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Microsatellite Instability in the Management of Stage II Colorectal Patients

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Rezumat

Instabilitatea microsatelită în managementul cancerului colorectal stadiu II

Introducere: La momentul actual există controverse legate de chimioterapia adjuvantă în cazul pacienților cu cancer colorectal stadiul II. Prezența unor factori de risc (T4, CEA > 5 ng/dl, mai puțin de 12 limfoganglioni examinați) reprezintă o indicație pentru regimuri bazate pe Oxaliplatin. În absența lor, nu există consens: 5 Fluorouracil sau doar urmărire fiind egal recomandate de oncologi. Instabilitatea microsatelită este asociată cu un prognostic bun în stadiul II și cu o lipsa de răspuns la terapia cu 5 Fluorouracil, trebuind utilizat ca marker predictiv.
Metodă: Am realizat un studiu prospectiv pe 115 pacienți consecutivi operați în clinica noastră pentru cancer colorectal în 2011 și 2012, folosind un algoritm de stratificare a riscului bazat pe stadializarea TNM, markeri clinico-patologici și moleculari.
Rezultate: Din cei 44 de pacienți cu stadiul II, 10 cazuri au fost clasificate cu risc înalt, în 26 de cazuri s-au practicat teste imunohistochimice, ce au identificat 8 pacienți cu fenotip de instabilitate microsatelită, cu risc scăzut, fără indicație pentru chimioterapie adjuvantă; 26 pacienți cu risc intermediar au urmat regimuri bazate pe 5 Fluorouracil.
Concluzii: Considerăm că testarea instabilității microsatelite oferă un instrument util în vederea unei mai bune caracterizări a pacienților cu cancer colorectal stadiu II, privind prognosticul și responsivitatea la chimioterapie.

Cuvinte cheie: instabilitate, microsatelită, cancer, colorectal

Abstract

Background: Up-to-date it is unclear whether stage II colorectal cancer patients should receive adjuvant chemotherapy. The presence of high risk features (T4, CEA > 5 ng/dl, less than 12 lymph nodes examined) is an indication for Oxaliplatin based treatment. In their absence, there is no consensus, 5 Fluorouracil regimens, or observation only being equally recommended by oncologists. Microsatellite instability is associated with good prognosis in stage II colorectal cancer and also with poor response to 5 Fluorouracil and should be used as a predictive marker.
Methods: We performed a prospective descriptive study on 115 consecutive patients who received surgical resection for colorectal cancer in our clinic during 2011 and 2012 using a risk stratification algorithm based on TNM staging, clinicopathologic and molecular markers.
Results: From the 44 stage II colorectal cancer patients, 10 cases were classified as high risk, in 26 cases we performed Immunohistochemical analysis that identified 8 patients with low risk microsatellite instability phenotype, with no indication for adjuvant chemotherapy; 26 intermediate risk patients received 5-FluoroUracil regimens.
Conclusion: We believe that microsatellite instability testing provides a useful tool in the goal of better characterizing

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patients with stage II colorectal cancer in matters of risk of recurrence and likelihood of benefit from chemotherapy.

Key words: microsatellite, instability, colorectal, cancer

Introduction

One of the major controversies in the management of Colorectal Cancer (CRC) is regarding the use of postoperative chemotherapy in Stage II disease. Though stage II tumors are grouped together, there are subgroups that appear more likely to relapse and may, in turn, derive more benefit from adjuvant chemotherapy with targeted agents used in combinations as FOLFOX (5 Fluorouracil + Leucovorin + Oxaliplatin), Capecitabine or 5 F U + Leucovorin. Other subgroups, with good prognosis, do not benefit from adjuvant therapy at all. Thus, it becomes essential to identify these subgroups of patients, in order to avoid a potentially toxic overtreatment and an unprofitable financial burden for the health care system. Current consensus guidelines require a risk stratification of Stage II patients based upon the presence of clinical, pathological and molecular risk factors.

Microsatellite instability (MSI) is considered to be a prognostic and predictive molecular factor in stage II CRC. Germline mutations (as seen in Lynch Syndrome), or somatic hypermethylation of the DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, and PMS2) can result in MMR protein deficiency and MSI, which appears in 15% of all CRC. (1,2) Tumors showing the presence of MSI are classified as MSI, whereas tumors without this characteristic are classified as microsatellite-stable (MSS).

Because stage II MSI-H patients may have a good prognosis and do not benefit from 5-FU adjuvant therapy, the National Comprehensive Cancer Network (NCCN) panel recommends that MSI testing be considered for patients with stage II disease. In addition, MMR testing should be performed for all patients <50 years of age to assess for the possibility of Lynch syndrome. (3) MSI testing can be performed either by PCR amplification of extracted DNA from a tumor sample, or by immunohistochemical (IHC) analysis of MMR proteins. Both tests appear to be almost equivalent. (4)

The Aim of our study is to try to answer the "to treat or not to treat" question that very often arises in the management of stage II CRC. We present the preliminary results in our trying to implement a risk stratification algorithm in stage II CRC.

Materials and method

We performed a prospective descriptive study on a series of 115 consecutive patients who received surgical resection for colorectal cancer in our clinic during 2011 and 2012. All patients underwent a pre-established protocol of investigations, preoperative radiotherapy (for inferior rectal cancer), curative or palliative surgery, postoperative chemotherapy (depending on the TNM staging) and 1 year follow-up.

Patient records, standardized dictation operative summaries, a protocol for the pathological examination of specimens, synoptic pathological reporting and knowledge transfer protocols between the surgeon, the pathologist and the oncologist were established prior to study start.

Pathologic evaluations included the following: grade of the cancer; depth of penetration and extension to adjacent structures (T); number of regional lymph nodes evaluated; number of positive regional lymph nodes (N); an assessment of the presence of distant metastases to other organs, to the peritoneum or an abdominal structure, or in non-regional lymph nodes (M); the status of proximal, distal, and radial margins, lymphovascular invasion; perineural invasion, the presence/ absence of tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, mucin/ signet ring cell differentiation. We also followed the presence of bowel obstruction, bowel perforation and comorbidities. CEA levels were determined preoperatively, at 6 months and at 1 year follow-up.

We used the 7th edition of the American Joint Committee on Cancer's (AJCC) colorectal cancer staging system that included several modifications in 2011. (5) Stage II disease, characterized by full-thickness tumor invasion of the bowel wall and the absence of lymph node metastases (N0) is now subdivided into IIA (T3 lesions that invade through the muscularis propria into pericolorectal tissues), IIB (T4a lesions that directly penetrate to the surface of the visceral peritoneum), and IIC (T4b lesions where tumor directly invades or is adherent to other organs or structures). Based upon the pathological report, TNM staging was performed in all cases. Only Stage II patients were selected.

Using the risk stratification algorithm (shown in *Fig. 1*) patients were divided into high risk, intermediate risk and low risk categories with appropriate indication for adjuvant

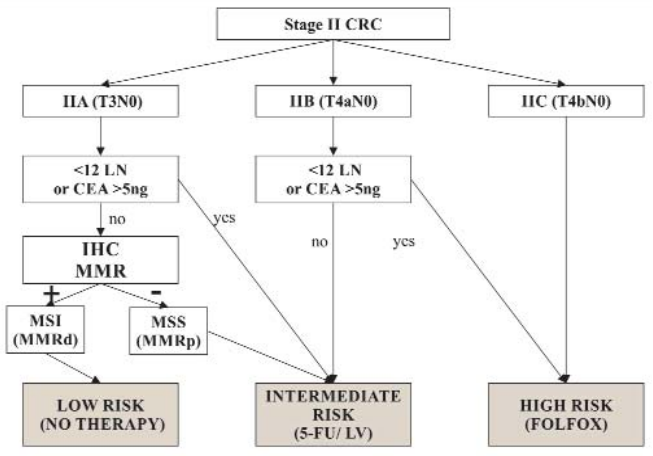

Figure 1. *Proposed risk stratification algorithm for adjuvant therapy in Stage II Colorectal Cancer*

LN: number of Lymph nodes examined; CEA: Serum Carcinoembryonic Antigen; IHC: Immunohistochemistry; MSI: Microsatellite instability; MMR: Mismatch Repair; MSS: microsatellite stable; FOLFOX: 5-Fluorouracil (5-FU) +Leucovorin (LV) + Oxaliplatin; XELOX: Capecitabine + Oxaliplatin

therapy: High risk – same regimens as stage III (FOLFOX); Intermediate risk: fluorouracil plus leucovorin or Capecitabine; Low risk: No therapy, observation only. Stage IIC is considered high risk in itself. Stage IIB with any other high risk feature associated (less than 12 lymph nodes examined, preoperative CEA > 5 ng/ml) are also classified as high risk. Stage IIB patients with no other high risk features are classified as intermediate risk. Stage IIA patients with high risk features are classified as intermediate risk. All stage IIA patients with no high risk features were tested for microsatellite instability by immunohistochemistry. These patients are further stratified into low risk (those who exhibit MSI/MMR-D phenotype) with good prognosis and no need for postoperative chemotherapy and intermediate risk (MSS phenotype), that were considered for fluorouracil plus leucovorin or Capecitabine.

Scheduled follow-up at 6 months and 1 year was performed. Follow-up included physical examination, abdominal ultrasound, CEA determination and colonoscopy (1 year).

Fisher's exact test (2 tailed) was used to assess the statistical significance of associations between high risk features of stage II CRC.

Results

During 2011 and 2012 a number of 115 consecutive patients were admitted in our clinic and received surgical resection for colorectal cancer. Using the TNM staging system we found 24 patients with Stage I (21%), 44 patients with Stage II (38%), 25 patients with Stage III (22%) and 22 patients with metastatic disease (19%). From the 44 patients with Stage II CRC, 30 patients were IIA (T3N0), 8 patients were IIB (T4aN0), and 6 patients were IIC (T4bN0).

The clinicopathological characteristics of this series of patients with Stage II CRC are shown in *Table 1*. All patients underwent complete surgical resection with clear histologic margins. All cases were adenocarcinomas.

We considered as high risk features: lesions where tumor directly invades or is adherent to other organs or structures (T4b), less than 12 lymph nodes examined and preoperative CEA > 5 ng/dl; the other potential risk factors (grading, obstruction, etc.), unconfirmed by meta-analysis, were taken into account but did not influence the decision making. The risk stratification algorithm was applied; patients were divided in the three risk categories. (See *Table 2*)

Immunohistochemical (IHC) analysis of MMR protein products was performed in 26 patients. IHC was abnormal in 8 cases (30.7%), suggestive for a MSI phenotype, that fell down in to the low risk subgroup, with no need for adjuvant chemotherapy. Microsatellite instability was associated with right colon tumor site (p=0.025), poor or undifferentiated histology (p=0.0028), mucinous or signet ring cell type adenocarcinoma (p=0.0138) and less than 60 years of age (p=0.0138).

It is worth mentioning the case of a 52 year-old female patient, with laparoscopic right hemicolectomy for a Stage IIA mucinous adenocarcinoma with signet ring cells and MSI phenotype at MMR-IHC (MSH6 loss of staining – see *Fig. 2*), that also fulfilled the Amsterdam II and revised Bethesda criteria. The patient was referred for specific Mismatch repair germline mutation testing that established the Lynch Syndrome diagnosis.

Table 1. *Clinicopathological characteristics of patients with Stage II colon cancer*

Factor	Category	No. of patients (n=44)
Age (yrs)	Median	65.6
	<60	12
	>=60	32
Gender	Male	26
	Female	18
Tumor location	Right	14
	Left	30
	Rectum	11
	Sigmoid colon	12
	Descending colon	3
	Splenic flexure	2
	Transverse Colon	2
	Ascending Colon	8
	Cecum	6
Surgical procedure	Right hemicolectomy	14
	Transverse colectomy	2
	Left hemicolectomy	7
	Sigmoidectomy	10
	Rectal/rectosigmoid colon (low anterior resection)	7
	Abdominoperineal resection	4
	Laparoscopic	11
Tumor size (cm)	<2	4
	2-5	26
	>5	14
T stage	T3 (IIA)	30
	T4a (IIB)	8
	T4b (IIC)	6
Number of Lymph Nodes examined	Median	13
	<12	10
	>=12	34
Grading	well-differentiated to moderately differentiated	31
	poorly differentiated to undifferentiated	13
Preoperative CEA	Median (ng/dl)	4.1
	<=5	32
	>5	12
Obstruction	Present	4
Perforation	Present	0
Lymphovascular invasion	Present	4
Perineural invasion	Present	4
Mucinous or signet-ring cell type	Present	12
Crohn-like reaction	Present	7
IHC analysis of MMR protein products	Total	26
	MSI phenotype	8
	MSS phenotype	18

CEA: Serum Carcinoembryonic Antigen; IHC: Immunohistochemistry; MSI: Microsatellite instability; MMR: Mismatch Repair; MSS: microsatellite stable;

Table 2. *Risk stratification in Stage II Colorectal cancer case series*

Risk Stratification	Low risk	No of pts	Intermediate risk	No of pts	High risk	No of pts
Category			Stage IIA CEA > 5 ng/dl or < 12LN	4		
	Stage IIA (T3AN0) with all of the following				Stage IIB CEA > 5ng/dl or < 12LN	4
	CEA < 5ng/dl > 12 LN MSI phenotype	8	Stage IIA CEA < 5ng/dl and > 12LN but MSS phenotype	18		
					Stage IIC	6
			Stage IIB CEA < 5ng/dl and > 12LN	4		
Total (n=44)		8		26		10
Adjuvant Therapy	No adjuvant therapy Observation only		5-Fluoro-uracil (5-FU)+Leucovorin (LV) Or Capecitabine		FOLFOX: 5-Fluoro-uracil (5-FU) +Leucovorin (LV) + Oxaliplatin	

CEA: Serum Carcinoembryonic Antigen; MSI: Microsatellite instability; MSS: microsatellite stable; LN: number of regional lymph nodes evaluated

We performed 11 laparoscopic interventions (5 low anterior resections, 4 right hemicolectomies and 2 sigmoidectomies) with no postoperative complications, and no cancer recurrence at 1 year follow-up. (*Fig. 3*)

Preoperative CEA > 5ng/dl was found in 12 cases in association with locally invasive T4 tumors – stage IIB and IIC (p=0.0004).

At 1 year follow-up we encountered 0 deaths and 7 cancer recurrences (15.9%). 5 patients developed liver metastases: 4 patients were part of the high risk subgroup that received FOLFOX adjuvant therapy, 1 patient was part of the intermediate risk subgroup that received Capecitabine. 2 patients with low anterior rectal resection suffered from local recurrence. Abdominoperineal resection was performed. They are currently following FOLFOX + Cetuximab chemotherapy.

We found liver metastases in association with T4 (p=0.0295), poor lymphatic resection - less than 12 lymph nodes examined (p=0.0068) and also lymphovascular invasion (p=0.05).

Elevated CEA was found in 9 cases at 6 months and in 10 cases at 1 year follow-up. All patients with elevated CEA levels at follow-up had also preoperative CEA > 5ng/dl; in 5 cases, cancer recurrence was diagnosed. In the other 5 cases, colonoscopy, abdominal ultrasound, computed tomography and chest X-ray did not show any sign of local recurrence or distant metastasis.

Low risk patients, in the observation only arm did not show any sign of recurrence at 1 year follow-up.

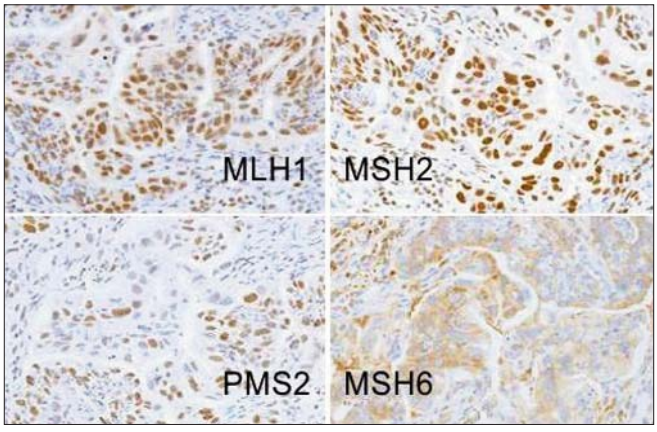

Figure 2. *Immunohistochemistry for Mismatch repair proteins on ascending colon cancer specimen. MLH1, MSH2, PMS2 show nuclear staining pattern; MSH6 (lower right) shows loss of staining*

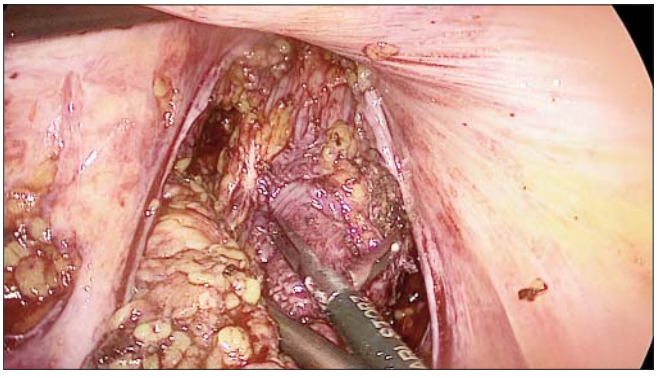

Figure 3. *Laparoscopic low anterior resection for rectal cancer – intraoperative image*

Discussion

Stage II colon cancer remains a very heterogeneous population, in which traditional staging systems are insufficient to accurately predict outcome and establish the indication for adjuvant therapy.

The impact of adjuvant chemotherapy for patients with stage II CRC has been addressed in several clinical trials. One important trial indicated a small but statistically significant survival benefit for patients with stage II disease treated with 5-FU/LV compared to patients not receiving adjuvant therapy (relative risk of recurrence at 2 years, 0.71; 95% CI, 0.54-0.92; P=.01). (6) A recent meta-analysis of 12 randomized

controlled trials from 1988 to 2010 in which surgery alone was the control arm found a significant benefit to adjuvant therapy in patients with stage II colon cancer. (7) Contrary, other studies showed no statistically significant difference in 5-year OS between the groups (78% vs. 75%, respectively), with a hazard ratio (HR) for survival of 0.91 (95% CI, 0.77-1.09) when patients receiving adjuvant treatment were compared with untreated patients. (6)

The 2011 TNM staging system changes were supported by an analysis of 109,953 patients with invasive colon cancer that found a 5-year survival rate considerably higher (79.6%) for node-negative patients with T4 tumors that penetrated the visceral peritoneum compared with patients with tumors that invaded or were adherent to other organs (58.4%). (8) Despite this modification, the TNM staging system remains imperfect because the same study found lower survival rate for both stages IIB and IIC than the more advanced Stage IIIA.

The current guidelines of the NCCN include the following high-risk features of Stage II CRC: bowel obstruction, grade 3-4 histology, T3 tumors with localized perforation, lymphatic or vascular invasion, T4 Tumors, close, indeterminate or positive margins, less than 12 lymph nodes examined. (9)

However, not all of them were confirmed by subsequent studies. In fact, in the QUASAR study, only T4 and inadequate lymph node sampling (<12) were confirmed as independent poor prognostic factors, while high grade of tumor, paradoxically, poorly differentiated histology is not considered a high-risk feature for patients with stage II disease whose tumors are MSI. Studies also confirmed that the presence of elevated preoperative CEA (>5 ng/ml) correlates with poor prognosis and reduced overall survival in Stage II colon cancer (10,11). CEA could therefore be used for identifying high-risk Stage II patients who might benefit from adjuvant therapy. (12) These high risk patients should follow the same postoperative protocols as in stage III CRC, as highlighted in one other trial (High risk Stage II patients' disease-free survival at 5 years was 82.1% with the use of FOLFOX by comparison with 74.9% with fluorouracil plus leucovorin). The same trial found that Stage II with none of the high risk features received no recurrence or survival benefit with the use of FOLFOX by comparison with fluorouracil plus leucovorin alone, so these patients should be spared from the toxicity of oxaliplatin treatment. (13) One of the key clinical features of MSI-CRC is their good prognosis and non-aggressive biology in spite of a commonly found undifferentiated histology. One of the consequences of this behaviour is that the prevalence of MSI is higher in earlier compared with later tumor stages. Specifically, MSI tumors account for up to 22% of all stage II colon cancers, but only for 12% in stage III and about 5% in metastatic cancer (14). In addition, MSI cancers are preferably right-sided with a decreasing prevalence of the MMR-D phenotype from proximal to distal locations, so that only around 4% of rectal cancers are MSI.

Recent results from large individual randomized adjuvant trials clearly validated the prognostic implication of microsatellite instability with hazard ratios for relapse-free survival (RFS), disease-free survival (DFS), and overall survival between 0.16 and 0.70. (15,16) Both trials demonstrated a very strong prognostic effect of MSI compared with MSS for stage II colon cancers but only an attenuated prognostic effect in stage III tumors. In conclusion, the MSI phenotype has unanimously been recognized as a marker of good prognosis. The risk of recurrence of an MSI-H stage II colon cancer is in the range of 3%–6% within the first 3 years, even without any adjuvant therapy.

Sargent et al. pooled the individual patient data from five randomized trials and compared a 5-FU–based adjuvant chemotherapy against surgery alone in stage II and stage III colon cancer. There was a statistically significant detriment in overall survival in stage II MSI-H tumors treated with 5-FU–based adjuvant chemotherapy compared to the untreated cohort (HR2.95, 95% CI 1.02–8.54, P00.04). This negative effect of adjuvant therapy was not found in stage III colon cancers. Therefore, the overall consensus has been that patients with stage II tumors exhibiting the MSI-H phenotype do not derive any benefit from 5-FU–based adjuvant chemotherapy, and might even have a detrimental effect (17).

Immunohistochemical assessment of the MMR protein products in selected patients managed to identify 8 cases of a MSI phenotype providing a clear and undisputed marker of good prognosis and no need for further adjuvant therapy. Thus, 18% of Stage II patients were assigned to the low risk category.

The risk stratification algorithm we implemented provides promising preliminary results, in identifying the low risk (8 patients) and high risk (10 patients) subgroups of stage II CRC. However, in between, there still remains a large grey intermediate risk category (26 patients) that will require to be further subdivided in order to avoid over- or under-treatment.

The standard of care in stage II CRC is surgical resection and a marker for its adequacy besides the clear tumoral margins is the number of lymph nodes removed by the surgeon and examined by the pathologist. (18) Patients considered to have N0 disease but for whom <12 nodes have been examined are suboptimally staged and should be considered to be at higher risk. In order to overcome this, three things can be done: a more extensive lymphadenectomy, by the surgeon, yielding more lymph nodes in a specimen, a more thorough examination by the pathologist that should go back to the specimen and submit more tissue of potential lymph nodes if fewer than 12 nodes were initially identified and last but not least, a better collaboration between the surgeon and the pathologist which can be obtained by introducing tumor boards, operative summaries, structured pathology reporting and knowledge transfer protocols.

The proposed algorithm of risk stratification is useful but still imperfect and requires further validation and constant optimization; perhaps the addition of new pathological markers such as perineural and lymphovascular invasion or molecular markers like allelic loss of chromosome 18q would help to further stratify stage II CRC patients, especially those in the intermediate risk category; clinical judgement must be exercised in all cases.

With this in mind, decision making regarding the use of adjuvant therapy for patients with stage II disease should

incorporate patient/physician discussions individualized for each patient, with explanations of the specific characteristics of his disease and its prognosis that include him in a certain risk category, and the evidence related to the efficacy and possible toxicities associated with treatment, centring on patient choice. (19,20)

Conclusions

Patients with stage IIA colorectal cancer with MSI phenotype should not receive adjuvant chemotherapy unless other factors such as preoperative CEA > 5ng/dl, a low number of lymph nodes retrieved convincingly put these patients into an intermediate-risk category.

We believe that microsatellite instability testing provides a useful tool in the goal of better characterizing patients with stage II colorectal cancer in matters of risk of recurrence and likelihood of benefit from chemotherapy. Immunohistochemical assessment of the MMR protein products should become a routine investigation in stage II CRC.

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