Chirurgia (2013) 108: 473-477
No. 4, July - August

Evaluation of Colorectal Adenocarcinomas at Single-Institution with Respect to Microsatellite Instability

S. Ozkara[1], B. Sari[1], A. Yesil[2], A. Aktekin[3], M. Sezikli[4], Z. Cetinkaya[4], F. Aker[1]

[1]Departments of Pathology, Haydarpasa Numune Education and Research Hospital, Istanbul,Turkey
[2]Departments of Gastroenterology, Haydarpasa Numune Education and Research Hospital, Istanbul,Turkey
[3]Departments of Surgery, Haydarpasa Numune Education and Research Hospital, Istanbul,Turkey
[4]Departments of Gastroenterology,Derince Research Education and Research Hospital Kocaeli, Turkey

Rezumat

Evaluarea adenocarcinoamelor colorectale într-un singur centru medical din punct de vedere al instabilității microsateliților

Premize şi scopul lucrării: Cancerul colorectal non-polipozic ereditar prezintă o frecvență mare a instabilității microsateliților (MSI). Studiile comparative privind stadiul şi alți parametri prognostici demonstrează un prognostic mai bun în prezența instabilității microsateliților comparativ cu cancerele colonice, fără instabilitatea microsateliților.
Material și Metodă: Studiul nostru a inclus 608 pacienți diagnosticați cu adenocarcinoame colorectale în laboratorul nostru între 2004-2010. Cazurile au fost reevaluate din punct de vedere al criteriilor definite pentru MSI, luând în considerare vârsta, localizarea anatomică şi criteriile histopatologice. S-a efectuat studiu imunohistochimic în blocurile alocate, utilizând MLH-1, MSH-2, MSH-6 şi PMS-2.
Rezultate: Specimenele au fost reevaluate din punct de vedere al criteriilor definite pentru instabilitatea microsateliților. Anticorpi anti MLH-1, anti MSH-2, anti MSH-6 şi anti PMS-2 s-au aplicat blocurilor de parafină a 27 de cazuri care au prezentat criterii morfologice sugestive pentru mutații în repararea ADN-ului şi care au avut un scor Mspath mare. Studiile imunohistochimice cu MLH-1, MSH-2, MSH-6 şi PMS-2, pentru analiza defectelor de reparație ADN au fost rafinate folosind cazurile cu scorurile Mspath mai mari.
Concluzii: În acest studiu am revizuit trăsăturile caracteristice clinice şi histopatologice a 608 cazuri de adenocarcinoame colorectale diagnosticate în laboratorul nostru între 2004 -2010 şi am evaluat caracteristicile patologice referitoare la instabilitatea microsateliților. Rezultatele au fost discutate în concordanță cu literatura de specialitate.

Cuvinte cheie: adenocarcinom colorectal, instabilitatea microsateliților, cancer colorectal nonpolipozic ereditar, imunohistochimie

Abstract

Background and aim: Hereditary non-poliposis colorectal cancers exhibit a high rate of microsatellite instability. Comparative studies involving stage and other prognostic parameters demonstrate a better prognosis in the presence of microsatellite instability versus colon cancers without microsatellite instability.
Methods: Our study included 608 cases diagnosed with colorectal adenocarcinoma by our laboratory between 2004-2010. The cases were re-evaluated with respect to criteria defined for MSI, taking into consideration age, anatomic localization, and histopathological criteria. Immunohistochemical study was performed in appropriate blocks for using MLH-1, MSH-2, MSH-6, and PMS-2.
Results: The specimens were re-evaluated according to the

Corresponding author: Selvinaz Ozkara MD
Tıbbiye Cad, 34668, Uskudar, Istanbul, Turkiye
Tel. +905052411647, +902164144502
E-mail: selvinazo@gmail.com

histological criteria defined for microsatellite instability. Anti-MLH-1, anti-MSH-2, anti-MSH-6, and anti-PMS-2 antibodies were applied to the paraffin blocks of 27 cases which presented morphological criteria suggestive of DNA repair mutation and had a high Mspath score. Immunohistochemical study with MLH-1, MSH-2, MSH-6, and PMS-2 for the analysis of mismatch repair was refined using the cases with higher Mspath scores.

Conclusions: In this study, we reviewed the clinical and histopathological features of 608 cases with colorectal adenocarcinoma diagnosed in our laboratory between 2004-2010 and assessed pathological features in terms of microsatellite instability. The results were discussed in view of the literature.

Key words: colorectal adenocarcinoma, microsatellite instability, hereditary non-poliposis colorectal cancer, immunohistochemistry

Introduction

Hereditary non-poliposis colorectal cancer (HNPCC) accounts for approximately 3-5% of colorectal malignancies. These cancers are seen in a younger age group, show multiple and right colon localization, and have a better prognosis than sporadic adenocarcinomas, with a majority showing genomic instability (1,2). This condition is defined as microsatellite instability (MSI) and has also been determined in 10% of sporadic colon adenocarcinomas.

It has been reported that HNPCC cases exhibit a high rate of MSI and show lower rates of lymph node metastasis even in advanced tumor stages. Comparative studies involving stage and other prognostic parameters demonstrate a better prognosis in the presence of MSI versus colon cancers without MSI (1,2,3).

Methods

Our study included 608 cases (320 males, 288 females; age range 28-84 years) diagnosed with colorectal adenocarcinoma in our laboratory between 2004 and 2010. The cases were re-evaluated with respect to criteria defined for MSI taking into consideration age, anatomic localization, and histopathological criteria (exophytic / polypoid growth, signet ring cell component, mucinous type, accompanying mucinous component, differentiation, pushing margins of medullary type, medullary component of $>10\%$, cribriform pattern, lymphocytosis, Crohn's-like reaction). In addition, other prognostic parameters were documented, including tumor size, degree of invasion, and lymph node metastasis. Immunohistochemical study was performed in appropriate blocks for genetic analysis of mismatch repair using MLH-1, MSH-2, MSH-6, and PMS-2.

For the statistical evaluation of data, SPSS for Windows 10.0 was used. In statistical analysis, descriptive statistical methods (mean, median, percentage and frequency) were used. Finally, for the comparison of qualitative data, Pearson's chi-square test was used with 95% confidence interval ($p<0.05$).

Results

The mean tumor size was 5.22 cm (range 1-15 cm). The tumors were located in the distal colon (n=271, 44.5%) (splenic flexure and distally), rectum (n=121, 20%), and proximal colon (n=216, 35.5%) (cecum, ascending colon, transverse colon). The tumor location is given in *Fig. 1*. There were 42 mucinous (6.9%) and 10 (%1.7) signet ring cell carcinomas. Of the adenocarcinomas, 66 were poorly (10.9%), 460 (75.6%) were moderately, and 30 (4.9%) were well differentiated. The tumor type and differentiation are given in *Fig. 2*. The specimens were re-evaluated with respect to the histological criteria defined for MSI (*Table 1*). Anti-MLH-1, anti-MSH-2, anti-MSH-6, and anti-PMS-2 antibodies were applied to the paraffin blocks of 27 cases, which presented morphological criteria suggestive of DNA repair mutation and having a high Mspath score defined by Jenkins et al. [IV]. Upon immunohistochemical examination, normal tissues showed immunoreactivity to these antibodies, while tumor tissues showed no reaction, which was accepted as a positive result. Immunohistochemical findings were as follows: 14 negative (51.85%) and 13 positive (48.15%) results to MLH-1; 23 negative (85.18%) and 4 positive (14.82%) results to MSH-2; 20 negative (74.07%) and 7 positive (25.03%) results to MSH-6

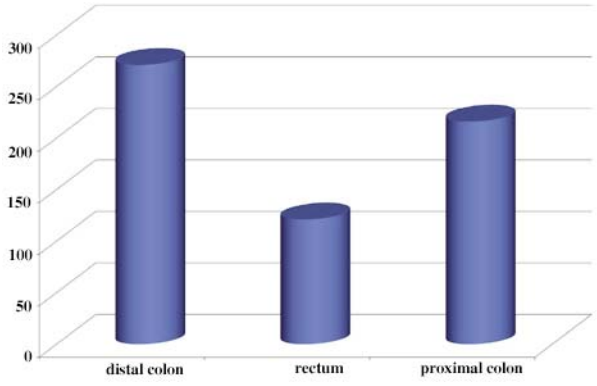

Figure 1. *The tumor location*

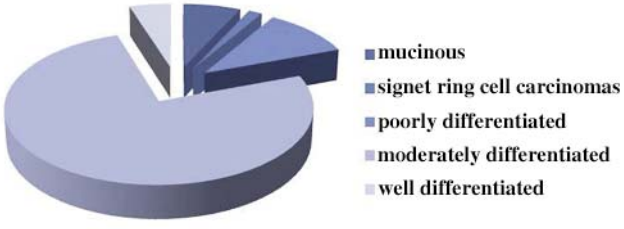

Figure 2. *The tumor type and differentiation*

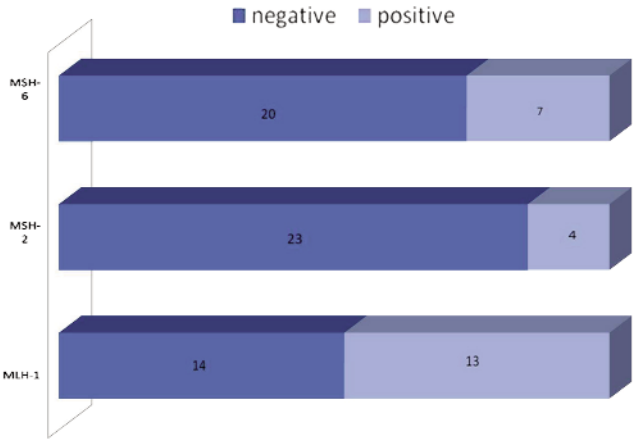

Figure 3. *Immunohistochemical findings*

(*Fig. 3*). Immunohistochemical results suggested MMR gene mutation (for MLH1, MSH2, MSH6, PMS2 genes alone or in combination) in a total of 17 cases (2.79%) (*Table 2*).

Discussion

The demographic characteristics of our patients are generally consistent with the literature. It has been reported in the literature that men and women are equally affected. Our cases were composed of 47.37% women and 52.63% men. Localization in our series was proportional to the frequency of the rectosigmoid region reported in the literature (5).

Two major pathways were identified for the development of colorectal adenocarcinomas. The first of these is the more common pathway leading to the sequence of inactivation of

Table 1. *Pathological features of colorectal carcinomas with high-frequency microsatellite instability*

Pathological features		Number (%)
Gender	female	288 (47.37)
	male	320 (52.63)
Age	<=45	54 (8.88)
	46-60	147 (24.18)
	61-75	236 (38.82)
	>75	171 (28.12)
Tumor location	proximal colon	216 (35,50)
	distal colon	392 (64,50)
Size	<4	262 (43.10)
	5—7	242 (39.80)
	>8	104 (17.10)
Stage / TNM (pT)	pT1	6 (0.98)
	pT2	56 (8.71)
	pT3	515 (85.22)
	pT4	31 (5.09)
Metastasis	Yes	14 (2.30)
	No	594 (97.70)
Synchronous tumor	Yes	13 (2.13)
	No	595 (97.87)
Accompanying component	Mucinous	23 (3.78)
	Medullary (>%10)	30 (54.93)
Cribriform pattern	Yes	48 (7.89)
	No	560 (92.11)
İnfiltration pattern	Expansive	34 (5.59)
	İnfiltrative	574 (94.41)
Crohn's-like reaction	Yes	137 (22,50)
	No	471 (77,5)
Tumor-infiltrating lymphocytes	yes / prominent	87 (14.30)
	no / minimally	521 (85.70)

Table 2. *Immunohistochemical results of MMR gene mutation (for MLH1, MSH2, MSH6, PMS2 genes alone or in combination)*

		MMR† gene mutation (+) n=17 (%)	MMR gene mutation (-) n=10 (%)	P
Gender	Female	5 (29.41)	6 (60.00)	0.816
	Male	12 (70.59)	4 (40.00)	
Age	< 50	2 (11.76)	2 (20.00)	0.523
	> 50	15 (88.24)	8 (80.00)	
Location	Left colon	8 (47.05)	7 (70.00)	0.168
	Right colon	9 (52.95)	3 (30.00)	
Stage	PT2	1 (5.88)	0 (0.00)	0.227
	PT3	16 (94.12)	8 (80.00)	
	PT4	0 (0.00)	2 (20.00)	
Differentiation	Poor	8 (47.05)	6 (60.00)	0.686
	Mucinous	1 (5.90)	0 (0.00)	
	Moderate	8 (47.05)	4 (40.00)	
İnfiltration	Expansive	14 (82.35)	7 (70.00)	0.080
	İnfiltrative	3 (17.65)	3 (30.00)	
Lymph node metastasis	No	17 (100.00)	4 (40.00)	0.024**
	Yes	0 (0.00)	6 (60.00)	
Mspath				
Scoring	Positive	17 (100.00)	9 (90.00)	0.326
	Negative	0 (0.00)	1 (10.00)	

** p<0.05 †: mismatch repair gene

tumor suppressor genes such as APC, p53 and the second pathway seen in a small number of patients with HNPCC (10-15%) is sporadically encountered in colon adenocarcinoma. In this alternative "mutator" pathway, increased MSI (MSI-H: instability in microsatellite focus > = 30%) associated with germ-line mutation in the "DNA mismatch repair" gene was observed. The cases in our series were re-evaluated in terms of pathological features defined with regard to MSI (6,7,8,9,10). In the study conducted by Jenkins et al. the pathological features of the tumor were compared to MSI or to a scoring system of high sensitivity-specificity which was proposed in order to determine the patients who should be directed to MSI or IHC tests. In the system defined as the Mspath score, the total value given according to the parameters varies between 0 and 6.6 (4). The cut- off value is 1, which is recommended for high sensitivity and maximum specificity in the system. The height of the Mspath score may show that the patients could have a better prognosis. However, further investigation (immunohistochemical and genetic) must be performed in these cases. When evaluating all patients in our study group, Mspath score was found >1 in 47.5%. In our series, the levels of the Mspath score were found high in poorly differentiated tumors. The levels of the Mspath score were found lower in well and moderately differentiated tumors. In our study, the difference between tumor stage and the level of the Mspath score was not statistically significant in poorly differentiated patients. However, there is a significantly strong, negative correlation between the lymph node score and the Mspath score (*Fig. 4*). In studies performed by Kim YH et al. and Lanza G et al. it was reported that lymph node metastases at a lower rate were compatible with better biological behaviour even in the MSI-H tumors which were at advanced stage (11,12).

In our series, the rate of patients in whom the Mspath score was found > 1 according to the method by Jenkins et al. (4) was 47.5%. Given the number of cases, this rate is too high to investigate the group comprising 10-15% patients, which can be defined as the target group. It seems to be both costly and time-consuming to perform the immunohistochemical and genetic analysis in all of them. The level of the Mspath score may be kept higher in order to make further reduction in the number of cases for whom further examination can be performed, or the patients that meet the current histopathological and clinical findings entirely could be included in the evaluation. For this purpose, the mean Mspath score of poorly differentiated cases was also measured.

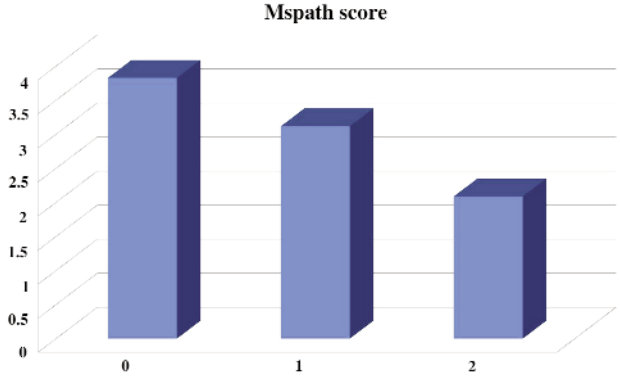

Figure 4. *The correlation of lymph node and Mspath score*

Of the microsatellite instability determinants, anti-MLH-1, anti-MSH-2, anti-MSH-6 and anti-PMS-2 antibodies were immunohistochemically applied to the paraffin blocks of cases that met the morphological criteria to point out the height of the level of the Mspath score (1,2,10,13). In 17 of our cases (62.96%) undergoing immunohistochemical examination one or more of these markers were stained. There was a strong negative relationship between any of the MLH-1, MSH-2, MSH-6, PMS-2 genes, or the associated immuno-reactivity and the lymph node metastasis score. No lymph node metastasis was observed in patients in whom no staining was seen on immunohistochemical examination. These findings are consistent with several studies in the literature and can be considered significant as a prognostic predictor even if they are not definitive. As is known, however, the basic method for detection of microsatellite instability is the PCR method, and positive prediction value of immunohistochemical examination is about 70% (1).

In the study by F Paraf et al. in cases that were MSI-H they found 20% mutations in MLH-1 or MSH-2 genes (14). In our study, the rate of observing mutation in MLH-1 and / or MSH-2 genes was found to be high because we performed immunohistochemical examination not only in patients with high MSI-H but also in those with high Mspath score level, and in whose DNA repair genes we expected clinical and histopathological mutations.

In studies carried out, in 22% of cases with colorectal carcinoma that were MSI-H, MSH-6 gene mutation was found. It has been reported that the risk of mutation increases at the ages of 70-80s and that in female patients who have colorectal carcinoma and MSH-6 gene mutation the coexistence of endometrial is 26% (15). The coexistence of mutation in MLH-1, MSH-2, MSH-6 genes is reported to be 1. In our study, the rate of MSH-6 mutation was 25.06%, which is consistent with the literature; the age range of the cases in which mutation was found varied between 62 and 85 years of age in accordance with the literature. The coexistence of mutation in the MLH-1, MSH-2, MSH-6 genes was found to be 3%.

The PMS-2 gene, which is one of the DNA repair genes, shows the lowest rate of mutation as compared to the other genes known in the literature. As PMS-2 antibody used in the immunohistochemical examination is very sensitive and requires special conditions for examination, and due to long fixation of the samples and the paraffin blocks of cases dating back to past years, the PMS-2 expression could not be examined ideally. In the immunohistochemical examination, performed by different methods (manual and Bond brand device) in 44% of the cases the internal control was not available and they were included in the uncertain group.

Mut S alpha complex (MSH-2 and MSH-6) detects faulty parts that occur during DNA replication and has

ATPase activity. Therefore, MSH-2 gene mutation was accompanied by MSH-6 gene mutation in patients with MSH-2 gene mutation (15,16,17). In our study, the rate of MSH-6 gene mutation was found significantly higher in cases with MSH-2 gene mutation, fact consistent with the literature ($p<0.05$). In these cases, a lifetime risk of endometrial carcinoma is 20-60%; the risk of ovarian carcinoma is approximately 10%. In our study, hospital records were re-evaluated in cases with MSH-2 and MSH-6 gene mutation. No data that suggests the existence of another carcinoma focus has been identified in these cases so far. However, the patients should be warned in this regard and the necessary precautions should be taken.

In conclusion, the presence of microsatellite instability has to be evaluated as an independent and important prognostic factor with reference to colon tumors. Based on some reports in the literature the presence of microsatellite instability both suggests a special group of carcinoma, and is considered to be a prognostic factor in determining the benefit of adjuvant chemotherapy (2). It was observed that although microsatellite instability-positive tumors had a better clinical course they were less responsive to adjuvant therapy, (5-fluorouracil) (6).

The genetic counselling and genetic testing should be started between the ages of 18-25 in the diagnosis of HNPCC. Molecular tests should be performed to confirm the diagnosis of HNPCC in patients who meet the clinical diagnostic criteria. Because in the majority of cases a satisfactory family history could not be taken, the Amsterdam criteria has become a scarce means for determining HNPCC cases. Today, among the patients who have just been diagnosed with colorectal carcinoma, the determination of the groups for MSI analysis seems to be a more accurate and faster method. The most appropriate tests to use are "microsatellite instability test" (MSI) and MLH1 / MSH2 "germ-line" test, after the immunohistochemical examination of the tumor. The individuals in whom genetic mutation is not detected, but who meet the clinical diagnostic criteria, should be considered at high risk for HNPCC and should be included in follow-up protocol. This way, it is predicted that the mortality and morbidity rates will be significantly reduced in patients with HNPC and in their relatives. Our results can show that the patients with high mean Mspath score may have a better prognosis. In our laboratory the scoring system and immunohistochemical panel have been used routinely in colon resections. However, further investigations must be implemented in cases with a high mean Mspath score (immunohistochemical and genetic).

References

1. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004 ;96(4):261-8.
2. Scartozzi M, Bianchi F, Rosati S, Galizia E, Antolini A, Loretelli C, et al. Mutations of hMLH1 and hMSH2 in patients with suspected hereditary nonpolyposis colorectal cancer: correlation with microsatellite instability and abnormalities of mismatch repair protein expression. J Clin Oncol. 2002;20(5):1203-8.
3. Hamilton SR, Vogelstein B, Kudo S, et al. Carcinoma of the colon and rectum. In: Hamilton SR, Aaltonen LA, eds. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Digestive System. Lyon, France: IARC Press; 2000. p. 103-143.
4. Jenkins MA, Hayashi S, O'Shea AM, Burgart LJ, Smyrk TC, Shimizu D, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology. 2007;133(1):48-56.
5. Redstone M. Epithelial Neoplasm of the Large ıntestine. In: Odze RD, Goldblum JR, Crawfordm JM, eds. Surgical Pathology of the GI Tract Liver Biliary Tract and Pancreas. Philadelphia: Elsevier Saunders; 2003. p. 597-637.
6. Gafà R, Maestri I, Matteuzzi M, Santini A, Ferretti S, Cavazzini L, et al. Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. Cancer. 2000;89(10):2025-37.
7. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer. 2001;91(12):2417-22.
8. Lanza G, Gafà R, Maestri I, Santini A, Matteuzzi M, Cavazzini L. Immunohistochemical pattern of MLH1/MSH2 expression is related to clinical and pathological features in colorectal adenocarcinomas with microsatellite instability. Mod Pathol. 2002; 15(7):741-9.
9. Nastase A, Pâslaru L, Niculescu AM, Ionescu M, Dumitraşcu T, Herlea V, et al. Prognostic and predictive potential molecular biomarkers in colon cancer. Chirurgia (Bucur). 2011;106(2):177-85.
10. Mureşan F, Simescu R, Domşa I, Buiga R, Cazacu M. Immunohistochemical screening of hMLH1 and hMSH2 gene mutations in patients diagnosed with colorectal cancer and microsatellite instability suspicion. Chirurgia (Bucur). 2011; 106(6):775-80. Romanian
11. Kim YH, Min BH, Kim SJ, Choi HK, Kim KM, Chun HK, et al. Difference between proximal and distal microsatellite-unstable sporadic colorectal cancers: analysis of clinicopathological and molecular features and prognoses. Ann Surg Oncol. 2010; 17(5):1435-41.
12. Lanza G, Gafà R, Matteuzzi M, Santini A. Medullary-type poorly differentiated adenocarcinoma of the large bowel: a distinct clinicopathologic entity characterized by microsatellite instability and improved survival. J Clin Oncol. 1999;17(8):2429-38.
13. Müller A, Giuffre G, Edmonston TB, Mathiak M, Roggendorf B, Heinmöller E, et al. Challenges and pitfalls in HNPCC screening by microsatellite analysis and immunohistochemistry. J Mol Diagn. 2004;6(4):308-15.
14. Paraf F, Gilquin M, Longy M, Gilbert B, Gorry P, Petit B, et al. MLH1 and MSH2 protein immunohistochemistry is useful for detection of hereditary non-polyposis colorectal cancer in young patients. Histopathology. 2001;39(3):250-8.
15. Baglietto L, Lindor NM, Dowty JG, White DM, Wagner A, Gomez Garcia EB, et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst. 2010;102(3):193-201.
16. Hendriks YM, Wagner A, Morreau H, Menko F, Stormorken A, Quehenberger F, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology. 2004;127(1):17-25.
17. Lagerstedt Robinson K, Liu T, Vandrovcova J, Halvarsson B, Clendenning M, Frebourg T, et al. Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics. J Natl Cancer Inst. 2007;99(4):291-9.