and pain, the presence of subtle, flat or depressed lesions that are difficult to detect with standard endoscopic equipment and lesion location in "blind spots" of colonic mucosa [13].

When it comes to improving patient comfort and increasing cecal intubation rate, water infusion techniques and cap-assisted colonoscopy have shown promising results.

For appropriate mucosa visualization, colon folds need to be separated. Standard air insufflation colonoscopy provides good visualization but also distends and elongates the colon, increasing the likelihood of patient discomfort. Water infusion techniques ("water immersion" and "water exchange") use water adjunct to air. Water helps open colon folds with less comparative distention, which significantly reduces pain during colonoscopy, decreases the need for sedation drugs and increases the completion rate in patients with prior difficult colonoscopies [19-22].

Cap-assisted colonoscopy uses transparent caps attached to the distal tip of the colonoscope thus helping in depressing colonic folds in order to improve visualization of their proximal aspects. It was initially suggested that the technique might be of benefit for polyp detection, however, studies did not confirm this hypothesis. They did however report reduced cecal intubation times and improved cecal intubation rates. The same accounted for procedures in patients in whom cecal intubation initially failed with standard colonoscopy [22,23].

Standard forward viewing colonoscopes visualise the colon from the flexible tip of the instrument, with an angle of view up to 170° and as mentioned above a high adenoma miss rate is partially due to their inability to detect lesions hidden in the “blind spots”, such as the internal lining of flexures and proximal aspects of folds. In order to surpass this obstacle and get a better overview of colonic mucosa, Full Spectrum Endoscopy (FUSE) colonoscope and Third-Eye Retroscope have been designed.

FUSE colonoscope allows a high resolution 330 degrees “full spectrum” viewing of the colonic lumen while maintaining technical features and capabilities of a standard 140 and 170 degrees colonoscope.

The FUSE system consists of a main control unit and a video colonoscope with three cameras on the left-side, front and rightside of the flexible tip. Video images are displayed on three contiguous monitors corresponding with each individual camera [24-26].

Third-Eye Retroscope is a probe based device that is retroflexed 180 degrees after being advanced through the working channel of the colonoscope. It provides a 135 degrees retrograde view of the colon on the same monitor as a forward viewing colonoscope. However there are some limitations inherent to the device that confine its use in every day practice. First, thorough suctioning of colonic debris must be done during insertion of the colonoscope due to a 50% reduced suctioning capacity when the retroscope is in position. A second disadvantage is that the Third-Eye Retroscope needs to be removed from the working channel in case an accessory device is required, such as a biopsy forces or a polypectomy snare, which is bothersome and increases the procedural time [22,27].

For both FUSE colonoscopy and Third-Eye Retroscope colonoscopy preliminary studies have reported significant reductions in the proportion of missed adenomas in comparison with standard 170 degrees view straight-forward colonoscopy, even though reductions were more modest with Third-Eye Retroscope than those described for FUSE colonoscopy [22,24-27].

IMAGING INNOVATIONS FOR DETECTION AND DIFFERENTIATION OF COLORECTAL NEOPLASIA

The aim of colonoscopy is to detect adenomatous, pre-cancerous polyps, remove them and thus reduce CRC related morbidity and mortality. However, not all polyps are adenomatous but reliable differentiation between adenomatous and hyperplastic lesions is not possible solely according to the macroscopic lesion characteristics on white light illumination (accuracy 59-84%). Therefore, it has been recommended for all encountered lesions to be resected and submitted to pathology. New technologi-

es enable better visualization (high-definition and high-magnification endoscopes) and enhanced characterization of lesions (conventional and virtual chromoendoscopy, confocal laser endomicroscopy, endocytoscopy). Besides macroscopic features such as size and surface characteristics, microvascular pattern and pit pattern are being evaluated. With introduction of new parameters, endoscopists can more reliably differentiate between neoplastic and non-neoplastic lesions, apply more economical "leave in situ" or "resect and discard" approach for small hyperplastic polyps and diminutive adenomas respectively, but also estimate the risk of submucosal invasion, crucial for guiding therapeutic decision on whether endoscopic or surgical operation will be carried out.

The video capabilities of color images of standard definition endoscopes are based on traditional television broadcast formats. Advances in technology have resulted in increased number of pixels and increased resolution. Current high-resolution or high-definition endoscopes produce images with resolutions that range from 850,000 pixels to more than 1 million pixels, allowing them to discriminate objects that are only 10 to 71 microns in diameter.

At baseline, standard-resolution and high-resolution endoscopes magnify the endoscopic images 30 to 35 times. High-magnification endoscopes are defined by the capacity to perform optical zoom using movable lens in the tip of the endoscope thus magnifying images up to 150 times [22,28].

Conventional chromoendoscopy (dye assisted colonoscopy) involves topical application of stains or pigments to highlight surface contours.

The procedure is carried out using standard endoscopic equipment in addition of a special spray catheter essential to deliver a fine mist of dye to the mucosa. In diagnosing colonic neoplasia methylene blue, crystal violet and, most commonly, indigo carmine, are used.

Indigo carmine is a contrast stain that pools into crevices between epithelial cells, highlighting small or flat lesions and defining irregularities in mucosal architecture, particularly when used with high-magnification or high-definition endoscopy.

Pancolonic chromoendoscopy, recommended for neoplasia surveillance in patients with long-standing colitis [14], significantly increases the rate of detection of small neoplastic and flat lesions, but this technique requires an excessive volume of dye and a significantly prolonged procedure. Therefore, in average-risk patients, colonoscopists use "selective" chromoendoscopy for further examination of surface structure and mucosal crypt (pit) pattern of any subtle mucosal irregularity detected during standard colonoscopy [29].

Virtual chromoendoscopy refers to the use of image enhancement technologies built into the colonoscope system to alter the white-light image and enhance visu-

alization of mucosal surface architecture and capillary pattern. All commercially available systems work differently, but have a key aim of reducing the amount of red light in the image and of narrowing the bandwidth of blue and green light.

Narrow band imaging (NBI) separates white light into red, green, and blue using a special optical filter. Red light is eliminated and the contribution of blue and green wavelengths increased. Since mucosal hemoglobin selectively absorbs blue light, and the mucosa surrounding blood vessels reflects it, the contrast of the image is increased and the mucosal micro-vessel architecture can be estimated in fine detail. In the colon and rectum, microvessels form a ring, and each ring surrounds its respective gland. A deformed microvessel suggests a deformed neoplastic gland. Neoplastic changes result in a change in the form, density, and size of microvessels, and colorectal lesions can therefore be diagnosed [31,32].

FUJINON Intelligent Color Enhancement (FICE) is a computed spectral estimation technology that enhances the visibility of mucosal and vascular details by narrowing the bandwidth of light. FICE enables the endoscopist to choose between different wavelengths for optimal examination of the colon mucosa [14,22].

I-SCAN utilizes the same spectral estimation technology as FICE technology, and applies a digital color filter to images to emphasize lesions [14,22].

Even though the effect of pancolonial virtual chromoendoscopy on adenoma and polyp detection seems limited studies have reported high accuracy of both conventional and virtual chromoendoscopy (NBI, FICE and i-SCAN) [14, 30-32] in differentiation between adenomatous and hyperplastic polyps, therefore, **European Society of Gastrointestinal Endoscopy** suggests that they can be used, under strictly controlled conditions, for real-time optical diagnosis of diminutive ($\leq 5\text{mm}$) colorectal polyps to replace histopathological diagnosis [14].

Autofluorescence imaging (AFI) endoscopy is based on real-time detection of natural tissue fluorescence emitted by endogenous molecules, after excited by a certain wavelength. Differences in fluorescence emission between neoplastic and non-neoplastic tissues are captured during endoscopy and visualized as magenta or green color, respectively. The device is activated by a push-button on the handle of the endoscope. Recently, AFI has been connected to a high-resolution endoscope and to the NBI system, giving rise to a combined modality called endoscopic trimodal imaging. However, as opposed to above mentioned technologies, studies on benefit of AFI reported conflicting results [14,22,33,34].

Confocal laser endomicroscopy (CLE) and endocytoscopy are emerging endoscopic technologies that permit high-resolution assessment of gastrointestinal mucosal histology.

Confocal laser endomicroscopy provides microscopic images during ongoing endoscopy that trained endoscopists are able to evaluate on-site. The technology is based upon the principle of illuminating a tissue of interest with a low-power laser and then detecting fluorescent light reflected from the tissue. Intravenous injection of fluorescein is used to highlight the vasculature, lamina propria, and intracellular spaces of tissue being examined, in addition of topical acriflavine for nuclei staining [35-37].

Similar to CLE, endocytoscopy aims to enable real-time microscopic imaging of the mucosa in vivo. The main difference between CLE and endocytoscopy is that endocytoscopy is based solely on high-level magnification using optical lenses and only the very superficial layer of the mucosa can be imaged. In addition, the lens must come into direct contact with the tissue being examined. As with confocal endomicroscopy, acquisition of quality images requires application of a contrast agent. Typically, topical application of methylene blue or a combination of methylene blue with crystal violet is used [35].

For both CLE and endocystoscopy, probe-based systems are commercially available. The indications are still being defined. In general, these procedures are used to target biopsies of abnormal tissue and decrease biopsies of normal tissue [35].

ENDOSCOPIC INNOVATIONS IN EXCISION PROCEDURES FOR COLORECTAL LESIONS

Endoscopic treatment is recommended for benign lesions and early colorectal tumors with no evidence of enlarged lymph nodes or lymph node metastasis.

There are various established polypectomy techniques for endoscopic removal of polypoid colorectal lesions carried out in most of the gastroenterology centers. When it comes to flat or depressed colorectal lesions and laterally spreading tumors (LSTs), before the introduction of endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), the latter currently performed only in tertiary centers, patients have been referred to surgery with outcomes such as hemicolectomy or stoma formation, that greatly impacted the quality of life.

Careful characterization of lesions is mandatory in order to avoid incomplete treatment or unnecessary surgery. While lesions confined to superficial mucosa and up to 20 mm in diameter can be successfully treated with EMR, larger ones with higher possibility of submucosal invasion require ESD with en bloc resection for an accurate pathological evaluation. In cases of submucosal invasion of less than 1000 microns where the risk of lymph node metastasis is less than 1%, ESD is considered therapeutical. On the other hand, for deeper invasion, the risk is substantially increased, therefore, in such cases ensuing surgery is warranted [15].

Predictors of submucosal invasion, besides size [38], evaluated using conventional or virtual chromoendoscopy with high definition-high magnification colonoscope, include invasive type pit pattern, distorted capillary architecture and nodular mixed or pseudo-depressed LST surface morphology [15,39].

EMR, even though technically more difficult than standard polypectomy, is considered its variation and has become a routine method for the treatment of early gastrointestinal mucosal lesions. Typically, after marking the margins with electrocautery, a solution is injected into the submucosa to lift the lesion for easier removal and to provide a cushion to help protect the deeper layers of the bowel wall from mechanical or electrocautery damage followed by lesion resection using a mucosectomy device (grasping forceps, electrocautery snare, ligation device). The improved version of EMR, EMR with pre-cutting, is applied when the tumor location is not convenient for the traditional EMR operation [15,16,40].

EMR en block resection is feasible for lesions up to 20 mm but for larger ones, EMR can only be conducted in piecemeal fashion. Since piecemeal resection carries a high risk of local recurrence (0-55%) and prevents pathologist from reliably determining the status of the resection margins, intensive post-EMR colonoscopic surveillance is required (follow up at 2 to 6 months) [16].

ESD is indicated for larger lesions in which en block resection using EMR is difficult. It is usually limited to lesions up to 5 cm in diameter but Japanese referral centers have reported performing ESD on even larger ones. Further indications for colorectal ESD include mucosal tumors with fibrosis, scattered tumors caused by chronic inflammation and residual early tumor lesions after endoscopic operation.

The procedure involves visualization of the edges using virtual chromoendoscopy, marking the edges with argon knife, applying multipoint injections of pre-prepared solution into submucosal tissue. The edge of the lesion is then cut and the lesion stripped using special ESD knives (various knives are commercially available e.g. IT knife, Hook knife, Flex knife, B-knife, Flush-knife, Scissor-type knife, tongue-type knife) [41]. The improved technique of ESD, ESD-S, uses a snare instead of the ESD knife to strip the lesion [15,16,40,41].

Complications of both EMR and ESD include bleeding, perforation and local recurrence. Various studies have reported the rates of EMR and ESD complications (perforation rate: EMR: 0-1.3%, ESD 0-8%; postoperative bleeding: ESD EMR 3.6-8.1%, ESD 0.4-11.5%; local tumor recurrence: EMR 0.8-18.1%, ESD 0.4-2%) as well as resection rates (en-block resection: EMR 42.4%, ESD 74-89%) [42-51]. However, the data are hard to compare since indications and the intrinsic aggressiveness of two techniques on bowel wall differ. The risk of complications, as well as procedure time (ESD 108±71 min, EMR 29±25) [45], increase with tumor size and unfavorable loca-

tion (procedures are more feasible in rectum compared to colon) [51], but also, over the years, complication rates reported are decreasing.

Overall, EMR is superior to ESD in treatment of benign colorectal lesions since it has low risk of complications, shorter operation time and the characteristics of the operation are relatively simple. However, in case of suspected early carcinoma it is very important to mandate complete resection. ESD has higher resection rate and lower recurrence compared with EMR and provides a better quality of life for patients compared to surgery.

CONCLUSION

New endoscopic techniques and technologies, compared to standard endoscopic equipment, have increased adenoma detection rate and enabled better in vivo characterization of lesions. Also, many experts believe that state-of-the-art techniques in treatment of early colorectal cancer will replace surgery and thus improve patient's quality of life. Endoscopic innovations are leading into new era of colorectal cancer screening and management, hopefully resulting in reduction of CRC incidence, morbidity and mortality.

References

- [1] Hrvatski zdravstveno-statistički ljetopis 2013. Hrvatski zavod za javno zdravstvo. Available from: <http://hzjz.hr/publikacije/statisticki-ljetopis/>
- [2] World health report. Press release. World health organization. Available from: http://www.who.int/whr/1997/media_centre/press_release/en/index1.html
- [3] Cancer Fact sheet No. 297. World health organization. [Updated November 2014]. Available from: <http://www.who.int/mediacentre/factsheets/fs297/en/>
- [4] Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. *Br J Surg.* 2002;89(7):845-60.
- [5] Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med.* 2012;366(8):687-96. doi: 10.1056/NEJMoa1100370.
- [6] Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al; American Cancer Society Colorectal Cancer Advisory Group; US Multi-Society Task Force; American College of Radiology Colon Cancer Committee. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin.* 2008;58(3):130-60. doi: 10.3322/CA.2007.0018.

- [7] Hixson LJ, Fennerty MB, Sampliner RE, Garewal HS. Prospective blinded trial of the colonoscopic miss-rate of large colorectal polyps. *Gastrointest Endosc.* 1991;37(2):125-7.
- [8] Rex DK, Cutler CS, Lemmel GT, Rahmani EY, Clark DW, Helper DJ, et al. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology.* 1997;112(1):24-8.
- [9] Pickhardt PJ, Nugent PA, Mysliwiec PA, Choi JR, Schindler WR. Location of adenomas missed by optical colonoscopy. *Ann Intern Med.* 2004;141(5):352-9.
- [10] van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ, Dekker E. Polyp miss rate determined by tandem colonoscopy: a systematic review. *Am J Gastroenterol.* 2006;101(2):343-50.
- [11] Heresbach D, Barrioz T, Lapalus MG, Coumaros D, Bauret P, Potier P, et al. Miss rate for colorectal neoplastic polyps: a prospective multicenter study of back-to-back video colonoscopies. *Endoscopy.* 2008;40(4):284-90. doi: 10.1055/s-2007-995618.
- [12] Leufkens AM, van Oijen MG, Vleggaar FP, Siersema PD. Factors influencing the miss rate of polyps in a back-to-back colonoscopy study. *Endoscopy.* 2012;44(5):470-5. doi: 10.1055/s-0031-1291666.
- [13] Rembacken B, Hassan C, Riemann JF, Chilton A, Rutter M, Dumonceau JM, et al. Quality in screening colonoscopy: position statement of the European Society of Gastrointestinal Endoscopy (ESGE). *Endoscopy.* 2012;44(10):957-68. doi: 10.1055/s-0032-1325686.
- [14] Kamiński MF, Hassan C, Bisschops R, Pohl J, Pellisé M, Dekker E, et al. Advanced imaging for detection and differentiation of colorectal neoplasia: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy.* 2014;46(5):435-49. doi: 10.1055/s-0034-1365348.
- [15] Cai S, Zhong Y, Zhou P, Xu J, Yao L. Re-evaluation of indications and outcomes of endoscopic excision procedures for colorectal tumors: a review. *Gastroenterol Rep (Oxf).* 2014;2(1):27-36. doi: 10.1093/gastro/got034.
- [16] Fisher DA, Shergill AK, Early DS, Acosta RD, Chandrasekhara V, Chathadi KV, et al; ASGE Standards of Practice Committee. Role of endoscopy in the staging and management of colorectal cancer. *Gastrointest Endosc.* 2013;78(1):8-12. doi: 10.1016/j.gie.2013.04.163. Erratum in: *Gastrointest Endosc.* 2013;78(3):559.
- [17] Spada C, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, et al. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy.* 2012;44(5):527-36. doi: 10.1055/s-0031-1291717.
- [18] Adler DG, Chand B, Conway JD, Diehl DL, Kantsevoy SV, Kwon RS, Mamula P, et al; ASGE Technology Committee. Capsule endoscopy of the colon. *Gastrointest Endosc.* 2008;68(4):621-3. doi: 10.1016/j.gie.2008.06.046.

- [19] Leung F, Friedland S, Leung J, Mann S, Ramirez F, Yen A. Water-aided methods for colonoscopy - a review of VA experience. *J Interv Gastroenterol*. 2013;3(2):43-48.
- [20] Garborg K, Kaminski MF, Lindenburger W, Wiig H, Hasund A, Wronska E, et al. Water exchange versus carbon dioxide insufflation in unsedated colonoscopy: a multicenter randomized controlled trial. *Endoscopy*. 2014 Nov 20. Epub ahead of print.
- [21] Leung FW, Amato A, Ell C, Friedland S, Harker JO, Hsieh YH, et al. Water-aided colonoscopy: a systematic review. *Gastrointest Endosc*. 2012;76(3):657-66. doi: 10.1016/j.gie.2012.04.467.
- [22] Dik VK, Moons LM, Siersema PD. Endoscopic innovations to increase the adenoma detection rate during colonoscopy. *World J Gastroenterol*. 2014;20(9):2200-11. doi: 10.3748/wjg.v20.i9.2200.
- [23] de Wijkerslooth TR, Stoop EM, Bossuyt PM, Mathus-Vliegen EM, Dees J, Tytgat KM, et al. Adenoma detection with cap-assisted colonoscopy versus regular colonoscopy: a randomised controlled trial. *Gut*. 2012;61(10):1426-34. doi: 10.1136/gutjnl-2011-301327.
- [24] Gralnek IM, Carr-Locke DL, Segol O, Halpern Z, Siersema PD, Sloyer A, et al. Comparison of standard forward-viewing mode versus ultrawide-viewing mode of a novel colonoscopy platform: a prospective, multicenter study in the detection of simulated polyps in an in vitro colon model (with video). *Gastrointest Endosc*. 2013;77(3):472-9. doi: 10.1016/j.gie.2012.12.011.
- [25] Gralnek IM, Segol O, Suissa A, Siersema PD, Carr-Locke DL, Halpern Z, et al. A prospective cohort study evaluating a novel colonoscopy platform featuring full-spectrum endoscopy. *Endoscopy*. 2013;45(9):697-702. doi: 10.1055/s-0033-1344395.
- [26] Gralnek IM, Siersema PD, Halpern Z, Segol O, Melhem A, Suissa A, et al. Standard forward-viewing colonoscopy versus full-spectrum endoscopy: an international, multicentre, randomised, tandem colonoscopy trial. *Lancet Oncol*. 2014;15(3):353-60. doi: 10.1016/S1470-2045(14)70020-8.
- [27] Leufkens AM, DeMarco DC, Rastogi A, Akerman PA, Azzouzi K, Rothstein RI, et al; Third Eye Retroscope Randomized Clinical Evaluation [TERRACE] Study Group. Effect of a retrograde-viewing device on adenoma detection rate during colonoscopy: the TERRACE study. *Gastrointest Endosc*. 2011;73(3):480-9. doi:10.1016/j.gie.2010.09.004.
- [28] Kwon RS, Adler DG, Chand B, Conway JD, Diehl DL, et al; ASGE Technology Committee. High-resolution and high-magnification endoscopes. *Gastrointest Endosc*. 2009;69(3 Pt 1):399-407. doi: 10.1016/j.gie.2008.12.049.
- [29] Wong Kee Song LM, Adler DG, Chand B, Conway JD, Croffie JM, Disario JA, et al; ASGE Technology Committee. Chromoendoscopy. *Gastrointest Endosc*. 2007;66(4):639-49.

- [30] Koo JS. Equipment-based image-enhanced endoscopy for differentiating colorectal polyps. *Clin Endosc.* 2014;47(4):330-3. doi: 10.5946/ce.2014.47.4.330.
- [31] Nagorni A, Bjelakovic G, Petrovic B. Narrow band imaging versus conventional white light colonoscopy for the detection of colorectal polyps. *Cochrane Database Syst Rev.* 2012;1:CD008361. doi: 10.1002/14651858.CD008361.pub2.
- [32] Iwatate M, Ikumoto T, Hattori S, Sano W, Sano Y, Fujimori T. NBI and NBI Combined with Magnifying Colonoscopy. *Diagn Ther Endosc.* 2012;2012:173269. doi: 10.1155/2012/173269.
- [33] Inomata H, Tamai N, Aihara H, Sumiyama K, Saito S, Kato T, et al. Efficacy of a novel auto-fluorescence imaging system with computer-assisted color analysis for assessment of colorectal lesions. *World J Gastroenterol.* 2013;19(41):7146-53. doi: 10.3748/wjg.v19.i41.7146.
- [34] Moriichi K, Fujiya M, Sato R, Watari J, Nomura Y, Nata T, et al. Back-to-back comparison of auto-fluorescence imaging (AFI) versus high resolution white light colonoscopy for adenoma detection. *BMC Gastroenterol.* 2012;12:75. doi: 10.1186/1471-230X-12-75.
- [35] Meining A. Confocal laser endomicroscopy and endocytoscopy. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on January 15, 2015.). Available from: <http://www.uptodate.com/>
- [36] Goetz M. Confocal Laser Endomicroscopy: Applications in Clinical and Translational Science—A Comprehensive Review. *ISRN Pathol.* 2012;2012:387145. doi:10.5402/2012/387145.
- [37] Ussui VM, Wallace MB. Confocal endomicroscopy of colorectal polyps. *Gastroenterol Res Pract.* 2012;2012:545679. doi: 10.1155/2012/545679.
- [38] Kudo SE, Takemura O, Ohtsuka K. Flat and depressed types of early colorectal cancers: from East to West. *Gastrointest Endosc Clin N Am.* 2008;18(3):581-93. doi: 10.1016/j.giec.2008.05.013.
- [39] Kim KO, Jang BI, Jang WJ, Lee SH. Laterally spreading tumors of the colorectum: clinicopathologic features and malignant potential by macroscopic morphology. *Int J Colorectal Dis.* 2013;28(12):1661-6. doi: 10.1007/s00384-013-1741-6.
- [40] Kantsevov SV, Adler DG, Conway JD, Diehl DL, Farraye FA, Kwon R, et al; ASGE Technology Committee. Endoscopic mucosal resection and endoscopic submucosal dissection. *Gastrointest Endosc.* 2008;68(1):11-8. doi: 10.1016/j.gie.2008.01.037.
- [41] Saito Y, Yamada M, So E, Abe S, Sakamoto T, Nakajima T, Otake Y, Ono A, Matsuda T. Colorectal endoscopic submucosal dissection: Technical advantages compared to endoscopic mucosal resection and minimally invasive surgery. *Dig Endosc.* 2014;26 Suppl 1:S52-61. doi: 10.1111/den.12196.

- [42] Nakajima T, Saito Y, Tanaka S, Iishi H, Kudo SE, Ikematsu H, et al. Current status of endoscopic resection strategy for large, early colorectal neoplasia in Japan. *Surg Endosc.* 2013;27(9):3262-70. doi: 10.1007/s00464-013-2903-x.
- [43] Wang J, Zhang XH, Ge J, Yang CM, Liu JY, Zhao SL. Endoscopic submucosal dissection vs endoscopic mucosal resection for colorectal tumors: a meta-analysis. *World J Gastroenterol.* 2014;20(25):8282-7. doi: 10.3748/wjg.v20.i25.8282.
- [44] Lee EJ, Lee JB, Lee SH, Youk EG. Endoscopic treatment of large colorectal tumors: comparison of endoscopic mucosal resection, endoscopic mucosal resection-precutting, and endoscopic submucosal dissection. *Surg Endosc.* 2012;26(8):2220-30. doi: 10.1007/s00464-012-2164-0.
- [45] Saito Y, Fukuzawa M, Matsuda T, Fukunaga S, Sakamoto T, Uraoka T, et al. Clinical outcome of endoscopic submucosal dissection versus endoscopic mucosal resection of large colorectal tumors as determined by curative resection. *Surg Endosc.* 2010;24(2):343-52. doi: 10.1007/s00464-009-0562-8.
- [46] Niimi K, Fujishiro M, Kodashima S, Goto O, Ono S, Hirano K, et al. Long-term outcomes of endoscopic submucosal dissection for colorectal epithelial neoplasms. *Endoscopy.* 2010;42(9):723-9. doi: 10.1055/s-0030-1255675.
- [47] Tanaka S, Terasaki M, Hayashi N, Oka S, Chayama K. Warning for unprincipled colorectal endoscopic submucosal dissection: accurate diagnosis and reasonable treatment strategy. *Dig Endosc.* 2013;25(2):107-16. doi: 10.1111/den.12016.
- [48] Tanaka S, Terasaki M, Kanao H, Oka S, Chayama K. Current status and future perspectives of endoscopic submucosal dissection for colorectal tumors. *Dig Endosc.* 2012;24 Suppl 1:S73-9. doi: 10.1111/j.1443-1661.2012.01252.x.
- [49] Kiriya S, Saito Y, Yamamoto S, Soetikno R, Matsuda T, Nakajima T, et al. Comparison of endoscopic submucosal dissection with laparoscopic-assisted colorectal surgery for early-stage colorectal cancer: a retrospective analysis. *Endoscopy.* 2012;44(11):1024-30. doi: 10.1055/s-0032-1310259.
- [50] Repici A, Hassan C, De Paula Pessoa D, Pagano N, Arezzo A, Zullo A, Lorenzetti R, et al. Efficacy and safety of endoscopic submucosal dissection for colorectal neoplasia: a systematic review. *Endoscopy.* 2012;44(2):137-50. doi: 10.1055/s-0031-1291448.
- [51] Saito Y, Uraoka T, Yamaguchi Y, Hotta K, Sakamoto N, Ikematsu H, et al. A prospective, multicenter study of 1111 colorectal endoscopic submucosal dissections (with video). *Gastrointest Endosc.* 2010;72(6):1217-25. doi: 10.1016/j.gie.2010.08.004.

Sažetak

Endoskopske inovacije u dijagnostici i liječenju kolorektalnog karcinoma

Kolonoskopija je zlatni standard u dijagnostici kolorektalnog karcinoma koji u većini slučajeva nastaje iz prekursorske lezije, adenoma. Međutim, standardna kolonoskopija nije savršena metoda; prema rezultatima tandem studija čak četvrtina adenoma ostaje neotkrivena. Stoga se razvijaju nove tehnike i tehnologije koje omogućuju bolju detekciju adenoma uvećanjem i boljom rezolucijom slike ("high-definition", "high-magnification" endoskopi) te boljim pregledom sluznice debelog crijeva ("Full Spectrum Endoscopy" kolonoskop, "Third-Eye Retroscope"). Nove tehnologije također omogućuju i napredniju karakterizaciju kolorektalnih promjena i in vivo razlikovanje ne-neoplastičnih i neoplastičnih lezija (konvencionalna kromoendoskopija, virtualna kromoendoskopija, konfokalna laserska endomikroskopija, endocitoskopija). Osim dijagnostičkih, napreduju i terapijske endoskopske metode. Do sada su sve ne-polipoidne kolorektalne promjene liječene kirurški, a danas je endoskopska mukozna resekcija postala rutinska metoda za lezije do 2 cm u promjeru. U slučaju većih promjena indicirana je endoskopska submukozna disekcija, state-of-the-art tehnika koja se trenutno izvodi samo u tercijarnim centrima.

Inovacije u endoskopiji vode u novu eru dijagnostike i liječenja kolorektalnog karcinoma te nagovijestaju bolju prevenciju i smanjenje incidencije ove česte maligne bolesti.

Ključne riječi: kolorektalni karcinom; dijagnostika; liječenje kolorektalnog karcinoma; kolonoskopija; inovacije.

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SYSTEMIC THERAPY FOR COLORECTAL CANCER – OVERVIEW OF RECENT TRIALS

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Summary

Colorectal cancer (CRC) is the most common malignancy of the gastrointestinal tract worldwide, and also one of the leading causes of cancer-related mortality, accounting for more than 600000 deaths every year. Depending on the stage of the disease some patients who were surgically treated for CRC with curative intent require adjuvant chemotherapy (fluorouracile-based), which reduces the risk of recurrence and death in patients with stage III CRC, but its role in stage II is still controversial. Pathogenesis of CRC is a result of two different genetic pathways: chromosomal instability (CIN), and inactivation of mismatch repair (MMR) genes. Stage II patients with deficient MMR tumor status do not benefit from adjuvant therapy and should receive surgery alone. For patients with metastatic CRC optimal sequence of chemotherapy regimens and targeted therapy is still debated. Epidermal growth factor receptor (EGFR) has been validated as a therapeutic target in several human tumors including CRC. Recently it has been reported that activating mutations in exons 2, 3, and 4 of both KRAS and NRAS all predict a lack of response to EGFR targeted agents. Recent studies suggest BRAF is another prognostic and potential predictive biomarker of CRC. It is necessary to identify new prognostic and predictive markers, to acquire maximal benefit from every therapy line and to improve prognosis of these patients.

Keywords: colorectal cancer; personalized medicine; chemotherapy; targeted therapy; biomarkers.

Colorectal cancer (CRC) is the most common malignancy of the gastrointestinal tract worldwide, and also one of the leading causes of cancer-related mortality [1], accounting for more than 600,000 deaths every year [2]. Treatment options include surgery, chemotherapy, immunotherapy and radiotherapy. In localized disease, primary

surgery is the most widely used primary treatment, and these patients are treated with curative intent. Depending on the stage of the disease, some of these patients require adjuvant (fluoropyrimidine-based) chemotherapy, which reduces the risk of recurrence and death in patients with stage III CRC, but its role in stage II is still controversial. Sargent et al. have collected individual patient data from 18 trials and more than 20,800 patients to investigate efficacy of adjuvant therapy in patients with stage II and III CRC [3]. According to their results, adjuvant chemotherapy for colon cancer (CC) provides significantly longer progression-free survival (PFS) and overall survival (OS), what reflects the curative role of chemotherapy in the adjuvant setting. In stage II, 72.2% of patients who underwent surgery and received adjuvant chemotherapy were alive at 8 years follow-up, in contrast to 66.8% of patients who received surgery alone. In stage III, 53% of patients who underwent surgery and received adjuvant chemotherapy and 42.7% of patients who received surgery alone were alive at 8 years follow-up.

Pathogenesis of CRC is a result of two different genetic pathways. Chromosomal instability (CIN) involves changes in number and structure of chromosomes and accounts for 85% of CRC cases, and inactivation of mismatch repair (MMR) genes, which take part in the repair of incorrectly connected DNA fragments, results in microsatellite instability (MSI) and accounts for 15% of CRC cases. MMR tumor status can be deficient (dMMR), with high content of MSI (MSI-H), and proficient (pMMR), with low-stable content of MSI (MSS i MSI-L). Previous retrospective and observational studies showed that dMMR phenotype (in contrast to CIN-tumors) is associated with lower stage of the disease, right-sided tumor localization, high grade, mucinous/medullar type, more favorable prognosis, and lower efficacy of adjuvant fluorouracil-based adjuvant chemotherapy. Sargent et al. analyzed data from 7,803 stage II and III patients enrolled in 17 trials [4]. In adjuvant setting, 571 (7.3%) of these patients received surgery alone, 3878 (49.7%) 5-fluorouracil (5-FU) monotherapy, 2299 (29.5%) 5-FU and oxaliplatin, and 1055 (13.5%) 5-FU and irinotecan. Results have confirmed prognostic utility of MMR status in stage II CC, since dMMR tumor status was shown to be strongly associated with improved OS (90% of dMMR-positive patients who did not receive adjuvant chemotherapy were alive at 5-year follow-up). Based on these results, determination of tumor MMR status should be a routine test in all stage II CC patients, if adjuvant treatment is considered, since patients with dMMR tumor status do not benefit from adjuvant therapy and should receive surgery alone. Impact of MMR status on the outcome in stage III patients was also confirmed, but altering the management based on the MMR status was not recommended. Hence, determination of MMR tumor status should not be a routine test in these patients – all stage III patients should receive adjuvant

chemotherapy. In patients who were treated with fluorouracil- based adjuvant chemotherapy, prognostic value of MMR status was attenuated in both stage II and stage III patients compared to patients who received surgery alone, and after disease recurrence MMR status was no longer prognostically significant in the sense of OS in neither stage II nor stage III patients.

In rectal cancer (RC), fluorouracil- based radiochemotherapy is nowadays a standard preoperative treatment for locally advanced disease, with continuous infusion of 5-FU being equally efficient to peroral capecitabine. Adjuvant chemotherapy for rectal cancer should be optimized, since up to 30% of these patients who are treated with preoperative radiochemotherapy and subsequent mesorectal excision (TME) ultimately develop distant metastases, irrespective of the application of adjuvant chemotherapy. In CC, fluorouracil-based monochemotherapy is a standard in high- risk stage II patients, and FOLFOX or CapeOx are preferred in stage III. To integrate the most effective treatment of stage II and III rectal cancer, in a clinical trial by German Rectal Cancer Study Group a total of 637 patients were randomly assigned to preoperative radiotherapy (tumor dose (TD) =50.4 Gy) plus infusional 5-FU (1 g/m² on days 1-5 and 29-33), followed by TME and 4 cycles of bolus 5-FU (500 mg/m² for 5 days) or preoperative radiotherapy (TD =50.4 Gy) plus infusional 5-FU (250 mg/m² on days 1-14 and 22-35) and oxaliplatin (50 mg/m² on days 1, 8, 22, and 29), followed by TME and subsequent 8 cycles of adjuvant FOLFOX6 regimen [5]. Results showed that adding oxaliplatin to 5-FU-based neoadjuvant chemoradiotherapy and adjuvant chemotherapy in locally advanced rectal cancer significantly improved DFS. In contrast to these results, several other trials showed that adding an oxaliplatin to fluorouracil-based preoperative chemoradiotherapy does not improve tumor regression (there is no radiosensitizing effect). In a study by Schmoll et al., in capecitabine plus radiotherapy arm minimal, moderate, good, and complete tumor regression were achieved in 22%, 37%, 20%, and 13% of patients, respectively, and in capecitabine plus oxaliplatin and radiotherapy arm in 16%, 40%, 22%, and 15% of patients, respectively [6]. Not only that tumor regression was not improved, patients' compliance was also significantly compromised due to toxicity in a study arm with added oxaliplatin. After TME in these patients, the first study arm continued to receive capecitabine alone, and the second arm received capecitabine and oxaliplatin, same as in the preoperative setting. Again, no difference in PFS was observed, and thus preoperative fluorouracil-based monochemotherapy remains the standard treatment in this setting for rectal cancer, however, adjuvant oxaliplatin-based chemotherapy (FOLFOX regimen) improves 3-year PFS in patients with residual T3-4 and/or N1-2 disease, after completed chemoradiotherapy and curative rectal cancer resection [7]. Results regarding OS in this setting are still awaited.

Epidermal growth factor receptor (EGFR) has been validated as a therapeutic target in several human tumours including CRC. Previous studies have shown that anti-vascular endothelial growth factor (VEGF) monoclonal antibody bevacizumab in non-selected patients and EGFR inhibitors cetuximab and panitumumab in patients with KRAS (exon 2) wild-type tumors improve OS (median survival > 24 months) when given with chemotherapy. Ten percent of these patients survive more than 5 years. Prevalence of KRAS exon 2 wild-type mutation is approximately 40%, and it is a predictor of EGFR inhibitors' efficacy. Activating mutations in KRAS exon 2 have long been known to blunt the response to EGFR-targeted agents in metastatic CRC. Lately, more extensive genetic testing for RAS gene mutations beyond routine analysis of exon 2 is becoming a new standard of care, to pinpoint which patients stand to benefit from anti-EGFR therapy in the treatment of metastatic CRC, since it was reported that activating mutations in exons 2, 3, and 4 of both KRAS and NRAS all predict a lack of response to panitumumab in the second-line setting, corroborating similar recent findings from the PRIME and PEAK trials regarding panitumumab's efficacy in the first-line setting [8, 9]. In PRIME trial, statistically significant benefit in OS was shown with addition of panitumumab to FOLFOX regimen in „all- RAS“ wild-type patients, when compared to KRAS exon 2 wild-type patients, what is in accordance with results that show no benefit of panitumumab being added to FOLFOX regimen in patients with KRAS exon 2 wild-type and other RAS mutation present. These results were also confirmed in PEAK trial, in which bevacizumab and panitumumab were directly compared, both in combination with FOLFOX6 regimen. When looking at the original KRAS exon 2 wild-type patients only, there was no statistically significant difference in PFS between study arms ($p=0.35$), although prolonged OS was observed in the panitumumab arm (34.2 vs. 24.3 months, $p=0.009$), however, in the „all-RAS“ wild-type patients both PFS and OS were prolonged in the panitumumab study arm. Again, there was no benefit of addition of panitumumab to FOLFOX regimen in patients with KRAS exon 2 wild-type and other RAS mutations. Similar results were reported in FIRE-3 trial, in which FOLFIRI plus cetuximab showed benefit in OS over FOLFIRI plus bevacizumab of 3.7 months in KRAS exon 2 wild-type patients and 7.5 months in „all-RAS“ wild-type patients [10]. In contrast, benefit in OS in CALGB/SWOG 80405 clinical trial was also in favor of the FOLFOX regimen plus cetuximab versus FOLFOX plus bevacizumab, but difference did not reach statistical significance. Expanded „all-RAS“ analysis of KRAS and NRAS exons 2, 3, and 4 is crucial for the optimal selection of first-line therapy in CRC. According to ASPECCT trial, panitumumab is not less effective than cetuximab in chemorefractory patients in terms of OS [11].

Another investigated marker in CRC is BRAF [12, 13]. BRAF mutation is a poor prognostic factor. Van Cutsem and colleagues concluded that lacking randomised tri-

als in this specific molecular subgroup, FOLFOXIRI plus bevacizumab might be a reasonable option for the first-line treatment of BRAF mutant metastatic CRC patients.

To conclude, optimal sequence of chemotherapy regimens and targeted therapy in patients with metastatic CRC is still debated. It is necessary to identify new prognostic and predictive markers to acquire maximal benefit from every therapy line, to improve prognosis of these patients.

References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011;61:69-90.
- [2] Ahlquist DA, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszat LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sorensen HT, Berger BM, Lidgard GP. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology.* 2012;142(2): 248-56.
- [3] Sargent D, Sobrero A, Grothey A, O'Connell MJ, Buyse M, Andre T, Zheng Y, Green E, Labianca R, O'Callaghan C, Seitz JF, Francini G, Haller D, Yothers G, Goldberg R, de Gramont A. Evidence for Cure by Adjuvant Therapy in Colon Cancer: Observations Based on Individual Patient Data From 20,898 Patients on 18 Randomized Trials. *J Clin Oncol.* 2009;27:872-7.
- [4] Sargent D, Shi Q, Yothers G, Tejpar S, Bertagnolli MM, Thibodeau SN, Andre T, Labianca R, Gallinger S, Hamilton SR, Monges G, Pogue-Geile KL, Paik S, Klingbiel D, Roth A, Pavey ES, Kim GP, Sinicrope FA, ACCENT Collaborative Group. Prognostic impact of deficient mismatch repair (dMMR) in 7,803 stage II/III colon cancer (CC) patients (pts): A pooled individual pt data analysis of 17 adjuvant trials in the ACCENT database. *J Clin Oncol (Meeting Abstracts).* 2014;32(15_suppl):3507.
- [5] Preoperative chemoradiotherapy and postoperative chemotherapy with 5-fluorouracil and oxaliplatin versus 5-fluorouracil alone in locally advanced rectal cancer: Results of the German CAO/ARO/AIO-04 randomized phase III trial. Rodel C, Liersch T, Fietkau R, Hohenberger W, Graeven U, Hothorn T, Arnold D, Raab HR, Wittekind C, Hess CF, Staib L, Becker H, Sauer R, German Rectal Cancer Study Group; *J Clin Oncol (Meeting abstracts).* 2014;32(15_suppl):3500.
- [6] Schmoll HJ et al. *Eur J Cancer.* 2014;50(suppl 1):S6.
- [7] Hong YS, Nam BH, Kim KP, Lee JL, Park JO, Park YS, et al. Adjuvant chemotherapy with oxaliplatin/5-fluorouracil/leucovorin (FOLFOX) versus 5-fluorouracil/leucovorin (FL) for rectal cancer patients whose postoperative yp stage 2 or 3 after preoperative chemoradiotherapy: Updated results of 3-year disease-free survival from a randomized phase II study (The ADORE). *J Clin Oncol.* 2014;32(5s) Suppl; abstr 3502.

- [8] Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humbelt Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocakova I, Ruff P, Blasinska-Morawiec M, Smakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Randomized phase 3 study of panitumumab with FOLFOX4 vs FOLFOX4 alone as first-line treatment in patients with metastatic colorectal cancer: The PRIME trial. *Eur J Cancer*. 2009;7(suppl): 6.
- [9] Schwartzberg LS, Rivera F, Karthaus M, Fasola G, Canon JL, Hecht JR, Yu H, Oliner KS, Go WY. PEAK: A Randomized, Multicenter Phase II Study of Panitumumab Plus Modified Fluorouracil, Leucovorin, and Oxaliplatin (mFOLFOX6) or Bevacizumab Plus mFOLFOX6 in Patients With Previously Untreated, Unresectable, Wild-Type KRAS Exon 2 Metastatic Colorectal Cancer. *J Clin Oncol*. 2014;32:2240-7.
- [10] Heinemann V, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmuller C, Kahl C, Seipel G, Kullmann F, Stauch M, Scheithauer W, Hielscher J, Scholz M, Muller S, Link H, Niederle N, Rost A, Hoffkes HG, Moehler M, Lindig RU, Modest DP, Rossius L, Kirchner T, Jung A, Stintzing S. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet*. 2014;15:1065-75.
- [11] Price TJ, Peeters M, Kim TW, Li J, Cascinu S, Ruff P, Suresh AS, Thomas A, Tjulandin S, Zhang K, Murugappan S, Sidhu R. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol*. 2014;15:569-79.
- [12] Van Cutsem E, Kohne CH, Lang I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol*. 2011;29(15):2011-9.
- [13] Loupakis F, Cremolini C, Salvatore L, Masi G, Sensi E, Schirripa M, Michelucci A, Pfanner E, Brunetti I, Lupi C, Antoniotti C, Bergamo F, Lonardi S, Zagonel V, Simi P, Fontanini G, Falcone A. FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer. *Eur J Cancer*. 2014 Jan;50(1): 57-63.

Sažetak

Sustavna terapija raka debelog crijeva – pregled novih kliničkih studija

Rak debelog crijeva (CRC) najučestalija je maligna neoplazma gastrointestinalnog trakta, te je sa preko 600.000 smrti godišnje jedan od vodećih uzroka smrtnosti od zloćudnih bolesti. Ovisno o stadiju bolesti, neki od bolesnika koji su kirurški liječeni radi raka debelog crijeva s ciljem izlječenja zahtijevaju primjenu adjuvantne kemoterapije (bazirane na fluorouracilu) koja smanjuje rizik povrata bolesti i smrti u III. stadiju bolesti, no njena uloga u stadiju II još je uvijek nedovoljno jasna. Patogeneza u CRC rezultat je dvaju različitih molekulsko-genskih mehanizama: kromosomske nestabilnosti (CIN) te inaktivacije gena koji sudjeluju u popravku pogrešno spojenih fragmenata DNA (MMR). Na temelju provedenih istraživanja, u bolesnika stadija II u kojih je prisutan deficijentan MMR status ne postoji dobit od primjene adjuvantne terapije te je u toj skupini bolesnika kirurško liječenje dostatno. U bolesnika s metatastatskim CRC još uvijek nema konsenzusa o optimalnom redoslijedu kemoterapijskih protokola i ciljane biološke terapije. Receptor za epidermalni čimbenik rasta (EGFR) potvrđen je kao terapijski cilj u više humanih tumora, uključujući CRC. Odnedavno, istraživanja su pokazala da aktivirajuće mutacije na eksonima 2, 3 i 4 KRAS i NRAS gena dovode do izostanka odgovora na ciljanu terapiju usmjerenu na EGFR. BRAF je još jedan od istraživanih biljega u raku debelog crijeva, te je pokazano da je povezan sa lošijom prognozom. Potrebna su daljnja istraživanja i identifikacija novih prognostičkih i prediktivnih biljega kako bi se postigla maksimalna učinkovitost svake terapijske linije te poboljšala prognoza ovih bolesnika.

Ključne riječi: rak debelog crijeva; personalizirana medicina; kemoterapija; ciljana terapija; biomarkeri.

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CONTEMPORARY TREATMENT OF RECTAL CARCINOMA

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Summary

In the last few decades we have witnessed a great advancement in the treatment of rectal carcinoma. This advancement has partly been enabled by the development of new surgical techniques, magnetic resonance imaging as an optimal diagnostic tool and of new histopathological techniques for resected specimen evaluation. Other important contributions include the development of sophisticated radiotherapy treatment techniques which, applied with new cytostatics and smart drugs, induce a better tumor response while reducing toxicity. Although each discipline has contributed to a better understanding of the disease, it is the multidisciplinary approach that has yielded success in treatment so far.

Keywords: neoadjuvant therapy; *short course* radiotherapy; *long course* radiotherapy; concomitant chemotherapy; pathologic complete response.

INTRODUCTION

Carcinoma of the rectum, together with colon cancer, is the third most common malignancy. It appears frequently and at the same rate in women and men - 9.4% of the newly detected patients and 7.9% of all causes of death from malignant disease.

In the last two decades we have witnessed great changes in the detection and treatment of rectal carcinoma. These changes have been mainly founded on improvements in the fields of preoperative classification of the disease, surgical techniques, histopathological evaluation of the resected specimen and application of multidisciplinary procedures with the aim of ensuring a better long-term outcome for patients. A crucially important concept is the "mesorectal excision" based on a precise dissection of the anatomical plane surrounding the mesorectal adipose tissue. Numerous studies, including randomized controlled trials, clearly show the

importance of the total mesorectal excision as a surgical technique which has led to a statistically significant decrease in local recurrence rates in tertiary referral hospitals. Surgery has been so far the most important therapeutic procedure in the treatment of rectal cancer, although multidisciplinary and individualized approaches have been gaining more significance recently. Magnetic resonance imaging (MRI) has become the primary diagnostic tool for preoperative evaluation of patients due to its ability to precisely display the mesorectal fascia. Establishing of a tumor infiltration has been suggested as a mandatory stage in the radiological processing of every patient. The development of radiological classification of rectal carcinoma is closely connected with changes in the histopathological evaluation techniques for measuring the microscopic distance of the tumor from the circumferential resection margin (CRM) of the resected specimen. CRM represents the distance from the edge of the soft tissue preparation/soft tissue margin to the deepest penetration of the tumor. After the operation, the description of the CRM margin presents an essential component of the pathological finding. This procedure provides surgeons with the choice of the optimal surgical plane. Low-lying tumors pose a major challenge for successful treatment. The restricted space between the bony structures of the pelvis reduces the possibility of radical surgical removal, resulting in a high risk of local recurrence [1]. Most cases of recurrence (85%) appear within the first three years after the end of the treatment [2].

THE ROLE OF RADIOTHERAPY IN THE RECTAL CANCER TREATMENT

Research on modalities and timing of chemoradiotherapy application departs from the science-based facts about better local control and prolonged survival in patients with T3-4 N+ tumors treated with adjuvant chemoradiotherapy (NSABP R – 01 [3] and NSABP R – 02 [4]).

Multiple European phase III randomized studies have assessed the role of radiotherapy and chemotherapy in the neoadjuvant treatment of rectal cancer. Based on the results of these studies, neoadjuvant radiotherapy was accepted by ESTRO in 2007 as a standard approach in the treatment of patients with locally advanced disease (T3-4 N+). Preoperative treatment benefits range from the reduction of the tumor, to pathologic complete regression/pathologic complete response (pCR) in 10-25% of patients. Radical local resection is facilitated and the likelihood of a margin-free tumor resection is increased.

Short course preoperative radiotherapy (SCPRT) has proved superior in decreasing local recurrence in operable tumors compared to adjuvant radiotherapy (5% vs. 11%) [5]. Studies comparing *long* and *short course preoperative radiotherapy* have shown no

difference in the local recurrence rate, total survival rate, dissemination risk and late toxicity between these two groups [6]. Less side effects and fewer treatment fractions make SCPRT cheaper and more accessible than the adjuvant chemoradiotherapy. The SCPRT is optimal in the treatment of T3 tumors located in the middle third of the rectum with a negative CRM. In “potentially” operable, CRM positive or low-lying tumors concomitant preoperative chemoradiotherapy based on *long course schedule* (LC) is the preferred choice.

Following the first application of *short course preoperative* radiotherapy in England, two major studies have confirmed its significance in the treatment of rectal cancer. Between 1980 and 1993, more than 2,000 patients were included in the Swedish Rectal Cancer trials, of which the most important one is the Swedish Rectal Trial 1 conducted between 1987 and 1990 [7].

The Swedish study showed a statistically significant decreased local recurrence rate in *short course* radiotherapy followed by surgery compared to surgery alone. The local recurrence rate decreased from 27% to 11% ($p < 0.001$), 5-year survival rate increased from 48% with surgery alone to 58% with SCRT followed by surgery ($p = 0.004$). A recent report confirmed that the benefits shown were sustained after a 13-year follow-up. The applied radiation benefit (a reduced local recurrence rate) was briefly called into question after published results of only 8.2% local recurrence in patients treated with the total mesorectal excision technique (TME) only. A crucial question which arose in that period was whether a *short course* radiotherapy only compensated the poor radiotherapy technique. For that reason further studies were conducted to examine the role of *short course* radiotherapy combined with total mesorectal excision.

The study from the Dutch Colorectal Cancer Group randomized patients into a group subjected to preoperative pelvic irradiation at a dose of 5x5 Gy and a control group operated without any irradiation. It was shown that the local recurrence rate after 5 years could be further reduced to 2.4 % even after TME ($p = 0.001$) [8]. Unlike the Swedish study, the Dutch study did not show any improvement in the total survival rate by adding radiotherapy to the treatment.

The study from the Medical Research Council CR07 compared the application of SCRT and surgery to initial surgery and postoperative chemoradiotherapy, the latter being limited to patients with the tumor found on the circumferential resection margin. The percentage of local recurrence was reduced by almost half – from 11% using TME only to 5% by adding SCRT before TME. In this study, pathologists assessed the plane obtained in the resected specimen (mesorectal, intramesorectal and muscularis propria). A larger percent of local recurrence was found in the muscularis propria plane, while a decrease in local recurrence was observed in all

three planes when SCPR was applied [5]. Later studies have confirmed the success of preoperative radiotherapy in reducing local recurrence, even in cases with the optimized surgical technique.

LITERATURE EVIDENCE SUPPORTING APPLICATION OF *LONG COURSE* PREOPERATIVE RADIOCHEMOTHERAPY

This approach was initially developed as a postoperative adjuvant therapy and introduced in Northern American official guidelines since 1990 when adjuvant postoperative chemotherapy and concomitant chemoradiotherapy with 5-FU were recommended for all patients with T₃₋₄ or node-positive rectal cancer.

In Europe, two phase III randomized studies were conducted between 1993 and 2003, comparing long course preoperative radiotherapy (45 Gy in 25 fractions) to concurrent 5-FU/LV with the same radiotherapy protocol. The studies mentioned were FFCD 9203 and EORTC 22921 [9,10]. Both have shown that the addition of concurrent 5-FU/LV causes an acceptable increase of acute toxicity and leads to the pathologic down-staging of the disease. Furthermore, both studies have displayed a decrease in local recurrence rate from 15% to 8-10%, with no difference in the disease free or overall survival.

A third study was conducted by GRCSG (German Rectal Cancer Study Group). According to the treatment results in 823 patients with T₃₋₄ or N+ rectal cancer 5-year survival was 76% and 74% in the groups treated before or after the operation. Local recurrence was 6% in the group with neoadjuvant radiochemotherapy and 13% in the control group with adjuvant radiochemotherapy (p=0.006). Significantly fewer side effects grade 3 and 4 were in neoadjuvant group – 27% than in the adjuvant group – 40% (p=0.001) [11]. Later published results of the same group (GRCSG) after a 11-year follow-up kept the level of statistical significance in terms of local recurrence, with no impact on the length of survival [12].

These results have led to significant changes, the consequence of which is a shift in treatment from postoperative to preoperative chemoradiotherapy. This treatment strategy has been further supported by the results of the NSABP R-03 study.

In that trial, a similar comparison of pre- and postoperative radiotherapy has been carried out which enrolled 267 patients [13]. After a median follow-up of 8.4 years, a significantly better 5-year disease free survival (DFS) in neoadjuvantly treated patients was observed – 64.7 % versus 53.4% (p=0.011). There was no difference in overall survival (OS) of 74.5% vs. 65.6% (p=0.065).

This treatment is widely applied today. Concurrent chemotherapy can be based on the combination of 5-FU/LV or on oral fluoropyrimidines, such as capecitabine.

DIRECT COMPARISON OF SHORT COURSE VS. LONG COURSE RADIOTHERAPY

Two studies have compared *short course* preoperative radiotherapy with *long course* preoperative chemoradiotherapy. Bujko et al. have compared the outcomes of a short-course (5x5 Gy) and long-course (28x1,8 Gy) neoadjuvant chemoradiotherapy. The 5-year local recurrence rates were 9% and 14% ($p = 0.17$) for SCPRT and CRT, respectively. The rate of late complications did not differ significantly – 10% (*short-course*) vs. 7% (*long-course*) [14]. The Trans Tasman Group (TROG) trial also randomized 326 patients with resectable rectal cancer to receive either SCPRT or preoperative CRT to compare the local recurrence rates [6]. Between these two groups no differences were noted in the occurrence of local recurrence, overall survival rate, dissemination and late toxicity.

The radiation technique necessary to reduce the early and late side effects risk has recently not significantly changed. It assumes radiation simulation with the patient in prone position placed on the table with a hole for the abdomen. The small gut should be shown by applying the contrast media and modern tools for radiation planning should be used (3-D).

OXALIPLATIN, IRINOTECAN AND BIOLOGICAL AGENTS IN PREOPERATIVE CHEMORADIOTHERAPY OF RECTAL CANCER

5-FU is fundamental in all chemoradiotherapy and adjuvant chemotherapy protocols used in the treatment of rectal cancer. It is believed that the effectiveness of these protocols can be improved by introducing new drugs successful in the treatment of colorectal cancer and that this can lead to a better control of distal metastases, as well as a higher survival rate. Oxaliplatin administered together with 5-FU and leucovorin has a significant effect in the treatment of metastatic colorectal cancer. This finding has caused an interest in its use as a part of a combined protocol for the treatment of locally advanced disease.

Aschele et al. have conducted a study on the synergistic effect of oxaliplatin (STAR-01) in 747 patients in Italy [15]. The pathological complete response rate was the same (16%) in two randomized groups, and the rate of grade 3 and 4 adverse reactions was 24% in the group with oxaliplatin and 8% in the control group ($p=0.001$).

In the ACCORD study, 598 patients with T₃₋₄N+ rectal cancer were randomized into groups of 45 Gy of radiation in 25 fractions with concomitant capecitabine or 50 Gy in 25 fractions with concomitant capecitabine and oxaliplatin. Complete pathological response was recorded in 19.2% of patients receiving oxaliplatin vs. 13.9% in the control group ($p=0.09$), while grade 3 or 4 toxicity was observed in 25% vs. 11%

in the control group ($p=0.001$). There was no difference in sphincter preservation during surgery (75% vs. 78%) [16]. Somewhat conflicting results were reported in a study performed by Rödel et al. CAO/ARO/AIO-04 where 1236 patients were randomized into groups of neoadjuvant radiotherapy with 5-FU or 5-FU with oxaliplatin [17]. A significantly higher complete pathological regression rate was recorded (17% vs. 13%) in the control group ($p=0.038$) with no significant difference in the incidence of grade 3 and 4 side effects.

Although the incidence of acute side effects was monitored in most studies, they cause only short-term discomfort and stop spontaneously or with symptomatic medication completely. Based on the results of these studies, the adding of oxaliplatin does not contribute to the reduction of the primary rectal tumor in neoadjuvant chemoradiotherapy, but increases the rate of acute side effects [15-17]. Currently, the application of oxaliplatin in the neoadjuvant treatment setting with radiation is not warranted outside of research protocols. Therefore is necessary to wait and observe the results from these studies, particularly in terms of disease free survival (DFS).

Irinotecan administered together with 5-FU and leucovorin has a significant effect in the treatment of metastatic colorectal cancer and it improves both progression-free and overall survival [18]. This finding, the same as in the use of oxaliplatin, has resulted in an interest in developing its use as part of a combined protocol for the treatment of a locally advanced disease. Iles et al. have conducted a study on the synergistic effect of irinotecan and 5-fluorouracil (NCCOG-1) on 31 patients with inoperable locally advanced rectal cancer [19]. Preoperative restaging with MRI showed a reduction in tumor stage in 79% of patients after the treatment, while 81% had clear circumferential resection margins. The regimen was well tolerated.

Gollins et al. have conducted a study on the synergistic effect of capecitabine and irinotecan (NCCOG-2) on 110 patients with MRI-defined locally advanced rectal cancer. The regimen appears more effective in down-staging by using a single-agent, fluoropyrimidine, than historical reports suggest. The pathologic complete response rate was 22% and the study showed a strong correlation between the efficacy of CRT down-staging as expressed by reduction of the histologic stage to ypCR or microfoci and superior overall survival [20]. Currently, the application of irinotecan in the neoadjuvant treatment setting with radiation is being further investigated.

The integration of EGFR and VEGF targeting therapies in preoperative chemoradiation protocols was based on vast theoretical knowledge, relatively good results of preclinical studies and significant effect in the treatment of metastatic colorectal cancer. Phase II studies showed no clear clinical benefit and detected a problem in the use of pCR as the final endpoint [21-23].

The rate of tumor complete response rate (pCR) was somewhat higher in bevacizumab containing protocols, but the incidence of general, bevacizumab-related adverse effects (hypertension, proteinuria, mucosal bleeding, arterial thrombosis) and postoperative complications (pelvic infection, delayed wound healing, anastomotic leak, fistulas) was relatively high in several trials [21-23]. A better understanding of the mechanisms responsible for the disappointing results is necessary before proceeding to phase III trials.

ASSESSMENT OF TREATMENT EFFECT

Nowadays there are great expectations on the treatment outcome forecast based on biological evidence of heterogeneity which determines the tumor (presence of EGFR expression, mutation status of BRAF, dMMR and KRAS).

Studying KRAS, BRAF and PI3KCA mutations in rectal cancer, Derbel et al. have failed to determine the predictors of the outcome of neoadjuvant treatment in a group of 98 patients [21]. They concluded that further research on a larger number of patients and greater statistical power was needed.

In the last decade, the search for biological markers that predict clinical outcomes has shifted toward the microenvironment in which the tumor grows. Gallon et al. have found that complete histologic regression occurred significantly more frequently in patients with a relative number of lymphocytes in the WBC >26% before treatment ($p=0.023$), absolute lymphocyte count $>1,634 \times 10^9/L$ ($p=0.004$), hemoglobin levels >12.0 g/dL before treatment and clinical findings of N – stage disease ($p=0.018$) [22].

It is possible to find similar claims in the literature about the beneficial effects of high levels of circulating lymphocytes on the outcome of the treatment of colorectal cancer [25,23]. A biological marker cluster which correlates to clinical outcomes is still unknown.

A further question is if we can obtain an adequate characterization of tumor heterogeneity based on clinical observation. According to recently published pooled data of randomized trials for locally advanced rectal cancer, there are four groups of patients with different tumor heterogeneity (very good, good, bad, ugly) [24]. In clinical trials, all of them were included and there is no reliable endpoint that could be used to estimate a beneficial impact of treatment on the overall survival. With rectal cancer there are no reliable surrogate endpoints as with other cancers (for example: prostate specific antigen is a good surrogate endpoint for survival in prostate cancer). The fractions of ugly and bad tumors conceal the treatment benefits of the group of good and best, thus preventing us from tailoring therapy on individual ba-

sis. Due to this inability, many characteristics of colorectal cancer were investigated with the intention of discovering possible factors which could predict the outcome of neoadjuvant chemoradiotherapy: tumor volume, the level of carcinoembryonic antigen (CEA), tumor distance from the anal verge and the period of time between the completion of radiotherapy and the definitive surgery [25,26].

A complete pathological response is associated with a significantly longer survival, while the N+ lymph node finding after neoadjuvant treatment is an unfavorable prognostic indicator regardless of the extent of the primary tumor regression [27,28]. The meta-analysis done by Lee et al. has demonstrated that the partial regression of tumor is associated with a 50% improvement in the length of disease free survival (DFS) and should be considered a favorable prognostic factor [29].

PET/CT imaging and the accumulation of FDG in tumor tissue (SUV) could be a predictor of tumor regression with 81 % sensitivity and 100 % specificity, with a 90% of overall reliability, as demonstrated by Bampo et al. [30]. Early recording showed no significant regularity, but the results of the SUV later recording was different between patients with complete and incomplete regression at the level of $p=0.006$. The possibility of distinguishing complete from incomplete tumor regression before histological examination after 6 weeks of radiotherapy (ie. before surgery) could lead to a less radical operation (the gospel only crop scar on the intestine and establish continuity) or even to a wait-and-see attitude and omitted surgery. The PET/CT with biological predictors could help in screening patients and determining the optimal timing for accessing surgery or even cancel the previously planned surgery [31].

While it is scientifically proven that complete regression after neoadjuvant treatment significantly prolongs the length of the disease free survival, the prognostic value of partial regression is less reliable. Several authors propose their own ways of scoring, eg. "tumor regression grade" (TRG) or "rectal cancer regression grade" (RCRG) in order to facilitate the further research of this issue. These schemes, if proven their reliability in the future, may become more important than the current TNM system introduced before the invention of neoadjuvant treatment approach. By then, the classification of tumor characteristics according to the TNM system should mark the way data are collected (eg, the suffix "c" – clinically, "mr" – by NMR, "p" – histologically, "ct" by CT scan). It can somewhat reliably predict the course of illness in patients with complete tumor regression or those with no response to neoadjuvant treatment.

We aim to achieve individual treatment by using the primary and surrogate endpoints based on the data obtained from clinical characteristics of patients and tumor heterogeneity.

Statistical prediction models have found multivariate correlations between the patient and the tumor characteristics with the outcome. One of the best and simplest methods with possible wide application is the interpretation of the prediction model with the help of a nomogram or calculator. It is a visual and numerical representation of mathematical relations between individual variables important for predicting patients' outcomes. The first step in compiling nomograms/calculators is gathering epidemiological data from large registries of outcomes of malignancies.

Bowles et al. have developed a mathematical model of interactive likelihood of 5- and 10-year survival by using the data on age, sex, race, tumor differentiation and the type of surgery [32]. Covariances were preoperative or postoperative radiotherapy, patients without treatment or stage IV disease. They have created the online calculator that can be used with reference to page www.mdanderson.org/rectalcalculator. Assessment results are valuable, but only orienteering aid in predicting the course of disease and the planning of further treatment or intensity of monitoring.

The possible subject of further research will be the prognostic significance of pelvic lymph node status after partial tumor regression, which will probably help us to further stratify the same group of patients and contribute to a better understanding of the disease.

DO ALL THE PATIENTS NEED SURGICAL PROCEDURE AFTER NEOADJUVANT THERAPY?

The optimal time for surgery after completion of a neoadjuvant radiotherapy is difficult to be unambiguously determined. On the basis of published results, there are indications that an interval longer than the generally accepted (6 weeks) could lead to higher rates of complete regression and operations with sphincter preservation. Arguments for the expectative attitude could result from the research that has already begun. In the group of 70 patients with low rectal tumors located stage T_2 , N_{0-2} , M_0 , Habr-Gama et al. have applied 54 Gy of radiation plus chemotherapy 5-FU/LV for 6 cycles every 21 days [33]. Complete clinical remission has been observed in 68 % of patients. Local recurrence occurred in this group to 17% in the first year, 10% after the second year, and 57 % is healthy after 5 years of follow-up. A total of 50 % of the patients was never operated. In these stages of the disease and the specific position of the tumor it is a significant contribution to the quality of life. Careful follow-up enabled a timely "salvage" operation in case of need.

An interesting Rapido study has just started in which two groups were randomized: a control group with conventional neoadjuvant therapy (50 Gy with capecitabine) and an experimental group where the introductory 5x5 Gy of radiation were

followed by six cycles of chemotherapy with capecitabine and oxaliplatin. Upon completion of the neoadjuvant treatment, all patients will be operated according to the principle of TME. The studied outcome will be not only the rate of local recurrence, but especially the impact on overall survival without signs of disease [34].

In a certain way, the above mentioned studies attempt to detect and separate the group of patients who have tumors with favorable clinical and pathological characteristics, and those who have already in the epidemiological studies been classified into the group of patients with very good and good tumors, as well as ensure them a more conservative treatment approach.

RESULTS OF NEOADJUVANT TREATMENT IN UNIVERSITY HOSPITAL FOR TUMORS IN ZAGREB, CROATIA

Between January 2011 and December 2014 at the University Hospital for Tumors, we have treated 78 patients with T₂₋₄N₊ rectal tumors by using two preoperative treatment approaches. We have used *short course* (SCRT) radiotherapy and *long course* chemoradiotherapy (LCCRT) protocols. Clinical and pathological characteristics of all of the patients were defined: gender, age, T and N stages of the disease, and tumor position (distance measured from the anal verge – “low” (0-5 cm), “medium” (5-10 cm) and “high” 10-15 cm)). Mesorectal fascia infiltration was assessed with the use of magnetic resonance imaging (MRI). Fifteen patients have received a total dose of 25 Gy in 5 fractions (*short course*). In accordance with current guidelines, the total treatment duration, including surgical procedure, was 10 days. The remaining 63 patients have received a total dose of 45-50.4 Gy in 25-28 fractions with concomitant chemotherapy based on fluoropyrimidines. The patients underwent an operation in 6 to 8 weeks after the combined treatment was completed. We have performed postoperative analyses of the type of surgical procedure, the degree of histological response according to the Ryan classification (degrees of regression – 0, 1, 2, 3) and the application of postoperative systemic treatment.

Concomitant chemoradiotherapy induced a complete response in 14.3% patients and a partial response in 52.4% patients. In the group of patients treated with *short course* protocol, there was only one patient with a partial response, and none with a complete response. In the group of patients treated with a *long course* chemoradiotherapy, a statistically significant difference was noted in the T and N stages of the disease and in the circumferential resection margin infiltration.

These results are a consequence of treating a larger percent of patients in advanced stages of the disease with concomitant chemoradiotherapy. Out of 25 patients with “low” tumors (< 5cm), a local recurrence occurred in one patient treated

with SC and in one patient treated with long course chemoradiotherapy. Among the patients treated with SCRT, one patient developed local recurrence after 15 months of follow-up, while in the LCCRT group six patients developed local recurrence after a median follow-up of 14.7 months (range 3–26 months). No statistically significant difference in the cumulative incidence of local recurrence (HR = 2.2 CI= 0,3581-10,273, $p=0.4469$) was noted when comparing patients treated with SCRT to those treated with LCCRT. There was no statistically significant correlation between positive lymph nodes and distal metastases, although a positive trend was noted. Anal sphincter preservation amounted to 73.3% in the group treated with SCRT, as compared to 31.7% in the group treated with LCCRT.

So, a longer patient follow-up, an individualized approach and gathering experience will enable further improvements in the treatment of these patients.

CONCLUSION

Despite the fact that previous studies have answered some very important questions, the problem of taking care of patients belonging to neither group remains. Consequently, two consensus documents (EURECCA and ESMO) have been produced, with guidelines on different strategies for staging and treatment with the aim of helping clinicians in their everyday work [35,36].

References

- [1] Arredondo J, Baixauli J, Beorlegui C, Arbea L, Rodríguez J, Sola JJ, et al. Prognosis factors for recurrence in patients with locally advanced rectal cancer preoperatively treated with chemoradiotherapy and adjuvant chemotherapy. *Dis Colon Rectum*. 2013;56(4):416-21. doi: 10.1097/DCR.0b013e318274d9c6.
- [2] Chauvenet M, Lepage C, Jooste V, Cottet V, Faivre J, Bouvier AM. Prevalence of patients with colorectal cancer requiring follow-up or active treatment. *Eur J Cancer*. 2009;45(8):1460-5. doi:10.1016/j.ejca.2009.01.012.
- [3] Fisher B, Wolmark N, Rockette H, Redmond C, Deutsch M, Wickerham DL, et al. Postoperative adjuvant chemotherapy or radiation therapy for rectal cancer: results from NSABP protocol R-01. *J Natl Cancer Inst*. 1988;80(1):21-9.
- [4] Wolmark N, Wieand HS, Hyams DM, Colangelo L, Dimitrov NV, Romond EH, et al. Randomized trial of postoperative adjuvant chemotherapy with or without radiotherapy for carcinoma of the rectum: National Surgical Adjuvant Breast and Bowel Project Protocol R-02. *J Natl Cancer Inst*. 2000;92(5):388-96.
- [5] Sebag-Montefiore D, Stephens RJ, Steele R, Monson J, Grieve R, Khanna S, et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a mul-

- ticentre, randomised trial. *Lancet*. 2009;373(9666):811-20. doi: 10.1016/S0140-6736(09)60484-0.
- [6] Ngan SY, Burmeister B, Fisher RJ, Solomon M, Goldstein D, Joseph D, et al. Randomized trial of short-course radiotherapy versus long-course chemoradiation comparing rates of local recurrence in patients with T3 rectal cancer: Trans-Tasman Radiation Oncology Group trial 01.04. *J Clin Oncol*. 2012;30(31):3827-33. doi: 10.1200/JCO.2012.42.9597.
- [7] Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. *N Engl J Med*. 1997;336(14):980-7.
- [8] Kapiteijn E, Marijnen CA, Nagtegaal ID, Putter H, Steup WH, Wiggers T, et al; Dutch Colorectal Cancer Group. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. *N Engl J Med*. 2001;345(9):638-46.
- [9] Gérard JP, Conroy T, Bonnetain F, Bouché O, Chapet O, Closon-Dejardin MT, et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol*. 2006;24(28):4620-5.
- [10] Bosset JF, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, et al; EORTC Radiotherapy Group Trial 22921. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med*. 2006;355(11):1114-23.
- [11] Sauer R, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, et al; German Rectal Cancer Study Group. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004;351(17):1731-40.
- [12] Sauer R, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. *J Clin Oncol*. 2012;30(16):1926-33. doi: 10.1200/JCO.2011.40.1836.
- [13] Roh MS, Colangelo LH, O'Connell MJ, Yothers G, Deutsch M, Allegra CJ, et al. Preoperative multimodality therapy improves disease-free survival in patients with carcinoma of the rectum: NSABP R-03. *J Clin Oncol*. 2009;27(31):5124-30. doi: 10.1200/JCO.2009.22.0467.
- [14] Bujko K, Nowacki MP, Nasierowska-Guttmejer A, Michalski W, Bebenek M, Kryj M. Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. *Br J Surg*. 2006 Oct;93(10):1215-23.
- [15] Aschele C, Cionini L, Lonardi S, Pinto C, Cordio S, Rosati G, et al. Primary tumor response to preoperative chemoradiation with or without oxaliplatin in locally advanced rectal cancer: pathologic results of the STAR-01 randomized phase III trial. *J Clin Oncol*. 2011;29(20):2773-80. doi: 10.1200/JCO.2010.34.4911.

- [16] Gérard JP, Azria D, Gourgou-Bourgade S, Martel-Laffay I, Hennequin C, Etienne PL, et al. Comparison of two neoadjuvant chemoradiotherapy regimens for locally advanced rectal cancer: results of the phase III trial ACCORD 12/0405-Prodige 2. *J Clin Oncol*. 2010;28(10):1638-44. doi: 10.1200/JCO.2009.25.8376.
- [17] Rödel C, Liersch T, Becker H, Fietkau R, Hohenberger W, Hothorn T, et al; German Rectal Cancer Study Group. Preoperative chemoradiotherapy and postoperative chemotherapy with fluorouracil and oxaliplatin versus fluorouracil alone in locally advanced rectal cancer: initial results of the German CAO/ARO/AIO-04 randomised phase 3 trial. *Lancet Oncol*. 2012;13(7):679-87. doi: 10.1016/S1470-2045(12)70187-0. Epub
- [18] Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med*. 2000;343(13):905-14.
- [19] Iles S, Gollins S, Susnerwala S, Haylock B, Myint S, Biswas A, et al. Irinotecan+5-fluorouracil with concomitant pre-operative radiotherapy in locally advanced non-resectable rectal cancer: a phase I/II study. *Br J Cancer*. 2008;98(7):1210-6. doi: 10.1038/sj.bjc.6604292.
- [20] Gollins S, Sun Myint A, Haylock B, Wise M, Saunders M, Neupane R, et al. Preoperative chemoradiotherapy using concurrent capecitabine and irinotecan in magnetic resonance imaging-defined locally advanced rectal cancer: impact on long-term clinical outcomes. *J Clin Oncol*. 2011;29(8):1042-9. doi: 10.1200/JCO.2010.29.7697.
- [21] Derbel O, Wang Q, Desseigne F, Rivoire M, Meeus P, Peyrat P, et al. Impact of KRAS, BRAF and PI3KCA mutations in rectal carcinomas treated with neoadjuvant radiochemotherapy and surgery. *BMC Cancer*. 2013;13:200. doi: 10.1186/1471-2407-13-200.
- [22] Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006;313(5795):1960-4.
- [23] Morris M, Platell C, Iacopetta B. Tumor-infiltrating lymphocytes and perforation in colon cancer predict positive response to 5-fluorouracil chemotherapy. *Clin Cancer Res*. 2008;14(5):1413-7. doi: 10.1158/1078-0432.CCR-07-1994.
- [24] Valentini V, van Stiphout RG, Lammering G, Gambacorta MA, Barba MC, Bebenek M, et al. Selection of appropriate end-points (pCR vs 2yDFS) for tailoring treatments with prediction models in locally advanced rectal cancer. *Radiother Oncol*. 2015 Feb 21. pii: S0167-8140(15)00074-2. doi: 10.1016/j.radonc.2015.02.001.
- [25] Das P, Skibber JM, Rodriguez-Bigas MA, Feig BW, Chang GJ, Wolff RA, et al. Predictors of tumor response and downstaging in patients who receive preoperative chemoradiation for rectal cancer. *Cancer*. 2007;109(9):1750-5.

- [26] Kalady MF, de Campos-Lobato LF, Stocchi L, Geisler DP, Dietz D, Lavery IC, et al. Predictive factors of pathologic complete response after neoadjuvant chemoradiation for rectal cancer. *Ann Surg.* 2009;250(4):582-9. doi: 10.1097/SLA.0b013e3181b91e63. PubMed PMID: 19710605.
- [27] Yeo SG, Kim DY, Kim TH, Chang HJ, Oh JH, Park W, et al. Pathologic complete response of primary tumor following preoperative chemoradiotherapy for locally advanced rectal cancer: long-term outcomes and prognostic significance of pathologic nodal status (KROG 09-01). *Ann Surg.* 2010;252(6):998-1004. doi: 10.1097/SLA.0b013e3181f3f1b1.
- [28] Kaminsky-Forreth MC, Conroy T, Luporsi E, Peiffert D, Lapeyre M, Boissel P, et al. Prognostic implications of downstaging following preoperative radiation therapy for operable T3-T4 rectal cancer. *Int J Radiat Oncol Biol Phys.* 1998;42(5):935-41.
- [29] Lee YC, Hsieh CC, Chuang JP. Prognostic significance of partial tumor regression after preoperative chemoradiotherapy for rectal cancer: a meta-analysis. *Dis Colon Rectum.* 2013;56(9):1093-101. doi: 10.1097/DCR.0b013e318298e36b.
- [30] Bampo C, Alessi A, Fantini S, Bertarelli G, de Braud F, Bombardieri E, et al. Is the standardized uptake value of FDG-PET/CT predictive of pathological complete response in locally advanced rectal cancer treated with capecitabine-based neoadjuvant chemoradiation? *Oncology.* 2013;84(4):191-9. doi: 10.1159/000345601.
- [31] Foster JD, Jones EL, Falk S, Cooper EJ, Francis NK. Timing of surgery after long-course neoadjuvant chemoradiotherapy for rectal cancer: a systematic review of the literature. *Dis Colon Rectum.* 2013;56(7):921-30. doi: 10.1097/DCR.0b013e31828aedcb. Review. PubMed PMID: 23739201.
- [32] Bowles TL, Hu CY, You NY, Skibber JM, Rodriguez-Bigas MA, Chang GJ. An individualized conditional survival calculator for patients with rectal cancer. *Dis Colon Rectum.* 2013;56(5):551-9. doi: 10.1097/DCR.0b013e31827bd287.
- [33] Habr-Gama A, Sabbaga J, Gama-Rodrigues J, São Julião GP, Proscurshim I, Bailão Aguilar P, et al. Watch and wait approach following extended neoadjuvant chemoradiation for distal rectal cancer: are we getting closer to anal cancer management? *Dis Colon Rectum.* 2013;56(10):1109-17. doi: 10.1097/DCR.0b013e3182a25c4e
- [34] Nilsson PJ, van Etten B, Hospers GA, Pählman L, van de Velde CJ, Beets-Tan RG, et al. Short-course radiotherapy followed by neo-adjuvant chemotherapy in locally advanced rectal cancer-the RAPIDO trial. *BMC Cancer.* 2013;13:279. doi: 10.1186/1471-2407-13-279.
- [35] van de Velde CJ, Boelens PG, Borras JM, Coebergh JW, Cervantes A, Blomqvist L, et al. EURECCA colorectal: multidisciplinary management: European consensus conference colon and rectum. *Eur J Cancer.* 2014;50(1):1.e1-1.e34. doi: 10.1016/j.ejca.2013.06.048.

- [36] Glimelius B, Tiret E, Cervantes A, Arnold D; ESMO Guidelines Working Group. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2013;24 Suppl 6:vi81-8. doi: 10.1093/annonc/mdt240.

Sažetak

Suvremeno liječenje karcinoma rektuma

Posljednjih desetljeća svjedoci smo velikog napretka u liječenju karcinoma rektuma. Razvoj novih kirurških tehnika, magnetske rezonance kao optimalnog dijagnostičkog alata i novih histopatoloških tehnika evaluacije reseciranog uzorka omogućili su dio ovih promjena. Ostatak je posljedica razvoja sofisticiranih radioterapijskih tehnika koje u kombinaciji s novim citostaticima i pametnim lijekovima postižu bolji odgovor tumora na primijenjeno liječenje uz smanjenje toksičnosti. Iako je svaka disciplina doprinijela boljem razumijevanju bolesti, tek je multidisciplinarni pristup omogućio sadašnju razinu uspjeha liječenja.

Ključne riječi: neoadjuvantna terapija; kratki radioterapijski protokol; dugi radioterapijski protokol; konkomitatna kemoterapija; patološki kompletni odgovor.

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CUTANEOUS SIDE EFFECTS OF TARGETED THERAPY FOR COLORECTAL CARCINOMA

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Summary

Research in cancer development has led to the new treatment strategies based on gene and protein changes in cells that cause cancer. Such targeted drugs work differently than standard chemotherapy agents, and have different and usually less severe side effects.

Targeted chemotherapies have, however, enormous cutaneous adverse events which may lead to poor adherence, dose interruption and discontinuation of these therapeutic regimens. Skin changes have a significant influence on the quality of life of affected patients.

In colorectal cancer, several targeted drugs are currently employed. Agents administered in colorectal cancer that have resulted in significant cutaneous side effects include primarily the epidermal growth factor receptor inhibitors (EGFRIs) and capecitabine.

Common EGFRIs dermatologic adverse events are acneiform rash and xerosis. Less common findings are paronychia, regulatory abnormalities of hair growth, maculopapular rash, mucositis and postinflammatory hyperpigmentation. Management of skin rash should be individualized for each patient, depending on the type, severity and location of the skin toxicity caused by EGFRIs.

Hand-foot syndrome (HFS), or palmar-plantar erythrodysesthesia, is the most common dose-limiting toxicity and the only clinically significant adverse event that frequently occurs with capecitabine compared to 5-FU/leucovorin intravenous administration.

Cutaneous side effects affect compliance, patients' quality of life, as well as the therapy regimen.

It is important to recognize and treat, as well as to administer efficacious prophylactic and therapeutic measures in a timely manner. This enables regular administration of adequate chemotherapy regimens and prolongs lives of oncology patients.

Studies have demonstrated a positive correlation between treatment efficacy and cutaneous side effects for both EGFRi and capecitabine. Therefore, cutaneous side effects can serve as a predictor of improved survival in these patients.

Since frequency and severity of skin lesions are dose-dependent, a gradual increase in dose until a cutaneous side-effect develops might be a good strategy to maximize the efficacy of EGFRi and capecitabine.

Keywords: targeted therapy; cutaneous side effects; colorectal cancer.

INTRODUCTION

Research in cancer development has led to the new treatment strategies based on gene and protein changes in cells that cause cancer. Such targeted drugs work differently than standard chemotherapy agents, and have different and usually less severe side effects. In colorectal cancer several targeted drugs are currently employed. Vascular endothelial growth factor (VEGF) inhibitors include bevacizumab (Avastin®) and ziv-aflibercept (Zaltrap®). Epidermal growth factor receptor (EGFR) targeted drugs include cetuximab (Erbix®) and panitumumab (Vectibix®). Rigosafenib (Stivarga®) is another targeted drug for advanced colorectal cancer and is known as kinase inhibitor [1].

Capecitabine is an oral fluoropyrimidine widely administered in patients with colorectal cancer [2].

Targeted chemotherapies have, however, enormous cutaneous adverse events which may lead to poor adherence, dose interruption and discontinuation of these therapeutic regimens. Skin changes have a significant influence on the quality of life of affected patients [3].

Agents administered in colorectal cancer that result in significant cutaneous side effects include primarily EGFRi and capecitabine. Therefore, this article will in particular focus on side effects occurring in these two therapy regimens.

EGFRi

The epidermal growth factor receptor (EGFR) is overexpressed or dysregulated in several solid tumors including lung, head and neck and colorectal carcinoma. Dysregulated EGFR may result in uncontrolled cell growth, proliferation and angiogenesis and is associated with poorer prognoses, manifested by increased metastatic potential and poorer overall survival (OS). Thus, EGFR is an ideal target for antitumor therapy. EGFR inhibitors (EGFRi) include tyrosin

kinase inhibitors (erlotinib and gefitinib) and the monoclonal antibodies (panitumumab, cetuximab) that competitively bind to the extracellular domain of EGFR and block cytoplasmic-domain phosphorylation. EGFR blockade results in growth arrest and apoptosis in cells dependent on EGFR for survival through the inhibition of downstream pathways [4,5].

As a class, EGFRIs are characterized by cutaneous adverse effects, mainly papulopustular reactions involving skin. In addition to leaving skin vulnerable to bacterial overgrowth and serious infection, skin rash can lead to dose modification or treatment discontinuation, potentially affecting the overall clinical benefits of the therapy [5].

EGFRI – cutaneous side effects

Pathobiology of EGFRI – associated cutaneous toxicity

EGFRIs affect basal keratinocytes which results in some of the cutaneous side effects. Inhibition of the EGFR mediated signaling pathways induces in keratinocytes growth arrest and apoptosis, decreasing cell migration, increasing cell attachment and differentiation and stimulating inflammation. All of these result in distinctive cutaneous manifestations [4].

Due to the interference of epidermal growth factor signaling in the skin, cutaneous adverse events are common. Common dermatologic adverse events are acneiform rash and xerosis [3].

Acneiform (papulopustular) rash

Acneiform rash is the most common EGFRI cutaneous adverse event reported in 50-100% of clinical trials. It can appear between day 2 to week 6 following initiation of therapy. Eruption is present in seborrheic areas and resembles lesions of acne, but comedones, primary acne lesions, are absent. Such lesions can be painful, burning, irritating or pruritic. The periorbital region as well as palms and soles are usually spared [3]. *Figures 1-3* present some of our patients receiving EGFRI with characteristic acneiform lesions. Acneiform rash is consistent with NCI CTCAE, version 4.0, graded into 5 grades, as shown in *table 1* [6]. Lynch et al. described the clinical severity of acneiform rash as presented in *table 2* [7].



Figures 1.-3. Acneiform rash in patients on EGFR therapy

Table 1. Acneiform rash grading*

Grade	Clinical status
1	Papules and/or pustules covering <10%BSA (body surface area), which may or may not be associated with symptoms of pruritus or tenderness
2	Papules and/or pustules covering 10-30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; associated with psychosocial impact; limiting instrumental ADL (activities of daily life)
3	Papules and/or pustules covering >30% BSA, which may or may not be associated with symptoms of pruritus or tenderness; limiting self-care ADL; associated with local superinfection with oral antibiotics indicated
4	Papules and/or pustules covering any % BSA, which may or may not be associated with symptoms of pruritus or tenderness and are associated with extensive superinfection with IV antibiotics indicated; life-threatening consequences
5	Death

*according to NCI CTCAE, version 4.0. [6]

Table 2. Acneiform rash severity*

Toxicity	Description
Mild	Generally localized Minimally symptomatic No impact on ADL No sign of superinfection
Moderate	Generalized Mild symptoms (pruritus, tenderness) Minimal impact on ADL No sign of superinfection
Severe	Generalized Severe symptoms (pruritus, tenderness) Significant impact on ADL Potential for superinfection

*according to Lynch et al. [7]

Acneiform rash treatment

Prophylactic treatment of acneiform rash: based on randomized clinical trials, tetracycline and tetracycline class antibiotics (doxycycline 100 mg bid) for the first 6 weeks are recommended [7-11]. Hydrocortisone 1% combined with moisturizer, avoidance of prolonged sun exposure and application of sunscreen, although lacking evidence, should still be considered as general patient recommendations [3,8].

Reactive treatment

If patients develop EGFRi-associated toxicity, treatment based on clinical severity of the rash is suggested. In mild toxicities only topical therapy such as hydrocortison (1% or 2,5%) cream or clindamycin (1%) gel is recommended. There is no need to alter the EGFRi dosage [7].

In patients who develop moderate toxicity, local therapy as mentioned above is recommended, with an addition of tetracycline class of antibiotics. Doxycycline 2x100 mg bid is mainly advisable due to its more favorable safety profile compared to minocycline [7].

In cases of severe toxicity, reduction in the EGFRi dose is recommended. Therapy recommended for moderate toxicity should be administered, with an addition of a methylprednisolone dose pack. If toxicity does not diminish within 2-4 weeks of therapy interruption of EGFRi treatment is recommended according to the prescribing information [7].

In order to avoid dermal toxicity as well as increased risk for bacterial or viral superinfection, pulsed application of topical corticosteroids is advisable [7].

EGFRi induced acneiform rash differs from acne vulgaris, therefore topical therapy advised for this disease, such as topical retinoids or benzoyl peroxide, is not recommended [12].

Xerosis cutis

Xerosis cutis is the second most common cutaneous adverse event from EGFRi, which occurs in over 35% in most reports. It is presented as a dry, itchy scaly area of the skin and can progress to painful fissuring and eczema (eczema craquelée). It may take place at sites where papulopustules have developed; however, more widespread involvement usually occurs. Symptomatic treatment including use of emollients is advisable [3,12].

In case of occurrence of fissures, which mainly develop on the hands and feet, creams or ointments with dexpanthenol are used to soften the skin and enhance healing [12].

Less common findings are paronychia, regulatory abnormalities of hair growth, maculopapular rash, mucositis and postinflammatory hyperpigmentation [3].

Paronychia

Paronychia is described in 5-20% of patients and usually develops after 1-2 months. In severe cases ingrown nail, periungual abscess and pyogenic granuloma-like lesions can occur [3].

Management strategies include wearing comfortable shoes, trimming nails but avoiding aggressive manicuring, and wearing gloves while cleaning. Topical corticosteroids and anti-inflammatory dose tetracyclines to decrease periungual inflammation and antibiotic soaks are advisable. Electrocautery, silver-nitrate and nail avulsion are recommended to eliminate excessive granulation tissue [8].

Hair growth disturbances

Abnormalities of hair growth occur infrequently and usually develop 2-5 months after initiating EGFRi. Trichomegaly and facial hypertrichosis (hirsutism) can be seen after the first 1-2 months of therapy, and they tend to persist for the duration of EGFRi therapy. Temporary or permanent hair removal may be advised. Aberrant eyelashes may lead to corneal irritation and ulceration. Trichomegaly can be treated with lash clipping every 2-4 weeks and referral to an ophthalmologist is indicated for patients with irritation or persistent discomfort [3,8].

Non-scarring alopecia may occur after 2-3 months of therapy. It may progress to diffuse alopecia and generally resolves after treatment discontinuation, although hair regrowth may be of varying quality. Minoxidil 2% and 5% may be used. Scarring alopecia has also been reported, and treatment options include topical corticosteroids (shampoos, creams and lotions), [8].

Mucositis

Oral mucositis is rare in EGFRi treated patients and may present as erythema, aphthous-like stomatitis or superimposed upon those of radiation and conventional chemotherapy. Prevention and treatment of oral complications include thorough oral care, aggressive pain management with topical analgesics and systemic analgesics as needed, adequate nutritional support, radiation treatment planning that optimizes therapeutic index, benzydamine oral rinse [8].

Postinflammatory hyperpigmentation

Postinflammatory hyperpigmentation occurs in approximately 10% of patients with acneiform rash. It is therefore recommended that patients take appropriate sun protective measures while receiving EGFRis [13].

Management of skin rash should be individualized for each patient, depending on the type, severity and location of the skin toxicity caused by EGFRis [5].

Patient should be referred to a dermatologist in following situations:

- 1) If the skin toxicity does not improve within 1-2 weeks of treatment;
- 2) If the patient is severely symptomatic;
- 3) If the skin toxicity has an uncharacteristic appearance or distribution [5].

The effect of EGFRi and concomitant radiotherapy

EGFRi are very often administered concomitantly with radiotherapy. Effects can be divided into the early and late phases. In the early phase, when EGFRi are introduced simultaneously with radiation compared to radiation alone, radiation dermatitis as well as EGFRi side effects increase. Skin eruption occurs predominantly in irradiated areas. This is due to synergistic cytotoxicity and the therapeutic response of to two agents [3].

In the late phase, however, prolonged radiation leads to the absence of skin toxicity to EGFRi in the preirradiated area. The reason for this is depletion of basal layer stem cells due to apoptosis induced by radiation [3].

EGFRi efficacy and severity of skin rash

It has been shown that the presence and severity of cutaneous side effects have a positive correlation with patient survival and tumor response. Since frequency and severity of skin lesions are dose dependent, a gradual increase in dose until skin eruption develops may be a good strategy to maximize the efficacy of EGFRi [3,7].

Capecitabine

Capecitabine is registered for the treatment of several tumor entities including colorectal-, gastric- and breast cancer. It is an orally administered 5-fluorouracil (5-FU) prodrug designed to mimic the pharmacokinetics of infusional 5-FU [14].

It is generally well-tolerated, with hand foot syndrome being the only clinical adverse event that commonly occurs with capecitabine treatment [2].

Hand-foot syndrome (HFS), or palmar-plantar erythrodysesthesia is the most common dose-limiting toxicity and the only clinically significant adverse event that frequently occurs with capecitabine compared to 5-FU/leucovorin intravenous administration [2]. HFS incidence in patients receiving capecitabine is 50-60%, while grade III toxicity appears in 10-17% of patients [15].

This side effect is common with administration of some other cytotoxic drugs such as continuous 5-FU infusion, doxorubicin, cytarabine or docetaxel [16].

The mechanism responsible for HFS is unknown, although it is frequently considered as a type of inflammation [15]. Recent data indicate that HFSR (Hand-foot-skin-reaction) may be caused by a reduction in the oxidative potential of the skin due to intensive radical formation [14].

The risk factors of HFS have been reported to be older age, female sex, improved performance, continuous infusion of chemotherapy agents as well as inclusion of docetaxel in chemotherapy regimen [15].

Patients usually present with a prodrome of dysesthesia affecting palms and soles. The condition progresses in a few days to burning pain sensations together with well-defined swelling and erythema. If the drug dose is not adjusted there may be skin breakdown, desquamation, bullous formation or secondary infections. This is the reason why HFS can lead to reduced compliance and quality of life [2]. Clinical aspects of hand foot syndrome in our patients are shown in *Figures 4-7*. The hand-foot syndrome grading scale is shown in *Table 3* [6]. Treatment-options for HFS are described in *Table 4* [2].



Figures 4.-7. Hand foot syndrome in patients receiving capecitabine.

Table 3. Hand-foot syndrome grading*

Grade	Clinical status
1	Minimal skin changes or dermatitis (erythema, edema, hyperkeratosis) <u>without pain</u>
2	Skin changes (peeling, blisters, bleeding, edema or hyperkeratosis) <u>with pain</u> , limiting instrumental ADL
3	Severe skin changes (peeling, blisters, bleeding, edema, hyperkeratosis) with pain; limiting self-care ADL

*according to NCI CTCAE, version 4.0.[6]

Table 4. Management of the hand- foot syndrome*

Grade I	Maintain dose Apply skin barrier cream and apply MEBO (moist exposed burn ointment)
Grade II	Maintain or reduce dose by 25% Apply MEBO Supportive care
Grade III	Interrupt one cycle Adjust dose Apply MEBO Supportive care
Supportive care	Avoid extremes in temperature, pressure and friction of hand and foot Cooling the hand and foot Cushioning sore skin Keeping the skin exposed to air Preventing excess sweating

*according to Son et al [2]

Zhang et al. demonstrated in a single-center, prospective, randomized phase II trial that celecoxib (COX-2 inhibitor) can prevent capecitabine-related hand foot syndrome in stage II and III colorectal cancer patients [15].

Several studies confirmed that HFSR is a predictor of improved survival in patients with colorectal cancer [14,17,18]. A study by Hofheniz et al. indicates that the development of HFSR may be regarded as an independent clinical predictor of improved survival in patients with colorectal cancer regardless of the time point of HFSR development. Testing individual dose optimization or titration of capecitabine according to development HFSR may be an appealing strategy for future studies [14].

Azuma et al. confirmed significant association between the hand-foot syndrome and the efficacy of capecitabine in patients with metastatic breast cancer [19].

CONCLUSION

Cutaneous side effects affect compliance, patients' quality of life, as well as the therapy regimen.

It is important to recognize and treat as well as to administer efficacious prophylactic and therapeutic measures in a timely manner. This enables regular administration of adequate chemotherapy regimens and prolongs the lives of oncology patients.

Studies have demonstrated a positive correlation between treatment efficacy and cutaneous side effects for both EGFRi and capecitabine. Therefore, cutaneous side effects can serve as a predictor of improved survival in these patients.

Since frequency and severity of skin lesions are dose-dependent, a gradual increase in dose until skin side-effect develops may be a good strategy to maximize the efficacy of EGFRi and capecitabine.

References

- [1] American Cancer Society. Targeted therapies for colorectal cancer. Available from: www.cancer.org/cancer/colonandrectumcancer.
- [2] Son HS, Lee WY, Yun SH, Chun H-K. Compliance and effective management of the hand-foot syndrome in colon cancer patients receiving capecitabine as adjuvant chemotherapy. *Yonsei Med J.* 2009;(50): 796-802.
- [3] Chanprapaph K, Vachiramon V, Rattanakaemakorn P. Epidermal growth factor receptor inhibitors: a review of cutaneous adverse events and management. *Dermatology Research and Practice.* 2014. <http://dx.doi.org/10.155/2014/734249>
- [4] Lacoutre ME. Mechanisms of cutaneous toxicities to EGFR inhibitors. Available from: <http://www.medscape.com>
- [5] Melosky B, Burkes R, Ryson D, Alcindor T, Shear N, Lacouture M. Management of skin rash during EGFR-targeted monoclonal antibody treatment for gastrointestinal malignancies: Canadian recommendations. *Curr Oncol.* 2009; 16(1):16-26.
- [6] Common Terminology Criteria for Adverse Events (CTCAE)/NCI CTCAE, version 4.0., 2010. Available from: http://www.evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14.
- [7] Lynch TJ, Kim ES, Eaby B, Garey J, West D, Lacouture ME. Epidermal growth factor receptor inhibitor – associated cutaneous toxicities: an evolving paradigm in clinical management. *The Oncologist.* 2007; 12:610-621.
- [8] Lacouture ME, Anadkat MJ, Bensadoun RJ, Bryce J, Chan A, Epstein JB, et al. Clinical practice guidelines for the prevention and treatment of EGFR inhibitor-associated dermatologic toxicities. *Support Care Cancer.* 2011; 19:1079-1095.

- [9] Scope A, Agero ALC, Dusza W et al. Randomized double-blind trial of prophylactic oral minocycline and topical tazarotene for cetuximab-associated acne-like eruption. *J Clin Oncol.* 2007; 25 (34): 5390-5396.
- [10] Jatoi A, Rowland K, Sloan JA, Gross HM, Fishkin PA, Kahanic SP, et al. Tetracycline to prevent epidermal growth factor receptor inhibitor-induced skin rashes: results of placebo-controlled trial from the North Central Cancer Treatment Group. *Cancer.* 2008; 113 (4): 847-853.
- [11] Lacouture ME, Mitchell EP, Piperdi B, Pillai MV, Shearer H, Iannotti N, et al. Skin toxicity evaluation protocol with panitumumab, a phase II open-label, randomized trial evaluating the impact of a pre-emptive skin treatment regimen on skin toxicities and quality of life in patients with metastatic colorectal cancer. *J Clin Oncol.* 2010; 28 (8): 1351-1357.
- [12] Ocvirk J, Cencelj S. Management of cutaneous side-effects of cetuximab therapy in patients with metastatic colorectal cancer. *J Eur Acad Dermatol Venereol.* 2010; 24(4):453-9.
- [13] Gutzmer R, Werfel T, Kapp A, Elsner J. Cutaneous side effects of EGF-receptor inhibition and their management. *Hautarzt.* 2006; 57:509-513.
- [14] Hofheinz RD, Heinemann V, von Weikersthal LF, Laubender RP, Gencer D, Burkhöfer I, et al. Capecitabine-associated hand-foot-skin reaction is an independent clinical predictor of improved survival in patients with colorectal cancer. *Br J Cancer.* 2012;107(10):1678-83.
- [15] Zhang RX, Wu XJ, Wan DS, Lu ZH, Kong LH, Pan ZZ, et al. Celecoxib can prevent capecitabine-related hand-foot syndrome in stage II and III colorectal patients: result of a single-center, prospective randomized phase III trial. *Ann Oncol.* 2012; 23(5): 1348-1353.
- [16] Webster-Gandy JD, How C, Harrold K. Palmar-plantar erythrodysesthesia (PPE): a literature review with commentary on experience in a cancer centre. *Eur J Oncol Nurs.* 2007;11:238-46.
- [17] Stintzing S, Fischer von Weikersthal L, Vehling-Kaiser U, Stauch M, Hass HG, Dietzfelbinger H et al. Correlation of capecitabine-induced skin toxicity with treatment efficacy in patients with metastatic colorectal cancer: results from the German AIO KRK-0104 trial. *Br J Cancer.* 2011; 105(2):206-211. 18. Twelves C, Scheithauer W, McKendrick J, Seitz JF, Van Hazel G, Wong A et al. Capecitabine versus 5-fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: final results from the X-ACT trial with analysis by age and preliminary evidence of a pharmacodynamic marker of efficacy. *Ann Oncol.* 2012; 23(5):1190-1197.
- [19] Azuma Y, Hata K, Sai K, Udagawa R, Hirakawa A, Tohkin M et al. Significant association between hand-foot syndrome and efficacy of capecitabine in patients with metastatic breast cancer. *Biol Pharm Bull.* 2012; 36(5):717-724.

Sažetak

Kožne nuspojave u bolesnika na ciljanoj terapiji raka kolorektuma

Istraživanja nastanka zloćudnih tumora dovela su do novih vrsta liječenja koje se zasnivaju na promjenama gena i proteina u stanicama zloćudnih tumora. Takvi ciljani lijekovi imaju drugačiji mehanizam djelovanja od standardnih citostatika, te obično blaže nuspojave.

Ciljani lijekovi međutim imaju značajne nuspojave na koži koje mogu dovesti do slabije suradljivosti bolesnika, izostavljanja pojedinačne doze ali i prekida liječenja. Promjene na koži znatno utječu na kvalitetu života bolesnika.

U liječenju kolorektalnog karcinoma primjenjuje se nekoliko ciljanih lijekova. Od njih značajne nuspojave na koži imaju inhibitori epidermalnog čimbenika rasta (EGFRI), te kapecitabin.

Česte dermatološke nuspojave EGFRI su akneiformni osip i suhoća kože, dok su manje česti paronihija, poremećaji u rastu dlaka, makulopapulozni osip, mukozitis i postinflamatorne hiperpigmentacije. Liječenje nuspojava na koži treba biti prilagođeno svakom bolesniku, ovisno o vrsti, težini i mjestu nastanka promjena.

Hand-foot syndrom (HFS, sindrom šaka i stopala) ili palmo-plantarna eritrodizesteziya je najčešća toksičnost koja ograničava doziranje lijeka i jedina klinički značajna nuspojava koja se često javlja tijekom liječenja kapecitabinom u usporedbi s intravenskom primjenom 5-FU/leukovorina.

Nuspojave na koži utječu na suradljivost, kvalitetu života bolesnika te provođenje terapije.

Važno je na vrijeme prepoznati, liječiti te primijeniti učinkovite profilaktičke i terapijske mjere. Na taj način se osigurava redovita primjena odgovarajuće terapije i produžava život onkoloških bolesnika.

Kliničkim studijama je dokazana pozitivna povezanost učinkovitosti liječenja onkoloških bolesnika i nuspojava na koži kod uporabe EGFRI i kapecitabina. Stoga nuspojave na koži mogu poslužiti kao prediktor boljeg ishoda bolesnika.

Budući su učestalost i težina nuspojava na koži ovisne o dozi lijeka, postupno povišenje doze do pojave nuspojava na koži može biti dobar način povećanja učinkovitosti kako EGFRI tako i kapecitabina.

Ključne riječi: ciljana terapija; nuspojave na koži; kolorektalni karcinom.

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PHARMACOGENETICS OF CYTOTOXIC THERAPY IN COLORECTAL CANCER

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Summary

Colorectal cancer is a relatively common tumor with incidence that has been increasing during recent decades. Despite the rapid advances in treatment of colorectal cancer, the best way to combine and sequence all available drugs to optimize treatment is not yet established. The majority of cytotoxic drugs used in therapy of colorectal cancer have a dose related effect and narrow therapeutic index. Hence, dosing of cytotoxic drugs is considered very important. Current modalities for therapy and dose selection give restricted possibilities to equalise inter-individual variations which are dependent on physiological, genetic and environmental factors. In this article, an overview of current possibilities of personalized medicine in therapy of colorectal cancer is presented. This includes review of the most important gene polymorphisms important for safety and efficacy of cytotoxic therapy in colorectal cancer, and evaluation of their importance for the current clinical practice. Despite raising knowledge, providing individual treatment with low toxicity and significant benefits is still an unsolved problem. Reasons for this are the lack of knowledge on distribution of these polymorphisms in the population, importance of non-genetic factors, price of genetic testing and still limited data from controlled clinical trials confirming the clinical usefulness of pharmacogenetic testing. Although the initial costs of cancer management and personalized medicine may be high, in the future they may result in significant benefits from both a clinical and economical perspective.

Keywords: colorectal cancer; pharmacogenetics; biomarkers; personalised medicine.

Background

Colorectal cancer is a relatively common tumor with incidence that has been increasing during recent decades, and the lifetime risk for colorectal cancer in industrialized countries is about 5% [1]. The last 5 to 10 years have seen unprecedented advances in the treatment of colorectal cancer. Therapeutic strategies involve seve-

ral different classes of drugs with significant anti-tumor activity in colorectal cancer: cytotoxic therapy which includes 5-fluorouracil, capecitabine (oral precursor of 5-fluorouracil), irinotecan and oxaliplatin, and targeted therapy which includes bevacizumab, aflibercept, regorafenib, cetuximab and panitumumab [2]. Despite the rapid pace of clinical research, the best way to combine and sequence all of these drugs to optimize treatment is not yet established.

The majority of cytotoxic drugs have a dose related effect and narrow therapeutic index. Hence dose selection is considered very important. Even small dose variations can lead to significant toxicity in some patients, and to hypodosing in others. There is an important inter-individual variability in the capacity of drug metabolism and elimination [3]. The choice of therapy is still based on standard clinical and pathological parameters represented by Dukes and TNM (Tumor Node Metastasis) grading systems. The dose is mostly chosen based on age, height and body mass by calculation of BSA (Body Surface Area) [4,5]. However, this gives restricted possibilities to equalise inter-individual variations which are dependent on physiological, genetic and environmental factors (drug-drug interactions, drug-food interactions). The influence of genetic factors on response variability is far greater than sex, age or interactions with other drugs. The distribution frequency of correct responses to drug usage in a population is far from normal distribution, which means that the presence of treatment non-responders and over-responders (increased toxicity) is much more common than has been assumed so far [6]. The clinical problems that arise from inter-individual variability in drug metabolism are mostly related to toxic effects of cytotoxic drugs, mainly affecting hair growth, gastrointestinal system and bone marrow, but also include resistance to therapy involving various mechanisms.

Personalised cancer medicine

Personalised medicine is a new concept arising from the need for more rational and effective treatment with less adverse reactions. It is based on the combination of therapeutic drug monitoring (TDM) and genotyping with the objective to evaluate metabolic capacity of the host and/or the characteristics of the tumor [7]. Cytotoxic anticancer drugs fit many of the criteria commonly defined as prerequisites for utilising TDM approaches. Firstly, the extent of inter-individual pharmacokinetic (PK) variability exhibited is large in the majority of cases. This large inter-individual PK variability is likely to be related to genetic differences as well as variations in the functional status of the cancer patients. Furthermore, relationships have been

described between plasma concentrations and pharmacodynamic (PD) end-points such as percentage decrease in neutrophil counts between pre-treatment and nadir values [8]. Genetic factors contribute to the phenotype of drug response. A significant proportion of variability in drug response can be attributed to genetic factors through modulation of drug PKs and/or PD. So, the rationale behind pharmacogenetic studies is to investigate genes encoding drug transporters, drug-metabolising enzymes and drug targets that can predict the usefulness of a particular drug so as to increase the number of responders and decrease the number of subjects affected by adverse drug reactions. Nevertheless, the sources of variability in drug response are multifactorial and apart from genetics, factors such as pathophysiology, environment, diet, drug-drug interactions, drug allergies, medication errors and poor compliance, may all have a profound impact on PKs and/or PDs, thereby affecting therapeutic outcome

Pharmacogenetics is not only important for targeted drugs which effectiveness is often dependent upon a genetic mutation. Inter-individual differences in toxicity and response are observed in practically all available anticancer treatment regimens. Identification of subgroups of patients which differ in their prognosis and response to treatment could be helpful to identify the best available therapy for individual patient.

The first studies on pharmacogenetics and colorectal cancer outcomes were conducted and published approximately 20 years ago [9]. Since then, many possible biodeterminants have been studied with many expectations, but the final step of clinical validation has remained an unmet objective for almost all putative biomarkers [10]. Consequently, providing individual treatment with low toxicity and significant benefit is still an unsolved problem. Reasons for this are the lack of knowledge on distribution of these polymorphisms in the population, importance of non-genetic factors, price of genetic testing and still limited data from controlled clinical trials confirming the clinical usefulness of pharmacogenetic testing.

Important gene polymorphisms in cytotoxic colorectal cancer therapy

Although significant research on polymorphisms important for the metabolism of cytotoxic drugs has been done, the translation of pharmacogenetic outcomes into clinical practice has proved to be surprisingly disappointing, with relatively few exceptions. These exceptions include genetic polymorphisms important for the metabolism of fluoropyrimidines (5-FU and capecitabine) and irinotecan.

Fluoropyrimidines

Fifty years after the first synthesis of 5-FU it is still a standard component of adjuvant and palliative therapy having a proven impact on survival time in patients with colorectal cancer [11]. Experimental studies have shown that 5-FU is converted to an active metabolite which is a potent inhibitor of DNA synthesis. It forms a complex with thymidylate synthase enzyme and 5,10-methylenetetrahydrofolate (CH₂THF) cofactor, responsible for the catalytic conversion of deoxyuridine monophosphate to deoxythymidine monophosphate which is a substrate for deoxythymidine triphosphate necessary for the process of DNA synthesis [12]. Despite significant progress in understanding the 5-FU activity mechanisms, the identification of molecular markers potentially clinically useful in predicting 5-FU treatment efficacy and toxicity and is still the subject of research. Approximately 10–40% of 5-FU develop severe, and sometimes life-threatening, toxicity (neutropenia, nausea, vomiting, severe diarrhoea, stomatitis, mucositis, hand–foot syndrome, and neuropathy) [13].

5-FU is the sole anticancer agent for which TDM has been validated in more than one randomised trial, in terms of improved therapeutic index [14-16]. Individual FU dose adjustment based on pharmacokinetic monitoring resulted in significantly improved objective response rate, a trend to higher survival rate, and fewer grade 3/4 toxicities. Approach that includes both patient's phenotype and genotype up to now Has not been studied and proven useful enough to be translated into everyday oncology practice. However, testing polymorphisms of 2 enzymes involved in fluoropyrimidine metabolism have found their way into clinical practice. These two enzymes are dihydropyrimidin dehydrogenase (DPD) and thymidylate synthase (TS).

Dihydropyrimidin dehydrogenase

5-FU as a prodrug, in order to achieve its intracellular cytotoxic activity, requires metabolic activation. Inter-individual variability in the response of patients to 5-FU treatment may in fact be associated with a decrease in the activity of enzymes responsible for catabolism of the drug, which will result in an increase in drug concentration and longer half-life, and thus an increased risk of serious toxic effects. Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme for fluoropyrimidine catabolism and eliminates >80% of administered 5-FU. The activity of DPD is dependent upon polymorphisms in the DPYD gene. Its activity is extremely variable in tumoral tissue and this variation might make a difference to the efficiency of 5-FU treatment, since intratumoral drug concentration is one of the most important factors for the determination of the antitumoral effect [17]. Deficiency in DPD activity, however, leads to severe toxicity correlated to 5-FU which may even be fatal. The partial or total lack

of this enzyme has, in fact, been associated with severe toxicity (mucositis, granulocytopenia, and neuropathy), and in several cases even death, after 5-FU administration (18). Partial DPD activity deficiency in the general population is about 5%-10%, and its total loss is very rare, about 0.2% [19]. However, it has been estimated that 23-38% of 5-FU toxicity can be attributed to DPD polymorphisms [20]. The acquired uncertain evidence is derived mostly from retrospective clinical studies and suggests that low expression of the *DPYD* gene may be a sensitivity marker in tumour cells for fluoropyrimidines and thus allow us to predict the degree of response to treatment. However, currently quality clinical data have become available that confirmed the predictive value of *DPYD* expression determination in order to predict the efficacy of 5-FU therapy in colorectal cancer patients [21].

An assay for DPD polymorphisms testing is commercially available as well as for TYMS polymorphisms. However, pre-emptive testing is not recommended.

The *US Food and Drug Administration* (FDA) has added statements to the drug labels for 5-fluorouracil and capecitabine that contraindicate use in patients with DPD enzyme deficiency. The FDA drug label also warns to use precaution with intravenous 5-fluorouracil in these patients. The *Dutch Pharmacogenetics Working Group* has evaluated therapeutic dose recommendations for 5-fluorouracil, capecitabine, and tegafur (5-fluorouracil prodrug combined with uracil; not available in United States). The Working Group recommends the use of an alternative drug for homozygous carriers of a decreased-activity allele and a reduced dose or alternative drug to capecitabine or 5-fluorouracil for heterozygous carriers of a decreased-activity allele [22]. Recently, *Clinical Pharmacogenetics Implementation Consortium Guidelines for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing* have been issued on dosing recommendations for fluoropyrimidines based on *DPYD* genotype, recommending that in heterozygous for high risk alleles the dose of fluoropyrimidines should be reduced by 50% and in homozygous instead of fluoropyrimidines alternative therapy should be used [23].

Tymidilate synthase

TYMS polymorphisms which result in increased expression of the enzyme are increasing the risk of 5-FU toxicity and decreasing the therapeutic response. In particular, TS overexpression has been found to be significantly associated with a low response to treatment based on 5-FU, both as adjuvant [24] and metastatic therapy [25]. Colorectal cancer patients with low levels of TYMS gene expression had a significantly higher rate of response to therapy and longer median survival compared to patients with higher TS expression in tumor tissue (leichman-19). Subsequent meta-

analyses confirmed the importance of TS expression on overall response rate and overall survival [26,27]. Available data suggest that high-risk TYMS polymorphisms are associated with 1.4-2.4 higher risk for 5-FU toxicity. Still, the positive predictive value of these tests is limited (50%) [28]. There is a need for further analyses to allow identification of *TYMS* transcription regulatory mechanisms including the role played by combinations of different genetic variants and their expression variability in populations. No recommendations have been issued on dosing of **fluoropyrimidines by TS phenotype**.

Other gene polymorphisms possibly important for fluoropyrimidine efficacy and toxicity for various enzymes have currently been explored (eg. dihydropyrimidinase, beta-ureidopropionase, methylenetetrahydrofolate reductase), but available research data are insufficient for conclusions on their potential clinical usefulness.

Irinotecan

Irinotecan is a synthetic analogue of a naturally occurring alkaloid, camptothecin. It was first approved for clinical use in Japan in 1994 for the treatment of small-cell lung cancer and hematologic malignancies, and then in 1995 in France for the treatment of advanced colorectal cancer. Tumour-specific somatic mutations and abnormal gene expression have been reported to be associated with irinotecan therapeutic efficacy and toxicity. However, the available studies do not provide unequivocal confirmation that somatic mutations have a significant impact on the outcome of irinotecan treatment, which prevents their usage as predictive markers. Generally, genetic variations may influence both the pharmacokinetics and pharmacodynamics of irinotecan [29,30].

It is recognised that there is large PK and PD inter-individual variability in patients receiving irinotecan with limited dependence on dosing based on BSA [31]. PK variability is linked to variability in biliary excretion and inherited variations in metabolic pathway which controls degradation of irinotecan.

Uridine diphosphate glucuronosyltransferase

The active metabolite of irinotecan, SN-38, is glucuronidated mainly in the liver by the uridine diphosphate glucuronosyltransferase enzymes (UGTs), primarily the *UDP-glycosyltransferase 1* family (UGT1As) isoenzyme, responsible in humans for bilirubin conjugation with glucuronic acid. UGTs are one of the most important classes of enzyme proteins participating in the coupling reaction phase II of xenobiotic metabolism. First evidence from clinical trials on the role of *UGT1A1*28* in the development of toxicity resulting from administration of irinotecan was published

by Ando et al [32]. It is estimated that there are 8-10% homozygous people for this allele in the population. Homozygous patients for the UGT1A1*28 allele have increased risk for severe neutropenia and severe delayed-type diarrhoea after treatment with irinotecan [33].

Early research and meta-analyses have suggested that the toxic effect of irinotecan is dose dependent and that this effect is not likely in patients receiving low doses of irinotecan (100-125 mg/m² weekly). Subsequent meta-analysis showed that UGT1A1*29 allele polymorphism is significantly associated with risk of neutropenia in all dose regimens. Still, this risk is significantly higher with higher doses of irinotecan ≥ 250 mg/m² (RR 7.0, 95%ci 3.10-16.78) as compared to lower doses (80-145 mg/m² weekly, RR 2.43, 95%CI 1.34-4.39) [34]. It is still unclear if neutropenia prevention would be possible with previous identification of homozygous patients. Only 1/10 are homozygous and it is unclear how much of the added risk for neutropenia can be attributed to irinotecan, especially in lower dosage. However, some studies have shown significant differences in prevalence of severe neutropenia in patients homozygous for UGT1A1*28 allele as compared to controls (48% vs 10%, respectively), higher hospitalization rate and higher mortality [35]. Subsequent systematic reviews and meta-analyses are generally supportive of the clinical utility of genotyping *UGT1A1**28 prior to commencement of irinotecan therapy in order to decrease the risk of severe neutropenia and diarrhoea through the pre-emptive dose reduction of irinotecan for *UGT1A1**28 homozygotes [36,37], and indicate that there is unlikely to be an important association between *UGT1A1* genotype and overall response rate with irinotecan, however, this does not provide direct evidence that a dose reduction for *UGT1A1**28 homozygous patients will not lead to an important reduction in overall response rate [38].

There is still no consensus on the need for dose reduction in homozygous patients. In conclusion, the clinical utility of pre-emptive UGT1A1*28 allele testing is not yet known. One proof-of-concept study has been conducted that showed that the maximum tolerated dose of irinotecan in UGT1A1*29 allele homozygous patients was significantly lower as compared to normal variants (400 mg vs. 840 mg). The authors concluded that identification of UGT1A1*28 genotype is useful for irinotecan dose individualisation [39]. An assay for UGT1A1 polymorphisms testing is commercially available (*Invader UGT1A1 Molecular Assay*), specifically for testing the *UGT1A1**1 (wild-type) and the *UGT1A1**28 genotype. However, the proposed benefit of testing colorectal cancer patients for *UGT1A1* genotype is that the risk for adverse drug-related side effects among patients found to be homozygous for the *UGT1A1**28 genotype can be reduced by lowering their initial and/or subsequent doses of irinotecan. The concomitant harm is that a reduction in irinotecan dosage

may also reduce the effectiveness of chemotherapy in tumour suppression and long-term survival [40].

There are other gene polymorphisms important for irinotecan metabolism that currently being explored (eg. carboxylesterase, topoisomerase-1, aprataxin, etc.) but the available data are insufficient for conclusions on their potential clinical usefulness.

Oxaliplatin

Combination therapy with 5-FU/leucovorin plus oxaliplatin (FOLFOX) is currently a standard in treating gastric cancer and colorectal cancer with a 40% positive response ratio during first relapse therapy [41]. Despite the efficiency of combined therapy, a high percentage of patients show drug resistance to a higher or lower degree, which suggest that the therapeutic efficiency of FOLFOX is characterised by high variability. Insufficient intra-tumour concentration of platinum compounds is a critical factor determining both primary and secondary resistance. Potential platinum uptake or influx transporters include copper transporter proteins [42], organic cation transporters belonging to the SLC22 family [43] and an undefined cis-configuration specific platinum influx transporter (44), in addition to drug transporters facilitating the active efflux of platinum compounds including adenosine triphosphate and additionally adenosine triphosphate (ATP) binding cassette (ABC) multidrug transporters, and copper-transporting P-type adenosine triphosphatases (ATPases) [45]. Clinical studies concerning transporters for platinum derivatives have concentrated on evaluation of the connection between intratumour expression of certain transporters and the results of treatment after chemotherapy based on platinum derivatives. *In vivo* clinical research is required to elucidate the meaning of genetic variability of membrane transporters and channels for gene expression and their influence on the pharmacokinetics and effectiveness of oxaliplatin-based therapy.

Other pathways important for oxaliplatin PK and PD are being evaluated (eg. glutathione S-transferases, nucleotide excision repair pathway apoptosis regulation). However, available research data are insufficient for conclusions on their potential clinical usefulness.

Barriers to the clinical implementation of pharmacogenomics

Although the testing context for pharmacogenetic tests is different from other genetic tests, decisions to use any new clinical tests in medical practice will require evaluation of not only the benefit linked to improved drug safety and efficacy, but also a host of ethical questions. Due to the uncertainties of future developments in the field, thoughts on the ethical aspects are somewhat preliminary at this point.

However, some aspects of pharmacogenetics are likely to have a potentially profound impact on medical practice, research and on society as a whole. Therefore, much thought has been invested in the anticipation of ethical, legal and social aspects of pharmacogenetics [46]. A number of other barriers remain with implementing clinical pharmacogenetics, including clinical utility, cost-effectiveness, professional education, and regulatory and reimbursement issues [47]. The greater the barriers to the clinical adoption of pharmacogenetics, the greater the evidence and size of the improvement in clinical outcome required. The problem to date is that the evidence and importance of most pharmacogenomic associations are not sufficient to overcome the barriers to the clinical implementation. Although long overdue, many of these potential barriers are now being subjected to closer examination and as a result, a framework for successful clinical uptake of pharmacogenomics is emerging.

Conclusion

The purpose of individualized therapy is to choose the most effective treatment and the optimal dosage for each patient, while minimizing toxicity and side effects of therapy. A limited number of pharmacogenetic markers are identified in colorectal cancer. In most studies they are explored individually which has led to somewhat conflicting results. The simultaneous testing of multiple markers predictive of response could help to identify more accurately the true role of these polymorphisms in colorectal cancer therapy [48]. Although the initial costs of cancer management and personalized medicine may be high, in the future they may result in significant benefits from both a clinical and economical perspective.

References

- [1] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin.* 2014;64:9-29.
- [2] http://www.uptodate.com/contents/systemic-chemotherapy-for-nonoperable-metastatic-colorectal-cancer-treatment-recommendations?source=search_result&search=colorectal+cancer+treatment&selectedTitle=2~150. Accessed on March 27,2015.
- [3] Bardin C, Veal G, Paci A, Chatelut E, Astier A, Leveque D, Widmer N, Beijnen J. Therapeutic drug monitoring in cancer- Are we missing a trick? *Eur J Cancer.* 2014;50(12):2005-9.
- [4] NCCN Clinical Practice Guidelines in Oncology: Colon cancer, Version 3.2014. Available from: <http://www.nccn.org>.

- [5] NCCN Clinical Practice Guidelines in Oncology: Rectal cancer, Version 3.2014. Available from: <http://www.nccn.org>.
- [6] Splawinski J, Kuzniar J. Clinical trials: active control vs placebo. What is ethical? *Sci Eng Ethics*. 2004;10:73-9.
- [7] Juretic A. Personalised cancer medicine in colorectal cancer- a short overview. *Medical Sciences*. 2014;40:33-9.
- [8] Paci A, Veal G, Bardin C, Leveque D, Widmer N, Beijnen J, Astier A, Chatelut E. Review of therapeutic drug monitoring of anticancer drugs part 1- Cytotoxics. *Eur J Cancer*. 2014;50(12):2010-9.
- [9] Lenz HJ, Hayashi K, Salonga D, Danenberg KD, Danenberg PV, Metzger R, Banerjee D, Bertino JR, Groshen S, Leichman LP, Leichman CG. p53 point mutations and thymidylate synthase messenger RNA levels in disseminated colorectal cancer: an analysis of response and survival. *Clin Cancer Res*. 1998;4:1243-50.
- [10] Loupakis F, Schirripa M, Zhang W, Falcone A, Lenz HJ. Pharmacogenetic concerns in Metastatic Colorectal Cancer Therapy. *Curr Colorectal Cancer Rep*. 2012;8:263-71.
- [11] Cassidy J, Saltz L, Twelves C, Van Cutsem E, Hoff P, Kang Y, Saini JP, Gilberg F, Cunningham D. Efficacy of capecitabine versus 5-fluorouracil in colorectal and gastric cancers: a meta-analysis of individual data from 6171 patients. *Ann Oncol*. 2011;22:2604-9.
- [12] Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinet*. 1989;16:215-37.
- [13] Amstutz U., Farese S., Aebi S., Largiadèr C.R. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics*. 2009;10:931-44.
- [14] Fety R, Rolland F, Barberi-Heyob M. Clinical impact of pharmacokinetically-guided dose adaptation of 5-fluorouracil: results from a multicentric randomized trial in patients with locally advanced head and neck carcinomas. *Clin Cancer Res*. 1998; 4: 2039-45.
- [15] Gamelin E, Delva R, Jacob J. Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: results of a multicenter randomized trial of patients with metastatic colorectal cancer. *J Clin Oncol*. 2008; 26: 2099-105.
- [16] Saif MW, Choma A, Salamone SJ, Chu E. Pharmacokinetically guided dose adjustment of 5-fluorouracil: a rational approach to improve therapeutic outcomes. *J Nat Cancer Inst*. 2009 (101): 1543-52.
- [17] McLeod HL, Sludden J, Murray GI, Keenan RA, Davidson AI, Park K, Koruth M, Cassidy J. Characterization of dihydropyrimidine dehydrogenase in human

- colorectal tumours. *Br J Cancer*. 1998;77:461-5
- [18] Milano G, Etienne MC, Pierrefite V, Barberi-Heyob M, Deporte-Fety R, Renée N. Dihydropyrimidine dehydrogenase deficiency and fluorouracil-related toxicity. *Br J Cancer*. 1999;79:627-30.
- [19] Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renée N, Schneider M, Demard F, Milano G. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol*. 1994;12:2248-53.
- [20] Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renée N, Schneider M, Demard F, Milano G. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol*. 1994;12:2248-53
- [21] Scartozzi M, Maccaroni E, Giampieri R, Pistelli M, Bittoni A, Del Prete M, Berardi R, Cascinu S. 5-Fluorouracil pharmacogenomics: still rocking after all these years? *Pharmacogenomics*. 2011;12:251-65.
- [22] Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin. Pharmacol. Ther*. 2011;89:662-73.
- [23] Caudle KE, Thron CF, Swen JJ, McLeod HL, Diasio RB, Schwab M. Clinical Pharmacogenetics Implementation Consortium Guidelines for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing. *Clin Pharmacol Ther*. 2013;94:640-5.
- [24] Leichman CG, Lenz HJ, Leichman L, Danenberg K, Baranda J, Groshen S, Boswell W, Metzger R, Tan M, Danenberg PV. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and resistance to protracted-infusion fluorouracil and weekly leucovorin. *J Clin Oncol*. 1997;15:3223-9.
- [25] Paradiso A, Simone G, Petroni S, Leone B, Vallejo C, Lacava J, Romero A, Machiavelli M, De Lena M, Allegra CJ. Thymidylate synthase and p53 primary tumour expression as predictive factors for advanced colorectal cancer patients. *Br J Cancer*. 2000;82:560-7.
- [26] Popat S, Matakidou A, Houlston RS. Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. *J Clin Oncol*. 2004;22:529-36.
- [27] Qiu LX, Tang QY, Bai JL, Qian XP, Li RT, Liu BR, Zheng MH. Predictive value of thymidylate synthase expression in advanced colorectal cancer patients receiving fluoropyrimidine-based chemotherapy: evidence from 24 studies. *Int J Cancer* 2008;123:2384-9.
- [28] Panczyk M. Pharmacogenetics research on chemotherapy resistance in colorectal cancer over the last 20 years. *World J Gastroenterology*. 2014;20(29):9775-827.
- [29] Cortejoso L, López-Fernández LA. Pharmacogenetic markers of toxicity for chemotherapy in colorectal cancer patients. *Pharmacogenomics*. 2012;13:1173-

1191.

- [30] Freyer G, Duret A, Milano G, Chatelut E, Rebischung C, Delord JP, Merrouche Y, Lledo G, Etienne MC, Falandry C. Pharmacogenetic tailoring of irinotecan-based first-line chemotherapy in metastatic colorectal cancer: results of a pilot study. *Anticancer Res.* 2011;31:359-66.
- [31] Mathijssen RH, Verweij J, de Jonge MJ, Nooter K, Stoter G, Sparreboom A. Impact of body-size measures on irinotecan clearance: alternative dosing recommendations. *J Clin Oncol.* 2002;20(1):81-7.
- [32] Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res.* 2000;60:6921-6.
- [33] Marcuello E, Altés A, Menoyo A, Del Rio E, Gómez-Pardo M, Baiget M. UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer. *Br J Cancer.* 2004;91:678-82.
- [34] Hoskins JM, Goldberg RM, Pingping Q, Ibrahim JG, McLeod HL. UGT1A1*28 Genotype and Irinotecan-Induced Neutropenia: Dose Matters. *J Natl Cancer Inst.* 2007;99(17):1290-95.
- [35] Shulman K, Cohen I, Barnett-Griness O, Kkuten A, Gruber SB, lejbnkowicz F, Rennert G. Clinical implications of UGT1A1*28 genotype testing in colorectal cancer patients. *Cancer.* 2011;117(14):3156-62.
- [36] Hu ZY, Yu Q, Zhao YS. Dose-dependent association between UGT1A1*28 polymorphism and irinotecan-induced diarrhoea: a meta-analysis. *Eur J Cancer.* 2010;46:1856-65.
- [37] Hu ZY, Yu Q, Pei Q, Guo C. Dose-dependent association between UGT1A1*28 genotype and irinotecan-induced neutropenia: low doses also increase risk. *Clin Cancer Res.* 2010;16:3832-42.
- [38] Dias MM, McKinnon RA, Sorich MJ. Impact of the UGT1A1*28 allele on response to irinotecan: a systematic review and meta-analysis. *Pharmacogenomics.* 2012;13:889-99.
- [39] Innocenti F, Schilsky RL, Ramirez J, Janisch L, Undevia S, House LK, Das S, Wu K, et al. Dose-finding and pharmacokinetic study to optimize the dosing of irinotecan according to the UGT1A1 genotype of patients with cancer. *J Clin Oncol.* 2014;32(22):2328-34.
- [40] Winder T, Lenz HJ. Molecular predictive and prognostic markers in colon cancer. *Cancer Treat Rev.* 2010;36:550-6.
- [41] Tournigand C, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study.

- J Clin Oncol. 2004;22:229-37.
- [42] Howell SB, Safaei R, Larson CA, Sailor MJ. Copper transporters and the cellular pharmacology of the platinum-containing cancer drugs. *Mol Pharmacol*. 2010;77:887-94.
- [43] Koepsell H, Endou H. The SLC22 drug transporter family. *Pflugers Arch*. 2004;447:666-76.
- [44] Helleman J, Burger H, Hamelers IH, Boersma AW, de Kroon AI, Stoter G, Nooter K. Impaired cisplatin influx in an A2780 mutant cell line: evidence for a putative, cis-configuration-specific, platinum influx transporter. *Cancer Biol Ther*. 2006;5:943-9.
- [45] Surowiak P, Materna V, Kaplenko I, Spaczynski M, Dolinska-Krajewska B, Garbarska E, Dietel M, Zabel M, Lage H. ABCC2 (MRP2, cMOAT) can be localized in the nuclear membrane of ovarian carcinomas and correlates with resistance to cisplatin and clinical outcome. *Clin Cancer Res*. 2006;12:7149-58.
- [46] Marx-Stolting L. Pharmacogenetics and ethical considerations: why care? *Pharmacogenomics J*. 2007;7(5):293-6.
- [47] McKinnon RA, Ward MB, Sorich MJ. A critical analysis of barriers to the clinical implementation of pharmacogenomics. *Ther Clin risk Manag*. 2007;3(5):751-9.
- [48] Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsao-Wei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB. Colorectal tumors responding to 5- fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. *Clin Cancer Res*. 2000;6:1322-7.

Sažetak

Farmakogenetika citotoksičnog liječenja kolorektalnog karcinoma

Rak kolorektuma relativno je čest tumor s rastućom incidencijom zadnjih desetljeća. Unatoč brzom napretku u liječenju kolorektalnog raka, još nije jasno na koji način je najbolje kombinirati i kojim redoslijedom koristiti dostupne lijekove. Većina citotoksičnih lijekova koji se primjenjuju u liječenju kolorektalnog raka imaju učinak ovisan o dozi i malu terapijsku širinu te je određivanje doze lijeka vrlo značajno. Važeći načini izbora terapije i određivanja doze daju ograničene mogućnosti ublažavanja interindividualnih varijacija ovisnih o fiziološkim, genetičkim i okolišnim čimbenicima. U ovom članku dan je pregled trenutačnih mogućnosti individualiziranja liječenja kolorektalnog raka. To uključuje pregled najznačajnijih genetskih polimorfizama bitnih za sigurnost i učinkovitost citotoksične terapije u liječenju kolorektalnog raka te evaluaciju njihove značajnosti za trenutačnu kliničku praksu. Unatoč sve većem znanju, omogućavanje individualiziranog liječenja uz ograničavanje toksičnosti a potenciranje učinkovitosti još je neriješeni problem. Razlog za to su manjak znanja o distribuciji polimorfizama u populaciji, važnosti negenetičkih čimbenika, cijena genetičkog testiranja i još uvijek ograničeni podaci iz kontroliranih kliničkih studija koji bi potvrdili kliničku korisnost farmakogenetičkog testiranja. Iako inicijalni troškovi liječenja kolorektalnog raka i personalizirane medicine mogu biti visoki, u budućnosti bi kombinacija ovih pristupa mogla rezultirati značajnim prednostima iz kliničke i ekonomske perspektive.

Ključne riječi: kolorektalni rak; farmakogenetika; biomarkeri; personalizirana medicina.

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THE IMPORTANCE OF MULTIDISCIPLINARY APPROACH IN THE TREATMENT OF RECTAL CANCER

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Summary

Colorectal cancer is the 3rd most common malignant disease worldwide and it represents the major public health issue. Nowadays, multidisciplinary approach is common in diagnostics and treatment of all malignant diseases, rectal cancer is an example for importance of various medical disciplines collaboration in achieving optimal treatment outcome. This paper is a short overview of most important clinical trials which defined optimal therapeutical approach to the patients with locally advanced rectal cancer. Actual guidelines and recommendations for treatment of patients with stage II or III rectal cancer include preoperative radiotherapy (RT) with or without concurrent chemotherapy, and radical surgical resection (with mandatory total mesorectal excision-TME). Such multidisciplinary approach to the therapy of these patients significantly reduced rates of local recurrence, and increased likelihood of pathologic complete response and sphincter-preserving surgery, with low rates of acute treatment toxicity. In order to further improve treatment outcomes, there are ongoing studies which investigate novel systemic drugs (immunotherapy-antibody-drug conjugates) as well as modern radiotherapy techniques (IMRT- intensity modulated RT, IGRT- image guided RT, VMAT- volumetric modulated arch RT) in patients with rectal cancer .

Keywords: rectal cancer; preoperative (neoadjuvant) chemoradiotherapy; total mesorectal excision; multidisciplinary treatment.

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females, with 1.4 million new cases and 690.000 deaths estimated to have occurred in 2012 worldwide [1]. Rates are substantially higher in males than in females. Nowadays, it is generally considered that significant percentage of rectal cancer is connected to lifestyle risk factors, such as obesity (especially abdominal), increased consumption of red and processed meat, physical inactivity, smoking and heavy alcohol use, and only about 10% is caused by inherited genetic syndromes (FAP, HNPCC, Turcots syndrome, Peutz-Jeghers syndrome). Some calculations predict that 2.4 million cases of colorectal cancer will be diagnosed annually worldwide by 2035. More than 95% of colorectal cancers are adenocarcinomas originating from epithelial cells of the colorectal mucosa, other rare versions are adenosquamous carcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, medullary, neuroendocrine and undifferentiated carcinoma [2]. The proportion of rectal cancer among cancers of the large intestine (colorectum) varies from 27% to 58%, depending on the cancer registry and classification of recto-sigmoid tumors.

Rectal cancer is a typical example for importance of multidisciplinary approach in the treatment of malignant disease. Disciplines involved in the care for rectal cancer patients (from diagnosis and treatment to posttreatment rehabilitation) include: radiology, pathology, molecular biology, abdominal surgery, anesthesiology, medical oncology, radiation oncology and rehabilitational medicine. This text focuses on multidisciplinary in the treatment of rectal cancer patients.

From the beginnings of rectal cancer treatment, it was evident that surgery alone is not sufficient treatment, since there was high proportion of patients with local or distant failure after tumor resection (with exception of patients with early, stage I tumors). Adjuvant radiotherapy with or without chemotherapy has been widely used for decades in order to improve outcomes in patients with rectal cancer. For locally advanced disease, postoperative chemoradiotherapy significantly improves both local control and overall survival as compared with surgery alone or surgery plus irradiation [3]. This information prompted a National Institutes of Health (NIH) consensus conference, convened in 1990, to recommend postoperative adjuvant chemoradiotherapy as standard treatment for patients with rectal cancer classified as stage II (i.e., a tumor penetrating the rectal wall, without regional lymph-node involvement) or stage III (i.e., any tumor with regional lymph-node involvement) [4].

In the last two decades numerous multidisciplinary clinical trials that addressed effects of preoperative (neoadjuvant) or postoperative (adjuvant) radiotherapy with or without chemotherapy for patients with rectal cancer have been conducted. Advantages of preoperative irradiation (as opposed to postoperative RT) are related to both tumor response and normal tissue preservation. First, reduction of tumor

volume prior to surgery may facilitate complete resection and increase the likelihood of sphincter-sparing procedure. Also, irradiation of intact tissue (surgery-naïve ie. better oxygenated) may result in increased sensitivity to RT. With preoperative radiotherapy, radiation-induced injury to small bowel trapped in the pelvis by postoperative adhesions, can be avoided. Preoperative RT targets tissues and structures that will be resected afterwards, which increases the likelihood of performing an anastomosis with healthy colon. Before the decision of neoadjuvant treatment, staging with pelvic magnetic resonance imaging (MRI) is obligatory. One potential disadvantage of using neoadjuvant approach is the possibility of over-treating early stage (stage I) rectal tumors which, based on definitive patohistological finding, don't require adjuvant therapy.

There are currently two main approaches of preoperative pelvic radiotherapy for resectable rectal cancer: short-course irradiation and long-course chemoradiotherapy. Although the radiation techniques and target volumes are similar, the fractionation and timing of surgery differ. In general, short-course radiation delivers 25 Gy (5 Gy/fraction in 5 fractions) of radiation followed by surgery 1 week later. Long-course chemoradiotherapy delivers 50.4 Gy (1.8 Gy/fraction in 28 fractions) of radiation concurrently with chemotherapy (usually fluoropyrimidines or its derivatives) followed by surgery 4 to 8 weeks later. These competing approaches evolved in parallel; short-course radiation developed in northern Europe and Scandinavia and long-course chemoradiotherapy (CRT) in the United States and selected European countries.

Two landmark trials support the use of short-course preoperative radiation. In 1997. Swedish Rectal Cancer Trial group published results of multicentric clinical trial investigating effects of preoperative neoadjuvant radiotherapy on local control and overall survival in rectal cancer patients [5]. 1168 patients were randomized into two arms: radiotherapy arm, where patients were preoperatively treated with short-course radiotherapy (25 Gy in 5 fractions; surgery was performed within one week after completion of RT) and control arm where patients received only surgical treatment (anterior or abdominoperineal resection). The irradiation did not increase postoperative mortality. After five years of follow-up, the rate of local recurrence was 11% in the group that received radiotherapy before surgery and 27% in the group treated with surgery alone ($P<0.001$). This difference was present in all subgroups of patients defined according to Dukes' classification. The overall five-year survival rate was 58% in the radiotherapy-plus-surgery group and 48% in the surgery-alone group ($P=0.004$). The cancer-specific survival rates at nine years among patients treated with curative resection were 74% and 65%, respectively ($P=0.002$). The Swedish Rectal Cancer Trial was one of the first clinical trials that showed clear benefit of

preoperative radiotherapy not only in terms of local recurrence rate reduction but in terms of improved survival as well. This was the only randomized trial which has revealed a significant advantage in survival.

The results prompted Dutch group of authors to conduct the CKVO 95-04 trial [6] which used the same design but mandated surgeons to use of total mesorectal excision (TME): 1861 patients with resectable rectal cancer and without evidence of metastatic disease were randomly assigned (ratio 1:1) to TME preceded by short-course (25 Gy in 5 fractions) pelvic irradiation or TME alone. 10-year cumulative incidence of local relapse was 5% in the RT+TME group and 11% in the TME-alone group ($p < 0.0001$). The effect of radiotherapy became stronger as the distance from the anal verge increased, but only in patients with a positive circumferential resection margin. Overall survival did not differ between the two arms. For patients with TNM stage III cancer with a negative circumferential resection margin, 10-year survival was 50% in the RT+TME arm versus 40% in the TME-alone group ($p = 0.032$). Both the initial and long-term reports revealed a significant improvement in local control with preoperative radiation, although no difference in overall survival was observed.

The German Rectal Cancer trial [7] long term follow up results, reported in 2012, compared preoperative and postoperative CRT. In this trial, 799 eligible patients with stage II/III rectal cancer were randomised to preoperative CRT (50.4 Gy in 28 fractions + FU 1000 mg/m² during the first and fifth weeks of radiotherapy), TME surgery, and adjuvant FU chemotherapy, or the same schedule of CRT used postoperatively (postoperative arm received an additional boost dose of 5.4 Gy on the tumor bed). 10-year overall survival was 59.6% in the preoperative arm and 59.9% in the postoperative arm ($P = 0.85$). The 10-year incidence of local relapse was 7.1% and 10.1% in the pre- and postoperative arms, respectively ($P = 0.048$). No significant differences were detected for 10-year cumulative incidence of distant metastases (29.8% and 29.6%; $P = 0.9$) and disease-free survival. Preoperative approach was superior in terms of treatment compliance, toxicity, tumor downstaging, sphincter preservation and 5-year local control. This trial changed the standard of care for patients with cT3-4 and/or N+ disease to preoperative longcourse chemoradiotherapy in Germany, most parts of Europe, and the USA.

There is still an ongoing controversy about optimal preoperative approach. The first randomized trial comparing short-course RT vs. Long-course CRT in patients with resectable cT3-4 rectal cancer was published by Bujko et al. [8]. The aim of the study was to compare overall survival, local control and late toxicity in 312 patients randomized in two treatment groups: short-course RT group (25 Gy in 5 fractions; surgery within 7 days) or long-course CRT group (50.4 Gy in 28 fractions of 1.8 Gy, bolus 5-FU/LV; surgery 4-6 weeks later). Acute toxicity was higher in the CRT group

(18.2% vs. 3.2%; $P < 0.001$). Although the long-course chemoradiotherapy arm had lower incidence of positive radial margins (4% vs. 13%; $P = .017$), there were no significant differences in crude local recurrence rates (9% vs. 14%; $P = 0.170$) or 4-year survival (66.2% vs. 67.2%; $P = 0.960$). Severe late toxicity was 10.1% vs. 7.1% ($P = 0.360$), respectively.

TROG 01-04 [9] is a multicenter randomized trial in which 326 patients with cT3Nx adenocarcinoma located in the lower 2/3 of the rectum were randomly assigned to short-course(SC) RT versus long-course (LR) CRT. Patients in both arms received 6 months of postoperative adjuvant chemotherapy. Rates of local recurrence after 3-year follow-up were 7.5% for SC vs. 4.4% for LC ($P=0.24$); 5-year distant recurrence rates were 27% for SC vs. 30% for LC ($P=0.92$). Overall survival rates at 5 years were 74% for SC vs. 70% for LC ($P=0.62$). Late toxicity rates were not substantially different (SC 5.8% vs. LC 8.2%; $P=0.53$). A subset analysis of the 79 patients with distal tumors revealed a cumulative incidence of local recurrence of 12.5% in SC arm vs. 0% in LC arm. Although LC chemoradiotherapy arm had a 3% lower local recurrence rate at 3 years and a 2% lower local recurrence rate at 5 years, neither were statistically significant. Authors conclude that the results of local recurrence rates have either no clinically important difference or speak in favor of LC chemoradiation. Also, LC could be more effective in reducing local recurrence rates for distal tumors.

Surgery of rectal cancer also experienced important improvements in the last two decades. TME (total mesorectal excision) is a surgical technique which was introduced into surgical practice and became widely used in rectal cancer surgery during 1990's. A significant length of the bowel around the tumor is removed, together with the belonging lymphatic drainage. The term *total mesorectal excision* strictly applies when performing a low anterior resection for tumors of the middle and the lower rectum, wherein it is essential to remove the rectum along with the mesorectum up to the level of the levator muscles. The principles of TME are also applied during an abdominoperineal excision of the rectum and for tumors of the upper rectum, although these are considered distinct from standard TME. In an abdominoperineal excision of the rectum where the tumor exists below the level of the levators, the lateral margins of the tumor are inferior to the mesorectum and the benefits of total mesorectal excision do not apply. Anterior resections involving the upper rectum may be completed with mobilization of the rectum to beyond 5 cm of the lower margin of the tumor, and which is often above the level of the levator and is sometimes referred to as *partial mesorectal excision*. TME results in a lower recurrence rate than traditional approaches and a lower rate of permanent colostomy. Postoperative recuperation is somewhat increased over competing methods. When practiced with diligent attention to anatomy there is no evidence of increased risk

of urinary incontinence or sexual dysfunction [10]. TME is now considered the gold standard for tumors of the middle and the lower rectum.

Chemotherapy used to treat rectal cancer can be categorized into one of two large groups: chemotherapy that is part of pre- or postoperative treatment (usually applied concomitantly or sequentially with pelvic irradiation) and chemotherapy for metastatic colorectal cancer. This text will be focused on chemotherapy in neoadjuvant and adjuvant setting in combination with radiotherapy.

A number of randomized clinical trials have evaluated and confirmed the effectiveness of adding chemotherapy to radiation in preoperative setting after clinical/radiological staging, as well as in postoperative adjuvant treatment, following pathological staging in patients with locally advanced rectal cancer. Benefits of addition of chemotherapy concurrent with pelvic irradiation include: local sensitization of tumor tissue (and normal tissue as well, but to a lesser extent), systemic control of the disease by eradicating possible micrometastases, increased likelihood of pathologic complete response and sphincter preservation. Drugs applied concurrently with radiotherapy are primarily radiosensitizing agents, with main intent to make irradiated (tumor) tissue more responsive to radiation-induced cell injury and/or death. 5-fluorouracil (5-FU), given either as an iv. infusion or orally (as prodrug capecitabine, that is enzymatically converted to (5-FU) in the body), appears to have the most favorable balance of efficacy and tolerability at the present time, so it is routinely used in concomitant chemoradiotherapy for rectal cancer. Oral administration without need for central IV access makes capecitabine an attractive and increasingly used option. Oxaliplatin and irinotecan have also been evaluated in neoadjuvant setting, but both demonstrated increased toxicity without substantial associated improvement in outcomes. There is currently a great need for finding new radiosensitizing agents and predictive biomarkers to help optimize the use of existing therapeutics.

A group of French authors published results of a clinical trial (FFCD 9203) which compared preoperative radiotherapy with and without concurrent chemotherapy [11]. 733 patients with T3-4NxM0 rectal adenocarcinoma were randomized into: RT group (preoperative radiotherapy with 45 Gy in 25 fractions; surgery planned 3-10 weeks after RT completion) and CRT group (same treatment protocol with addition of 5-FU 350 mg/m² + leucovorin (LV) 20 mg/m² during weeks 1 and 5 of RT). All patients received adjuvant chemotherapy with the same 5-FU/LV regimen. Although patients in the CRT group had significantly higher rates of pathologic complete response (11.4% vs 3.6%; $P < 0.05$) and less local recurrences (8.1% vs. 16.5%; $P < 0.05$) there was no benefit of adding chemotherapy to preoperative RT in terms of 5-year overall survival (67.9% vs. 67.4%; $P = 0.684$). Also, patients in CRT group had higher frequency of grade 3 and 4 acute toxicity (14.6% vs 2.7%; $P < 0.05$). Preoperative con-

current chemoradiotherapy despite a moderate increase in acute toxicity and no impact on overall survival significantly improves local control and is recommended for T3-4, N0-2, M0 adenocarcinoma of the middle and distal rectum.

EORTC 22921 phase III trial [12,13] evaluated effects of adding chemotherapy to preoperative pelvic irradiation and the value of postoperative chemotherapy on survival improvement in patients with T3-4 resectable rectal cancer. Preoperative trial design was similar to French study, except patients were also randomized into two groups depending on whether they received postoperative chemotherapy or not. Patients (n=1011) were allocated to the following four arms: arm 1, preoperative RT 45 Gy in 25 fractions; arm 2, preoperative CRT (addition of 5-FU 350 mg/m² + LV 20 mg/m² during weeks 1 and 5 of RT); arm 3, preoperative RT + 4 courses of postoperative chemotherapy; and arm 4, preoperative CRT + 4 courses of postoperative chemotherapy. Addition of chemotherapy to preoperative irradiation resulted in enhanced tumoricidal effect of radiotherapy: significant reductions in tumor size, pTN stage and lymphatic, vascular and perineural invasion were observed in preoperative CRT group compared to preoperative RT only. There was no significant difference in overall survival between the groups that received chemotherapy preoperatively (P=0.84) and those that received it postoperatively (P=0.12). The combined 5-year overall survival rate for all four groups was 65.2%. The 5-year cumulative incidence rates for local recurrences were 8.7%, 9.6%, and 7.6% in the groups that received chemotherapy preoperatively, postoperatively, or both, respectively, and 17.1% in the group that did not receive chemotherapy (P=0.002). Finally authors conclude that chemotherapy, regardless of whether it is administered before or after surgery, confers a significant benefit with respect to local control. Although chemotherapy had no significant effect on overall survival between the arms, its use in combination with preoperative RT is highly encouraged since it induces downsizing, downstaging, and significant changes in histologic tumor characteristics.

In 2012, Bonnetain et al. published results of pooled analysis of EORTC 22921 and FFCD 9203 trials [14]. Using meta-analysis methodology, this trial established that addition of 5FU-based chemotherapy to preoperative radiotherapy improves pathological complete response (pCR) and local control (LC) rates. Compared to only preoperative RT, CRT did not prolong overall survival (OS) or progression-free survival (PFS). This trial also evaluated strength of certain pathological and clinical parameters as potential surrogates for OS (identifying surrogate end points for long-term clinical outcomes such as OS would allow clinicians to reduce trial duration as well as assess their patients' prognosis during or shortly after the treatment). The authors stated that pathologic complete response (pCR) and local control (LC) cannot qualify as reliable surrogates for PFS or OS.

In the ACCORD-12 trial [15,16] two neoadjuvant chemoradiotherapy protocols are compared in terms of outcome parameters as well as toxicity. A total of 598 patients with T3-4N+ resectable rectal cancer were randomly assigned to preoperative concurrent chemoradiotherapy with CAP45 (45 Gy in 25 fractions with concurrent capecitabine) or CAPOX50 (50 Gy in 25 fractions with concurrent capecitabine + oxaliplatin). Surgery was performed 6 weeks after CRT completion. Pathologic complete response (sterilization of the operative specimen) was achieved in 13.9% vs. 19.2% of patients, respectively ($P=0.09$). After 3-year follow-up, there was no significant difference between local recurrence rates (6.1% vs. 4.4%), overall survival (87.6% vs. 88.3%) or disease-free survival (67.9% vs. 72.7%). More preoperative grade 3 to 4 toxicity occurred in the CAPOX50 group (25 v 1%; $P < .001$). In conclusion authors suggest that the benefit of oxaliplatin was not demonstrated and its use with concurrent irradiation is not recommended.

Interestingly designed RAPIDO trial [17] is a two-arm prospective randomized multicentric trial comparing conventional preoperative long-course concurrent chemoradiotherapy (45-50 Gy in 25 fractions with capecitabine) with introductory short-course radiotherapy (25 Gy in 5 fractions) followed by 6 cycles of chemotherapy (capecitabine and oxaliplatin) before surgery. The hypothesis is that short-course radiotherapy with neo-adjuvant chemotherapy increases disease-free and overall survival without compromising local control. The trial is still ongoing and oncologists worldwide are impatiently waiting for publication of the results.

There are still large variations between European countries in strategies of cancer care and subsequently, in cancer outcome. EURECCA (European Registration of cancer care) is a population-based colorectal cancer registry, but also involves multidisciplinary panel of experts, founded by leading professionals in all fields of medicine involved in cancer care. It's primary objective is to reduce treatment variances between countries or even within the same country and improve colorectal cancer care in Europe through registry, feedback and definition of core strategies in diagnostics, staging and treatment of colorectal cancer patients [18]. The panel formed a number of expert consensuses (with large to minimal equivocality); consensus is achieved for every step in diagnostic and treatment process of colorectal cancer patients, but panel strongly supports multidisciplinary team discussions in decision making for each patient. Also, diagnostic and treatment algorithms were developed and published by the panel, in order to provide up-to-date support to multidisciplinary teams involved in care for colorectal patients [18].

In conclusion, decision for the treatment of rectal cancer must mandatory be multidisciplinary. Surgical resection remains the therapeutic cornerstone, but the addition of neoadjuvant radiotherapy and chemotherapy is showing substantial be-

nefits. It is therefore necessary to subject all rectal cancer patients to multidisciplinary facility, where patients can be treated according to all actual recommendations and by that they receive best possible care and chance to fight the disease. Naturally, for every individual rectal cancer patient, individualized treatment plans based on a well-defined protocols should be designed.

References

- [1] Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2014. Available from: <http://globocan.iarc.fr>, accessed on 04/03/15
- [2] Hamilton SR, Bosman FT, Boffetta P, et al. Carcinoma of the colon and rectum. In: WHO Classification of Tumours of the Digestive System. Bosman FT, Carneiro F, Hruban RH, Theise ND, eds. Lyon: IARC Press, 2010:134-46.
- [3] Krook JE, Moertel CG, Gunderson LL, et al. Effective surgical adjuvant therapy for high-risk rectal carcinoma. *N Engl J Med*. 1991;324:709-15.
- [4] NIH consensus conference: adjuvant therapy for patients with colon and rectal cancer. *JAMA*. 1990;264:1444-50.
- [5] Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. [No authors listed] *N Engl J Med*. 1997;336:980-7.
- [6] van Gijn W, Marijnen CA, Nagtegaal ID, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. *Lancet Oncol*. 2011;12:575-82.
- [7] Sauer R, Liersch T, Merkel S, et al. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: Results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. *J Clin Oncol*. 2012;30:1926-33.
- [8] Bujko K, Nowacki MP, Nasierowska-Guttmejer A, et al. Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. *Br J Surg*. 2006;93:1215-23.
- [9] Ngan SY, Burmeister B, Fisher RJ, et al. Randomized trial of short-course radiotherapy versus long-course chemoradiation comparing rates of local recurrence in patients with T3 rectal cancer: Trans-Tasman Radiation Oncology Group Trial 01.04. *J Clin Oncol*. 2012;30:3827-33.
- [10] Ridgway PF, Darzi AW. The role of total mesorectal excision in the management of rectal cancer. *Cancer Control*. 2003;10:205-11.

- [11] Gérard JP, Conroy T, Bonnetain F, et al. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol.* 2006;24:4620-5.
- [12] Bosset JF, Calais G, Mineur L, et al. Enhanced tumorocidal effect of chemotherapy with preoperative radiotherapy for rectal cancer: preliminary results--EORTC 22921. *J Clin Oncol.* 2005;23:5620-7.
- [13] Bosset JF, Collette L, Calais G, et al.; EORTC Radiotherapy Group Trial 22921. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med.* 2006;355:1114-23.
- [14] Bonnetain F, Bosset JF, Gérard JP, et al. What is the clinical benefit of preoperative chemoradiotherapy with 5FU/leucovorin for T3-4 rectal cancer in a pooled analysis of EORTC 22921 and FFCD 9203 trials: surrogacy in question? *Eur J Cancer.* 2012;48:1781-90.
- [15] Gérard JP, Azria D, Gourgou-Bourgade S, et al. Clinical outcome of the ACCORD 12/0405 PRODIGE 2 randomized trial in rectal cancer. *J Clin Oncol.* 2012;30:4558-65.
- [16] Gérard JP, Azria D, Gourgou-Bourgade S, et al. Comparison of two neoadjuvant chemoradiotherapy regimens for locally advanced rectal cancer: results of the phase III trial ACCORD 12/0405-Prodige 2. *J Clin Oncol.* 2010;28:1638-44.
- [17] Nilsson PJ, van Etten B, Hospers GA, et al. Short-course radiotherapy followed by neo-adjuvant chemotherapy in locally advanced rectal cancer-the RAPIDO trial. *BMC Cancer.* 2013;13:279.
- [18] van de Velde CJ, Boelens PG, Borras JM, et al. EURECCA colorectal: multidisciplinary management: European consensus conference colon and rectum. *Eur J Cancer.* 2014;50:1.e1-1.e34.

Sažetak

Važnost multidisciplinarnog pristupa u liječenju karcinoma rektuma

Kolorektalni karcinom je treća maligna bolest po učestalosti u svijetu, te predstavlja značajan javnozdravstveni problem. Multidisciplinarni pristup danas je uobičajen kod dijagnostike i liječenja onkoloških bolesti, a karcinom rektuma tipičan je primjer za važnost suradnje različitih medicinskih disciplina u postizanju optimalnog terapijskog učinka. U ovom preglednom radu prikazan je kratak presjek najvažnijih studija koje su definirale optimalni pristup liječenju bolesnika sa lokalno uznapredovalim karcinomom rektuma. Današnje smjernice i preporuke za liječenje karcinoma rektuma stadija II i III uključuju preoperativnu radioterapiju sa ili bez konkomitantne kemoterapije, te radikalni kirurški zahvat (uz obaveznu totalnu mezorektalnu eksciziju-TME). Takvim se multidisciplinarnim pristupom kod tih bolesnika značajno reducirala stopa lokalnih recidiva, te povećala stopa patoloških kompletnih odgovora i zahvata sa očuvanjem analnog sfinktera, uz nisku stopu terapijske toksičnosti. Daljnje studije koje ispituju nove sistemske terapije (imunoterapija-konjugati antitijela i lijekova), kao i modernije radioterapijske tehnike (IMRT, IGRT, VMAT) kod bolesnika sa karcinomom rektuma, a kojima bi se dodatno unaprijedili terapijski ishodi, su u tijeku.

Ključne riječi: karcinom rektuma; preoperativna (neoadjuvantna) kemoradioterapija; kompletna mezorektalna ekscizija; multidisciplinarno liječenje.

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BOOK REVIEW

Dragan Dekaris

Ivo Vučković MD (editor)

A History of the Clinical Hospital Dubrava 1781 – 1991

Published by the Clinical Hospital Dubrava and
the Academy of Medical Sciences of Croatia,
November 2014.

The work is an 186 page history of military medicine in Croatia. It contains, I Introduction, II . Military hospitals in Zagreb 1781 – 1991; III . New Hospital in Zagreb 1988 – 1991; IV.A history of urology at the Clinical Hospital Dubrava 1930 to 1998; V. the story of colonel Alfred Nick MD, and VI. Conclusion. The book contains 22 pictures, 16 graphic representations, 7 layouts and 16 photocopies of important documents. The editor collected all relevant works concerning military medicine and military hospitals in Zagreb.

This collection of essays covers the history and activity of military hospitals and military medicine in Croatia from 1781 until 1991. In 1781 there were two military hospitals in Zagreb at Nova Ves. There was the Garrison Imperial-Royal Hospital and the Royal Croato-Hungarian Infantry hospital. In 1862 the Garrison Hospital moves into a newly completed building at Vlaška Street. In 1911 the Croato-Hungarian Infantry Hospital moves to a new building at Kuniščak. In 1918 both hospitals are combined in the Permanent Military Hospital of the Fourth army district of the Kingdom of Serbs, Croats and Slovenes. In 1941 the hospital is taken over by armed forces of Croatia (NDH). In 1945 a military hospital is established in the building of archdiocesan school for young men, at Voćarska Street. In 1945 civilians are admitted to the hospital; in 1953 the hospital joins emergency services. In 1986 the hospital develops its research department. In 1988 the hospital is moved to a newly completed facility in Dubrava.

It is useful to compare the growth and development of our military medicine with medical services of Great Britain, for instance. Regular armed services in Great Britain were established in 1660. Medical officers were attached to units. It is only during Napoleonic wars that a properly constituted medical service becomes part of the armed forces. After the disasters of the Crimean War The Medical Staff Corps

comes into being, (composed of «men able to read and write of regular habits and good temper and of kindly disposition»), but it will only be in 1898 that Queen Victoria establishes by decree Royal Army Medical Corps. Compared to the history of British military medicine, it seems that we have not done badly at all.

Considering the available histories of development of military medicine one can expect that this particular form of medicine ought to meet the following needs:

1. As a speciality, both preventive and clinical, it ought to meet the needs of armed services.
2. In the field of battle it ought to plan and execute surgical intervention in cases of massive wounding and organise ambulatory care.
3. In peace, it ought to provide medical care to members of armed forces and their families.
4. It must continue research in the field of military medicine.

Our Military Hospital in Zagreb meets all the above requirements.

The book under review is rich in articles and documents from a variety of sources. It is especially useful because it provides relevant sources accompanied by graphs, pictures, tables, layouts and photocopies of important documents.

It is important to mention two monographs: „Klinička bolnica Dubrava 1991-2001“ published in 2001 on the 10th anniversary of the „take over“ of the Military Hospital Zagreb, and „Klinička bolnica Dubrava 2001-2011“, published in 2011. The book under review contains works already published in „Acta historica medicinae, pharmaciae, veterinae“ (1964), and several works published in „Vojnosanitetski preglad“ (1975 and later). Detailed accounts of the development of military medicine and the military hospitals in Zagreb 1781-1945 by Alfred Nick, MD, and of the period 1945-1991 by Slava Blažević, MD, are significantly informative.

The story of how the Military Hospital in Zagreb was taken over painlessly and bloodlessly by the armed forces of the Republic of Croatia is interesting. It was the result of a conspirative action code named Dubrava. The crisis staff of the hospital was constituted by several department heads. The government of the Republic of Croatia appointed colonel Ivo Vučković, MD, head of the urology department, member of the professional group and chairman of the research council of the Military hospital. They managed to maintain the hospital fully functional, all equipment in order, and almost 85% of the staff on duty. The crisis group and 1100 loyal staff managed to take over the hospital from Yugoslav army (YPA). On the 16th of December Croatia took over the hospital and integrated it into the civilian system of

health care. The fate of the Military Hospital in Ljubljana, devastated when its staff left it, was avoided. During the Domovinski war more than 2000 severely wounded soldiers were treated in the hospital and more than 60 mobile surgical teams were in action.

The history of our medicine frequently gives military medicine short shrift. This work will remedy some of the deficiencies in our opus. It is good that we now have a sensible and accurate history of this particular segment of medical history.

Dragan Dekaris

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Addres of the Editorial Office
Razred za medicinske znanosti
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Rad 522 Medical sciences 41(2015)

Nakladnik

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Intergrafika TTŽ d.o.o.

Zagreb, svibanj 2015.

ISSN 1330-5301



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