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Molecular biology from bench-to-bedside – Which colorectal cancer patients should be referred for genetic counselling and risk assessment

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ABSTRACT

Lynch syndrome is associated with deficiency of the mismatch repair genes *MLH1, MSH2, MSH6 or PMS2*. However, most MLH1 deficient tumours are sporadic in origin, and they can be identified if harbouring a *BRAF* V600E mutation or hypermethylation of the *MLH1* gene promoter.

The aim of this study was to validate our previously suggested clinically applicable strategy based on molecular characteristics for identifying which patients to refer for genetic counselling.

The strategy was validated in an unselected cohort of 287 colorectal cancer patients. All tumours were tested for MLH1, PMS2, MSH2 and MSH6 protein expression with immunohistochemistry. DNA from MLH1 negative tumours was sequenced for *BRAF* mutations. If *BRAF* was wild-type, *MLH1* promoter was subsequently analyzed for promoter hypermethylation.

Most tumours, 251 (88%), stained positive for all four proteins. Six (2%) had negative MSH2 and one (<1%) isolated loss of MSH6. MLH1 and PMS2 were negative in 29 cases (10%). DNA quality allowed *BRAF* analysis in 27 of these with 14 mutations and 13 wild-type. DNA quality allowed methylation analysis in 11 of the 13 *BRAF* wild-type, and all but one were methylated. Subsequently, Lynch syndrome could not be ruled out in 12 patients. A follow-up at 8–10 years revealed four definite cases of Lynch syndrome and three highly suspicious.

An easy and clinically applicable step-wise approach with immunohistochemistry (100%), *BRAF* sequencing (10%) and methylation analysis (5%) identified several patients with hereditary cancer. It is feasible to perform a molecular screening to select patients for genetic counselling.

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1. Introduction

The most common hereditary colorectal cancer syndrome is hereditary non-polyposis colon cancer (HNPCC) or Lynch syndrome.[1] Phenotypically, Lynch syndrome is associated with a dominant trait where in a typical family every generation is affected and individuals are diagnosed at an earlier age compared to sporadic cancer.[2] On the molecular level, Lynch

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syndrome is caused by germ-line mutations in one of the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* or *PMS2*. The cumulative incidence of CRC at 70 years for gene mutation carriers is about 50%[3] indicating that affected patients acquire a second hit inactivating the other allele of the gene involved. Thereby the affected gene is inactivated and causes deficiency of the MMR system, hypermutability and eventually cancer. It has been shown that surveillance is very effective in preventing death from CRC in Lynch families.[4]

The important challenge to the surgeon, the gastroenterologist and the oncologist in daily clinic is therefore to identify patients who may have Lynch syndrome and then refer them for genetic counselling. So far, family history has been the gold standard for selecting patients for genetic counselling. Several sets of clinical criteria have been developed offering high specificity (Amsterdam criteria) or high sensitivity (revised Bethesda criteria).[5–8]

There are, though, several pitfalls in relying solely on the family history. The history may be severely insufficient[9] or the disease may be caused by new mutations or possibly by mutations with late onset or low penetrance. Furthermore, familial clustering is often overlooked in small families, known or unknown adoption and in alternate paternity.[10] Another pitfall with increasing importance is the effect of population screening for colorectal cancer with colonoscopy[11] where some patients will have adenomas removed that might eventually have led to cancer. Thereby some cases of Lynch syndrome may be prevented; cases that would otherwise have lead a positive family history. Although still imperative, family history is therefore not sufficient in several cases.

Recent years' advances in molecular techniques have now given instruments to improve the discovery of Lynch syndrome. Lynch syndrome is the only known cause of deficiency of MSH2, MSH6 or PMS2, whereas most MLH1 negative tumours are sporadic in origin and associated with epigenetic silencing of the gene by promoter hypermethylation[12] and a specific *BRAF* V600E mutation.[13] How can this basic knowledge be brought from bench-to-bedside?

We have previously suggested a clinically applicable strategy for the classification of CRC with respect to mismatch repair deficiency.[14] The strategy included immunohistochemistry (IHC), *BRAF* mutation analysis and methylation analysis as depicted in Fig. 1. Among 262 patients, we found four cases that were highly suspicious for Lynch syndrome. The aim of the present study was to validate the strategy in an independent cohort and thereby establish an algorithm for a feasible population screening to identify patients that would benefit from genetic counselling and testing.

2. Patients and methods

2.1. Screening algorithm and patients

The algorithm for screening is presented in Fig. 1.[14] In the present study, the strategy was validated in an unselected, retrospective cohort of 287 colorectal cancer patients. Patients undergoing surgery and diagnosed with colorectal adenocarcinoma at our institution during the years 1999 and 2000 were included retrospectively.

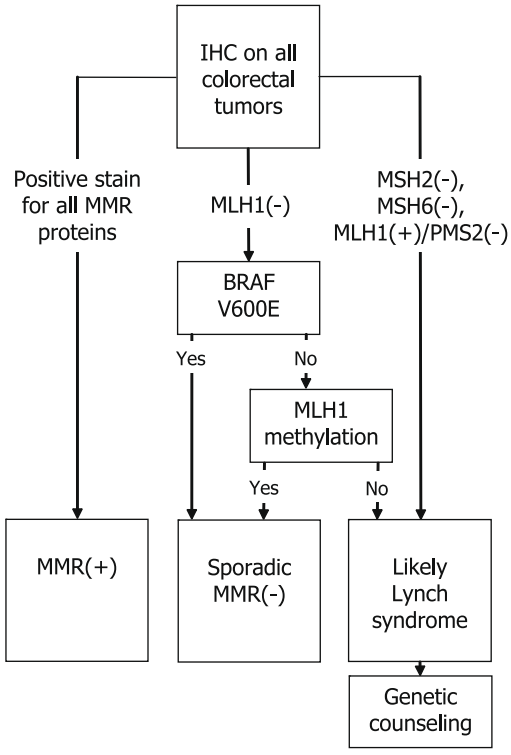

Fig. 1 – Algorithm for molecular based population screening of which patients to refer for genetic counselling because Lynch syndrome cannot be excluded. MMR(+), mismatch repair proficiency, MMR(–), mismatch repair deficiency.

2.2. Histopathology and BRAF

A standard histopathological examination was performed determining histologic type of cancer, differentiation, tumour (T) and node (N) classification, venous and perineural invasion. Stage was based on the T and N classification and data on metastases (M) derived from biopsies, surgical descriptions and radiologic examinations. Furthermore, all tumours were tested for protein expression of MLH1, PMS2, MSH2 and MSH6 with IHC. If tumour cells compared to normal cells had lost expression of the protein in question, it has been classified as negative for that protein. DNA from MLH1/PMS2 negative tumours was sequenced for the *BRAF* V600E mutation. All methods have been described earlier.[14]

2.3. Methylation analysis

If *BRAF* was not mutated, the *MLH1* promoter was analyzed with methylation-specific multiplex ligation-dependent probe amplification (MLPA). A commercially available kit was used and instructions according to the manufacturer were followed (kit ME011, MRC-Holland, Amsterdam, The Netherlands). In methylation-specific MLPA,[15] probes specific for several methylation sites in the *MLH1* gene promoter are annealed to denatured tumour-DNA and subsequently ligated. One of two reactions is then digested with a methylation-sensitive endonuclease that will digest un-methylated sites. After PCR, electrophoresis of both reactions is compared to indicate methylation at the corresponding methylation

site. It is possible to estimate the percentages of methylated versus un-methylated DNA, and methylation sites throughout the promoter region and intron 1 can be analysed simultaneously. Only methylation of proximal sites were considered positive for methylation induced inactivation of *MLH1*.[16] In cases with low quality DNA not suitable for MLPA, a more sensitive methylation-specific PCR was used. In this assay tumour-DNA was bisulphite treated according to the manufacturer's instructions (EZ DNA Methylation-Gold Kit, Zymo Research, CA, USA) and quality was tested with a methylation independent control (ALU-C4 primers and probe according to Weisenberger et al.[17]). Real-time PCR (Applied Biosystems 7900, Applied Biosystems Inc., CA, USA) subsequently compared the input of total bisulphite-treated DNA to the input of DNA with specific methylation of the proximal MLH1 promoter (MLH1 primers and probe according to Fiegl et al.[18]). Samples were run in doublets with adequate positive and negative controls.

2.4. Interpretation

Deficiency of MSH2, MSH6 or PMS2 was regarded as possibly due to a germ-line mutation. PMS2 and MSH6 are concomitantly lost on the protein level if the tumour is deficient of MLH1 or MSH2, respectively.[19] Therefore, loss of both MSH2 and MSH6 was suspicious for germ-line mutation in MSH2. Isolated loss of PMS2 or MSH6 indicated germ-line mutation in the affected gene. If both MLH1 and PMS2 were absent, MLH1 deficiency was suspected, but it was considered sporadic if *BRAF* V600E mutation or *MLH1* promoter hypermethylation was found.

2.5. Clinical records

Clinical records were obtained from patients with MSH2, MSH6 or PMS2 negative tumours and MLH1 negative cases not shown to be sporadic. The records were thoroughly examined for information about the family history and cancers related to Lynch syndrome. Initial records were defined as records covering the time from start of diagnostic setup to the first control after surgery. Available records during the 8–10 years follow-up were also obtained and histopathology records were drawn from a database. Furthermore, the patients were looked up in a registry covering known cases and families with Lynch syndrome.

2.6. Ethics

The study was approved by the responsible regional committee on human experimentation and was in concordance with the Helsinki Declarations.

2.7. Statistics

Descriptive statistics used median and range. Comparisons of proportions were done by the chi^2-test or Fisher's exact test. Ranked variables were compared with Wilcoxon rank sum test or Kruskal–Wallis test. The two-sided significance level was set at 5%. All analyses were performed using STATA version 8 (StataCorp LP, Texas, USA).

3. Results

3.1. IHC, BRAF and MLPA

Most tumours, 251 (87.5%), stained positive for all four proteins. Six (2.1%) had negative MSH2 and one (0.3%) isolated loss of MSH6. MLH1 and PMS2 were both negative in 29 cases (10.1%). Isolated loss of PMS2 was not observed.

DNA quality allowed *BRAF* analysis in 27 of the 29 MLH1/PMS2 negative tumours with 14 mutations and 13 wild-type found. DNA quality allowed MLPA in four of the 13 *BRAF* wild-type, and all four were methylated. Of the remaining nine cases, methylation-specific PCR was successful in seven with only one not showing methylation of the MLH1 promoter.

3.2. Screening outcome

The screening strategy thus resulted in six MSH2 negative, one MSH6 negative and five MLH1 negative cases without *BRAF* mutation or methylation or where the analyses failed due to low DNA quality. Subsequently, Lynch Syndrome could not be excluded in 12 patients.

3.3. Patient records

Among these 12 patients, information about the family history was documented in six of the initial patient records. Patient #1 (Table 1) had a large family with only a few cancers, so she was initially in the clinic regarded as a sporadic case, but a few years later she was referred to clinical genetics and an *MSH2* mutation was found. Patient #2 also had an *MSH2* mutation identified by the geneticists and she had previously suffered from a rectal cancer. Patient #3 had a sarcoma at the age of 26 and had a hysterectomy at the age of 40 after metrorrhagia and atypical cells in endometrial abrasion. Malignancy was not found in the surgical specimen. She had previously had her family history analysed without hereditary cancer being suspected, but received genetic counselling after the colon cancer at 53 years. An *MSH2* mutation was found and at risk family members were referred to relevant surveillance programmes. A cousin, patient #4 was subsequently diagnosed with an asymptomatic colon cancer. No familial details were given for patients #5 and #6 and they both have subsequently died from other causes. The family history of patient #7 was insignificant with unspecified cancers affecting her father and mother's father, but she had a few years later an endometrial cancer and died from metastatic disease. In patients #8 through #12, clinical data supporting Lynch syndrome were weak.

Table 1 shows individual data from the 12 patients. Based on retrospective data obtained 8–10 years postoperatively, four definite Lynch syndrome and three highly suspicious cases were identified.

3.4. Patient characteristics

Table 2 compares patients characteristics for the group of probably sporadic CRC ($n = 279$) to the group of verified and probably Lynch syndrome ($n = 12$). Lynch patients were youn-

Table 1 – Molecular screening of 287 patients identified 12 cases where Lynch Syndrome could not be excluded. This table shows individual patient data, the by immunohistochemistry affected mismatch repair gene, results of *BRAF* sequencing and methylation analysis, the probability of Lynch Syndrome, and key clinical information. Wt, wild-type; NA, not available due to low DNA quality; MLH1-CH_3, methylation of the MLH1 promoter.

No	Age	Sex	Tumour	Gene	*BRAF*	MLH1-CH3	Lynch	Clinical data	Dead
								Adjuvant chemotherapy (5-FU)	
1	31	Female	Right colon	*MSH2*	–	–	Yes	Years later Lynch was suspected and mutation was found	No
2	63	Female	Right colon	*MSH2*	–	–	Yes	Previous rectal cancer. Died from metastatic disease	Yes
								Mutation found	
3	53	Female	Right colon	*MSH2*	–	–	Yes	Mutation positive Lynch	No
								Adjuvant chemotherapy 5-FU	
4	60	Male	Right colon	*MSH2*	–	–	Yes	Mutation positive Lynch	No
								Adjuvant chemotherapy 5-FU	
5	82	Female	Right colon	*MSH2*	–	–	Probably	No information	Yes
6	79	Male	Left colon	*MSH6*	–	–	Probably	No relapse during follow-up	Yes
7	49	Female	Rectum	*MSH2*	–	–	Probably	Two years later metastatic cancer of the uterus	Yes
8	60	Female	Right colon	*MLH1*	NA	NA	Unknown	Metachronous MLH1 negative colon cancer 2 years later, no relapse	No
9	52	Male	Right colon	*MLH1*	wt	NA	Unknown	No relapse during follow-up	No
10	74	Male	Right colon	*MLH1*	wt	NA	Unknown	No information	Yes
11	66	Female	Right colon	*MLH1*	NA	NA	Unknown	Early liver relapse	Yes
12	79	Female	Left colon	*MLH1*	wt	No	Unknown	No familial clustering. Relapse at 2 years	Yes

Table 2 – Patient characteristics compared between screening negative (probably sporadic colorectal cancer) and screening positive (Lynch syndrome not excluded) patients.

		Screening negative (n = 275)	Screening positive (n = 12)	p
Age, years	Median (range)	73 (40–93)	62 (31–82)	0.04
Sex, n	Female	136	8	
	Male	139	4	0.38
Tumour, n	Right colon	68	9	
	Left colon	103	2	
	Rectum	104	1	<0.001
Stage, n	I	24	0	
	II	108	6	
	III	107	4	
	IV	36	2	0.76
Histology, n	Mucinous	21	5	
	Non-mucinous	254	7	<0.001
Differentiation, n	Moderate	243	6	
	Poor	32	6	<0.001

ger and had significantly more proximal tumours, mucinous histology and poor differentiation.

4. Discussion

Modern molecular techniques can add important information to the increasing use of immunohistochemistry staining for mismatch repair proteins in colorectal cancers. We wanted to unite these techniques and bring the combined approach into a clinical setting with the aim of improving the identification of colorectal cancer patients who would benefit from genetic referrals. This was done by validating a previously suggested screening strategy in an independent patient cohort. According to this algorithm, Lynch syndrome could not be excluded in 12 cases of which four after detailed genetic analyses were found to be definitely Lynch syndrome and another three cases were very likely also to suffer from this syndrome. This is comparable to the expected 3% rate of Lynch syndrome in unselected patients.[2] A retrospective design has been chosen in order to have a long follow-up, but due to this the DNA quality was impaired to allow methylation analysis in all of the MLH1 negative and *BRAF* wild-type cases. As a consequence four MLH1 deficient tumours could not be classified fully. DNA quality strongly depends on storage

time[20] and DNA analysis performed routinely at the primary diagnosis would be expected to be much more successful. To our knowledge, this is the first report on methylation-specific MLPA applied in molecular screening for Lynch syndrome. It is a promising method because methylation throughout the promoter region is analysed and the assay can thus distinguish insignificant distal methylation from the proximal methylation related to gene inactivation.[21,22] Furthermore, the semi-quantitative results allow discrimination between sporadic bi-allelic inactivation of the gene and methylation as a possible second hit inactivation of the non-mutated allele in Lynch syndrome.[23,24] A limitation in retrospective studies is the need for a higher amount of high quality DNA for MLPA than for *BRAF* mutation analysis or methylation-specific PCR as the amplified sequences are longer in MLPA.

Due to retrospective design, exhaustive clinical data including family history and results of genetic counselling and testing could not always be obtained. Therefore, our estimated number of definitively Lynch syndrome cases is rather conservative. However, it was not within the scope of this study to diagnose the syndrome, but to validate the feasibility of a screening algorithm. It is important to emphasize, that the algorithm is a screening tool and not a diagnostic test. It is a tool in daily practice to select which patients to refer for genetic counselling. The diagnosis of Lynch syndrome can only be properly given after genetic counselling and germ-line mutation analysis. In our opinion, family history, IHC, *BRAF*- and methylation analyses should all be part of a routine work-up of all colorectal cancer cases. Based on the results, the clinician can inform the patient properly and obtain consent before proceeding with genetic counselling and risk assessment. Information should contain considerations about the pros and cons of genetic testing for the patient as well as the family.

Compared to the family history alone, a molecular screening of all colorectal tumours for Lynch syndrome is both feasible and results in considerably more cases being identified.[25–27] In many cases a family history is either not obtained or its relevance is not assessed.[9,28] Furthermore, there is a considerable inaccuracy of patients' reporting.[29]

There are several ways to perform a molecular screening, but in general IHC and microsatellite instability analysis are used. The performance is comparable,[14,26] but because of the costs, the wide availability of IHC and extra information on the affected gene, IHC should be preferred. Supplemental microsatellite instability analysis can detect the few samples missed by variable IHC stain, and may thus be considered in selected cases. Whereas there is little doubt about this initial step, the great challenge in daily clinic is the large group of MLH1 deficient patients caused by sporadic hypermethylation. The high frequency of this group is likely to reduce the use of a molecular screening program, since it will be considered a waste of resources and it will induce unnecessary worry. Therefore, if IHC testing for MLH1 is done routinely, it is imperative to perform analysis for *BRAF* and/or methylation in order to get a clear picture.

The implementation of molecular screening for Lynch syndrome must rely on local conditions, but probably the driving force in daily clinic should be the pathologist to perform and report the results of IHC as suggested by Jahn.[30] Following our algorithm, the pathologist can also assure that tissue from MLH1 deficient patients is analysed for *BRAF* and/or methylation. Supplemental analyses can be performed within a few days and the results should be included in the written report together with a recommendation for genetic counselling, if indicated. Based on the present evidence, we will implement the screening strategy prospectively and describe the clinical outcome in a future study.

An easy and clinically applicable step-wise approach with IHC (100%), *BRAF* sequencing (10%), and methylation analysis (5%) identified several patients with hereditary cancer. It is feasible to perform a molecular screening to decide whom to send for genetic counselling.

Conflict of interest statement

None declared.

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REFERENCES

1. DeVita VT, Lawrence TS, Rosenberg SA. *Cancer principles and practice of oncology*. 8th ed. Lippincott Williams & Wilkins; 2008.
2. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med* 2003;**348**(10):919–32.
3. Barrow E, Alduaij W, Robinson L, et al. Colorectal cancer in HNPCC: cumulative lifetime incidence, survival and tumour distribution. A report of 121 families with proven mutations. *Clin Genet* 2008;**74**(3):233–42.
4. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary non-polyposis colorectal cancer. *Gastroenterology* 2000;**118**(5):829–34.
5. Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum* 1991;**34**(5):424–5.
6. Rodriguez-Bigas MA, Boland CR, Hamilton SR, et al. A National Cancer Institute workshop on hereditary non-polyposis colorectal cancer syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 1997;**89**(23):1758–62.
7. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the international collaborative group on HNPCC. *Gastroenterology* 1999;**116**(6):1453–6.
8. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary non-polyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004;**96**(4):261–8.
9. Trano G, Wasmuth HH, Sjursen W, Hofsli E, Vatten LJ. Awareness of heredity in colorectal cancer patients is insufficient among clinicians: a Norwegian population-based study. *Colorectal Dis* 2009;**11**(5):456–61.

10. Bellis MA, Hughes K, Hughes S, Ashton JR. Measuring paternal discrepancy and its public health consequences. *J Epidemiol Commun Health* 2005;**59**(9):749–54.
11. Rex DK, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American college of gastroenterology guidelines for colorectal cancer screening 2008. *Am J Gastroenterol* 2009;**104**(3):739–50.
12. Kane MF, Loda M, Gaida GM, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997;**57**(5):808–11.
13. Domingo E, Niessen RC, Oliveira C, et al. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. *Oncogene* 2005;**24**(24):3995–8.
14. Jensen LH, Lindebjerg J, Byriel L, Kolvraa S, Cruger DG. Strategy in clinical practice for classification of unselected colorectal tumours based on mismatch repair deficiency. *Colorectal Dis* 2008;**10**(5):490–7.
15. Nygren AO, Ameziane N, Duarte HM, et al. Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences. *Nucleic Acids Res* 2005;**33**(14):e128.
16. Capel E, Flejou JF, Hamelin R. Assessment of MLH1 promoter methylation in relation to gene expression requires specific analysis. *Oncogene* 2007;**26**(54):7596–600.
17. Weisenberger DJ, Campan M, Long TI, et al. Analysis of repetitive element DNA methylation by methylight. *Nucleic Acids Res* 2005;**33**(21):6823–36.
18. Fiegl H, Gattringer C, Widschwendter A, et al. Methylated DNA collected by tampons – a new tool to detect endometrial cancer. *Cancer Epidemiol Biomarkers Prev* 2004;**13**(5):882–8.
19. Chang DK, Ricciardiello L, Goel A, Chang CL, Boland CR. Steady-state regulation of the human DNA mismatch repair system. *J Biol Chem* 2000;**275**(24):18424–31.
20. Jensen LH, Cruger DC, Lindeberg J, Byriel L, Petersen GB, Jakobsen A. Laser microdissection and microsatellite analysis of colorectal adenocarcinomas. *Anticancer Res* 2006;**26**:2069–74.
21. Deng G, Chen A, Hong J, Chae HS, Kim YS. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. *Cancer Res* 1999;**59**(9):2023–9.
22. Capel E, Flejou JF, Hamelin R. Assessment of MLH1 promoter methylation in relation to gene expression requires specific analysis. *Oncogene* 2007(June 4).
23. Bettstetter M, Dechant S, Ruemmele P, et al. Distinction of hereditary non-polyposis colorectal cancer and sporadic microsatellite-unstable colorectal cancer through quantification of MLH1 methylation by real-time PCR. *Clin Cancer Res* 2007;**13**(11):3221–8.
24. Alemayehu A, Sebova K, Fridrichova I. Redundant DNA methylation in colorectal cancers of Lynch-syndrome patients. *Genes Chromosomes Cancer* 2008;**47**(10): 906–14.
25. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary non-polyposis colorectal cancer). *N Engl J Med* 2005;**352**(18):1851–60.
26. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. *J Clin Oncol* 2008;**26**(35):5783–8.
27. Julie C, Tresallet C, Brouquet A, et al. Identification in daily practice of patients with Lynch syndrome (hereditary non-polyposis colorectal cancer): revised Bethesda guidelines-based approach versus molecular screening. *Am J Gastroenterol* 2008;**103**(11):2825–35.
28. Grover S, Stoffel EM, Bussone L, Tschoegl E, Syngal S. Physician assessment of family cancer history and referral for genetic evaluation in colorectal cancer patients. *Clin Gastroenterol Hepatol* 2004;**2**(9):813–9.
29. Mitchell RJ, Brewster D, Campbell H, et al. Accuracy of reporting of family history of colorectal cancer. *Gut* 2004;**53**(2):291–5.
30. Jahn S, Minai-Pour MB, Speicher MR, Reiner-Concin A, Hoefler G. Comprehensive screening for Lynch syndrome: who can be the driving force in daily clinical practice? *J Clin Oncol* 2009;**27**(13):2292.