

# HEREDITARY NON-POLIPOMATOUS COLORECTAL CANCER: hereditary predisposition, diagnosis and prevention

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**ABSTRACT – Background** - Colorectal cancer is the third in frequency and the second in mortality in developed countries. In Brazil, it is among the six more common malignant neoplasias. About 20% of colorectal tumors have some hereditary component. **Aim** - This study presents a review of genetic and clinic aspects, as well as diagnosis and prevention of the hereditary non-polipomatous colorectal cancer, that is the more frequent form of hereditary colorectal cancer. This approach is important because, currently there are possibilities of management, prevention and surveillance specific to individuals at-risk for hereditary non-polipomatous colorectal cancer that can lead to a great improvement in patients' survival and their at-risk relatives.

**HEADINGS** – Colonic neoplasms, genetics. Colorectal neoplasms, hereditary nonpolyposis. Heredity.

## INTRODUCTION

Colorectal cancer (CRC) is the third tumor in frequency and the second in mortality in developed countries. In Brazil, it is among the six more common malignant neoplasias and is the third in mortality in both sexes<sup>(9,10)</sup>. Median 5 year-survival of CRC patients is of 60% and, currently, the most used prognostic factors are based on clinical findings<sup>(5,9)</sup>.

There are three different types of colorectal cancer with some overlapping of clinical features: sporadic, familial and hereditary cancer. Sporadic cancer is a result of the interaction of somatic mutations and environmental factors, and generally occurs isolated in a family and at older age. Familial cancer is clustered in families and probably occurs due to exposure to the same environmental risk factors or to the presence of low-penetrance mutations in susceptibility genes. On the other hand, high-penetrance germline mutations are found in hereditary cancer. The presence of these mutations in the involved families can lead to the occurrence of multiple cancers at an early age. Characterization of each pathway of their carcinogenesis sometimes is difficult, since several times the family history can not give all needed informations to

differentiation among these three types of cancer, specially in small families.

There is great importance in determining the hereditary nature of an intestinal neoplasia, since 20% of colorectal tumors have an hereditary component, and hereditary cancer predisposition syndromes usually predispose to the occurrence of more than one type of cancer in the same patient or family. In addition, currently there are available tools to achieve early diagnosis and then to individualize treatment in order to improve survival rates and to prevent the development of other tumors associated with hereditary colorectal cancer syndromes (HCRC)<sup>(1,48)</sup>.

Hereditary non-polyposis colorectal cancer (HNPCC), previously called Lynch syndrome, is the most common form of HCRC. HNPCC has an incidence of 1:1,000 in the general population in the United States<sup>(43)</sup>. In Brazil, there are no data about its incidence or prevalence<sup>(38)</sup>. HNPCC is an autosomal dominant disease of high penetrance (about 80%-90%) characterized also by the occurrence of extracolonic tumors (endometrium, ovaries, stomach, small bowel and others) in affected families. Disease morbidity and mortality can be significantly reduced if the benign and malignant tumors are removed in time<sup>(6,48)</sup>.

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## EPIDEMIOLOGY

Typical HNPCC families show successive generations affected by CRC in early age (about 45 years), occurring predominantly near to hepatic flexure ( $\approx 70\%$ ). There is an excess of synchronous and metachronic CRC<sup>(5,6)</sup>. Although affected patients can show polyps, usually they do not exceed 50 in number. Histopathological characteristics of HNPCC tumors are frequently distinguishable, although they are not pathognomonic. These characteristics include mucinous carcinomas with poor differentiation, presence of signet ring cells, peritumoral lymphocyte infiltration, Crohn's-like reaction and lymphocyte infiltration in tumoral tissue<sup>(26,41)</sup>.

HNPCC patients have an increased risk to develop several extracolonic tumors: endometrium (the most common after CRC), ovaries, stomach, small bowel, pancreas, hepatobiliary tract, brain and superior uroepithelial tract<sup>(2)</sup>. Association of CRC and benign or malignant sebaceous tumors constitute the Muir-Torre syndrome, and association of non-polyposis colorectal cancer with glioblastoma multiforme constitute the Turcot syndrome.

In comparison with sporadic CRC, HNPCC tumors are more frequently located in proximal colon, are more undifferentiated with an excess of mucus and signet ring cells. HNPCC adenomas tend to be villous and are more dysplastic at diagnosis than that detected in general population, showing an accelerated carcinogenesis process ("aggressive adenoma theory"). Thus, a small colonic adenoma can change into a carcinoma in 2 or 3 years, while in the general population this process lasts 8 to 10 years in average. Prognosis is better in HNPCC than in sporadic CRC cases (increased survival) and probably derives from better therapeutic response to chemotherapy and minor metastasis potential<sup>(11,27,29,48)</sup>.

## CLINICAL DIAGNOSIS

Positive family history is the more common risk factor to HCRC. Several epidemiological studies showed that an individual with one or more first degree relatives affected by CRC have an empiric risk 2 to 3-fold higher to develop the disease. However, the family history interpretation is very limited in small families<sup>(25)</sup>.

Amsterdam criteria (1991) was the first guideline of HNPCC clinical diagnosis, developed in an international consensus meeting. At that moment, HNPCC-associated genes and their functions had not been identified and characterized yet. For this reason, the guidelines were based only in family history and in the age at diagnosis (Figure 1)<sup>(45)</sup>.

This guideline was not very useful, due to its very strict criteria, and many families with HNPCC associated mutations do not fulfill all criteria. Therefore, the Amsterdam criteria were reviewed in 1998, giving rise to the Amsterdam criteria II, that included the extracolonic tumors associated with HNPCC<sup>(46)</sup>. The Amsterdam criteria were also modified in order to allow small families evaluation<sup>(46)</sup>.

Discovery of genes involved in HNPCC development, and the possibility of genetic testing to confirm clinical doubts lead to necessity of new guidelines. In this way, the Bethesda Guideline

was developed, in 1997, with the aim to determine when an individual that does not fulfill Amsterdam criteria should be submitted to genetic testing<sup>(37)</sup>. In 2003, the Bethesda Guideline was also reviewed<sup>(42,43)</sup>.

## MOLECULAR BIOLOGY

Molecular genetics of colorectal carcinomas is the better understood among human neoplasias<sup>(18)</sup>. Current models of carcinogenesis are based on experimental evidences that the accumulation of mutations leads to alterations of specific genes (oncogenes, tumor suppressor genes and other genes involved in regulation of cellular growth and proliferation), resulting in neoplastic clonal expansion<sup>(14,17)</sup>.

Colorectal cancer develops through a process of sequential steps recognized at histopathological level by the progression of the normal mucosa to an invasive carcinoma (adenoma-carcinoma sequence) (Figure 2). In majority of colorectal carcinomas, the inactivation of the APC gene (adenomatous polyposis coli; located at long arm of chromosome 5, 5q) starts the process leading to a dysplasia, generally as an adenoma. From then on, additional mutations in oncogenes, including *ras* family genes, and tumor suppressor genes located at chromosomes 18q (*DCC*, *SMAD2*, *SMAD4*) and 17q (*TP53*) carry the progression from initial adenoma to intermediate adenoma and, finally, to carcinoma. These alterations are found in different combinations in colorectal tumors<sup>(18)</sup>.

However, the number of alterations in oncogenes and tumor suppressor genes is too high to be explained only by spontaneous mutation rate. Thus, probably an unstable genotype is required to increase the spontaneous mutation rate leading to the tumor development<sup>(14,24,28)</sup>.

Therefore, two apparently distinct pathways of genomic instability can be identified. The first and more common is characterized by sequential inactivation of tumor suppressor genes (*APC*, *p53*, *DCC*, *SMAD2* e *SMAD4*). Tumors that arise by this "suppressor pathway" show chromosomal instability (CIN), with frequent cytogenetic abnormality and allelic loss. The exact mechanism that drives the CIN process is not well understood<sup>(20)</sup>. While mutations in oncogenes are generally single dominant events, the inactivation of tumor suppressor genes depends on functional loss of both copies of relevant genes. While the former occurs more frequently by gene mutations, the latter is more frequently a chromosomal event, usually a deletion. Since deletion generally involves simultaneous loss of gene *loci* close to tumor suppressor genes – and occasionally the loss of whole chromosome or a chromosome arm – these events are strongly associated with loss of heterozygosity (LOH) in hypervariable polymorphisms (minisatellites and microsatellites) located in the deleted region<sup>(19)</sup>.

The second pathway is typical of HNPCC tumors. This alternative "mutator pathway" is characterized by the microsatellite instability (MSI) spread along the genome. Recent studies point out that *hMLH1* gene inactivation by promoter hypermethylation can also cause a high instability genotype in sporadic CRC, and it is responsible by the majority of cases of sporadic CRC with this genotype<sup>(20)</sup>.

MODEL	CRITERIA	NUMBER OF CRITERIA NECESSARY FOR INCLUSION*
Amsterdam (*)	<ul style="list-style-type: none"> <li>Families with at least three CRC cases in which two affected individuals must be first degree relatives of the third;</li> <li>Families with CRC in at least two generations;</li> <li>Families with at least one CRC case diagnosed before the age of 50.</li> </ul>	All of the criteria must be fulfilled for clinical diagnosis of HNPCC and inclusion in the study
Modified Amsterdam (**)	<ul style="list-style-type: none"> <li>(1) If the family is very small, the presence of two affected individuals which are first degree relatives; presence of affected persons in at least two generations and at least one case diagnosed before the age of 55 may be considered sufficient</li> <li>OR</li> <li>(2) In families with two first degree relatives affected with CRC the presence of a third family member with endometrial cancer or other cancer diagnosed at an early age is sufficient.</li> </ul>	All of the criteria of only one of the items (1 or 2) must be fulfilled for clinical diagnosis of HNPCC and inclusion in the study
Bethesda (**)	<ul style="list-style-type: none"> <li>(1) Individuals that fulfill the Amsterdam criteria;</li> <li>(2) Individuals with two tumors associated with HNPCC (colonic or extra-colonic tumors);</li> <li>(3) Individuals with CRC and a first degree relative with CRC or another extra-colonic tumor associated with HNPCC and/or a colorectal adenoma (tumor diagnosed before the age of 45 and adenoma diagnosed before the age of 40 years);</li> <li>(4) Individuals with one or more CRC endometrial cancer cases in the family diagnosed before the age of 45 years;</li> <li>(5) Individuals with proximal CRC and a poorly differentiated pathology (or "signet ring" type) before the age of 45 years;</li> <li>(6) Individuals with adenomas diagnosed before the age of 40 years.</li> </ul>	All of the criteria of only one of the items (1 to 6) is sufficient for clinical diagnosis of HNPCC and inclusion in the study
Bethesda (Modified) (**)	<ul style="list-style-type: none"> <li>CRC diagnosed before the age of 50;</li> <li>Synchronous or metachronous CRCs or another HNPCC-related tumor independent of age at diagnosis;</li> <li>CRC with MSI-H before the age of 60 years;</li> <li>Individuals with CRC and one or more first-degree relatives affected with CRC or another HNPCC-related tumor, one of which at least diagnosed before the age of 50 years;</li> <li>Individuals with CRC and two or more relatives diagnosed with CRC or another HNPCC-related tumor independent of age at diagnosis.</li> </ul>	All of the criteria of only one of the items is sufficient for clinical diagnosis of HNPCC and inclusion in the study

FIGURE 1 – Different models to HNPCC diagnosis according to clinical criteria<sup>(49)</sup>

CRC = colorectal cancer; MSI-H = microsatellite instability-high  
 (\*) All criteria must be fulfilled to attain inclusion  
 (\*\*) All aspects of only one of the items must be fulfilled to attain inclusion

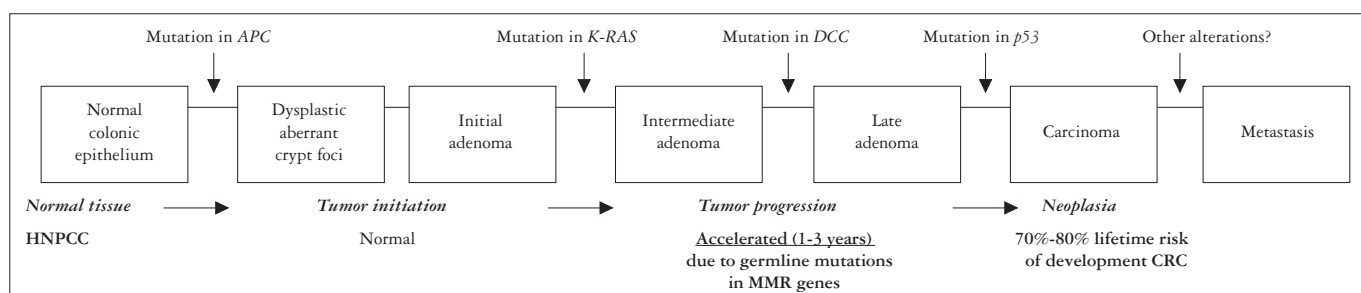


FIGURE 2 - The adenoma-carcinoma sequence in sporadic CRC and in HNPCC - adapted from FEARON and VOGELSTEIN<sup>(17)</sup>

Although these two mechanisms of genomic instability can be distinguished by their molecular features, there are several evidences suggesting that some degree of overlap among them can exist. LOH was described as an occasional mechanism of inactivation of wild allele of *hMLH1* in some MSI high tumors. It is also possible that CRCs initiates mechanisms that not involve persistent MSI or CIN. In this way, there are evidences indicating that an epigenetic alteration characterized by hypermethylation of promoter

regions of key tumor suppressor genes can play a crucial role in evolution and progression of many colorectal tumors. These findings also suggest that MSI and CIN can not represent completely distinct mechanisms and that multiple mechanisms can coexist in some tumors, since the arrangement give additional growth advantages<sup>(20)</sup>.

In normal states, the chromosomal mutation rate is higher than the genic one. Therefore, we expect to see frequent chromosomal deletions in tumors with an intact repair system undergoing

the classic pathway of tumorigenesis (suppressor pathway). In the mutator pathway, gene mutation rates are elevated by 100 or 1,000-fold and thus much more likely to occur than the chromosomal alterations that lead to LOH. However, there is no reason why LOH may not occur in tumors with MSI<sup>(19)</sup>.

MSI is caused by mutations in mismatch repair genes (MMR; *hMSH2* and *hMLH1*, specially) that result in failure of replication error repair (RER)<sup>(31)</sup>. In HNPCC, an allele of one of these repair genes shows a germline mutation and the other allele is inactivated or lost by somatic mutation, leading to accumulation of DNA replication errors, to the increase of mutations and, ultimately, to the acceleration of the carcinogenesis process<sup>(13, 32, 47, 48)</sup>.

About 70% of HNPCC families show germline mutation in one of the known MMR genes: *hMSH2*, *hMLH1*, *hMSH6*, *hPMS1* or *hPMS2*. Consequently, the majority of HNPCC tumors are MSI<sup>+</sup> (80%), while only 20% of sporadic tumors are MSI<sup>+</sup><sup>(13)</sup>. Among these MMR gene mutations, those occurring in *hMSH2* (2p) and *hMLH1* (3p) are more common and are found in 90% of HNPCC families with identified mutation<sup>(27, 47)</sup>.

In general, mutations described in these two genes are insertions, deletions, alterations in pre-mRNA splicing signals and no sense mutations. The majority of *hMSH2* mutations are frameshift mutations and no sense mutations. On the other hand, *hMLH1* mutations include frameshift mutations and missense mutations, a lot of them have not an established pathological role yet. Alterations of splicing site are common in *hMLH1* and less frequent in *hMSH2*. In addition, *hMSH2* mutations are randomly spread along of coding sequence while in *hMLH1* there is a cluster of mutations in exons 15 and 16<sup>(29)</sup>.

Mutations in other genes as *hGTBP* (2p16) and *hMLH3* (14q24.3) have also been associated with HNPCC<sup>(30)</sup>. In addition, mutations in specific regions of *RII* gene, that encodes the TGF II (transforming growth factor type II), are present in more than 90% of colon tumors with RER phenotype, sporadic or hereditary. Mutations in these genes are consistent with the tumoral suppressor model (Knudson theory)<sup>(12, 30)</sup>.

## MOLECULAR DIAGNOSIS

### Genetic Testing

The discovery of colorectal cancers MSI (+) and the hypothesis that these tumors can have a prognosis different from MSI (-) tumors lead to the study of several microsatellites through different protocols<sup>(16)</sup>. However, in 1998, an international workshop of the National Cancer Institute (USA) established a standard criteria to MSI detection in colorectal tumors<sup>(8)</sup>. Among these criteria, a minimum panel of 5 markers was suggested in order to diagnosis the MSI (+) phenotype: BAT25, BAT26 (mononucleotide repeats), D5S346, D2S123 and D17S250 (dinucleotide repeats). This reference panel lead to the classification of tumors as MSI high or MSI-H, if two or more markers show instability; MSI-L, if only one marker shows instability; and MSS, if there are no unstable markers. Standard protocols defined in the workshop were described by DIETMEIER et al.<sup>(15)</sup>. MSI analysis is performed through the

comparison between microsatellite sequences of normal and tumoral tissues of the same individual and MSI is characterized by the presence of different repeat sizes between them<sup>(48)</sup>.

When adequately detected, MSI shows a high sensibility to identify tumors with mutations in MMR genes. However, its specificity is lower, mainly because a great proportion of MSI (+) tumors is caused by *hMLH1* promotor hypermethylation (epigenetic silencing), that is a somatic event<sup>(28, 40)</sup>. A populational study in Finland reported that from 535 CRC individuals, not selected by family history, 66 (12%) showed MSI and 18 (3.4%) showed germline mutations in *hMLH1* or *hMSH2*<sup>(40)</sup>. Another study, also with CRC affected individuals not selected by family history, reported that 15% of analyzed patients showed MSI-H<sup>(17)</sup>.

Furthermore, the MSI test can give false-negative results due to technical limitations and to material scarcity of analyzed specimens. A possible reason for these false-negative results is that large *hMSH2* deletions, not associated with MSI, can contribute to more than 10% of all mutations<sup>(28, 40)</sup>.

All these data suggest that there are an underestimate of HNPCC incidence, since available methods still have low specificity, there are unknown mutations and some cases classified as sporadic, when adequately screened, show MSI and detectable germline mutation<sup>(40)</sup>. It is important to note that MSI analysis is a screening test and not a diagnostic one.

Another method of detection of altered expression of HNPCC-associated genes is immunohistochemistry (IHC), using monoclonal antibodies produced against the protein products of these genes. Preliminary studies reported a reduced expression of *hMSH2* and *hMLH1* encoded proteins in more than 90% of cases with germline mutations and RER phenotype, and also in the majority of cases RER+ without detectable mutations. There are no reports of reduced protein expression in cases without RER phenotype or mutation. The decrease in *hMLH1* encoded protein expression can also be explained by the hypermethylation of its promoter region, that can occur in sporadic CRC with RER phenotype but without mutations in coding regions<sup>(12, 30)</sup>. The majority of sporadic CRC that show MSI-H is explained by the hypermethylation of *hMLH1* promoter. Some works suggest a role of *hMLH6* in colorectal carcinogenesis and point out to the importance of including this gene in the molecular and immunohistochemistry analysis<sup>(34, 40)</sup>. IHC is a simple and cheap technique that can be used to orient the mutation screening when the gene product is not expressed in the tumor<sup>(34)</sup>. However, in cases where mutations lead to a truncated protein but not to its absence, this technique is not able to indicate the altered gene. Although MSI test is fundamental to evaluate tumor phenotype in relation to MMR genes inactivation, the IHC, at least for *hMLH1* and *hMSH2*, is also a sensitive, fast and cost-effective method<sup>(36)</sup>.

Furthermore, the presence of germline mutations in one of the MMR genes involved in colorectal carcinogenesis can be directly analyzed by dHPLC (denaturing high performance liquid chromatography) and sequencing. However, the previous screening through MSI analysis or IHC is very important to indicate the gene likely mutated, orienting the analysis to a specific gene and reducing the costs and time of analysis<sup>(48)</sup>. The promoter hypermethylation of MMR genes (specially *hMLH1*) can be investigated through a specific polymerase chain reaction (PCR).

The two main questions in relation to HNPCC diagnosis are the detection of large deletions and mRNA splicing errors, and the missense mutations interpretation, specially in *hMLH1* and *hMSH6* genes. These questions contribute more to the absence of diagnosis than to the lack of sensitivity of MSI and IHQ tests<sup>(43)</sup>.

### DIFFERENTIAL DIAGNOSIS

There are a lot of other hereditary syndromes that predispose to CRC. Familial adenomatous polyposis (FAP) is a rare autosomal dominant syndrome (incidence of 1:8000) characterized by the presence of hundreds to thousands colonic polyps. These polyps arise in childhood/adolescence or in early adult age and advance to colon cancer in virtually all cases. If prophylactic colectomy is not performed, virtually all FAP affected patients die at about 50 years of age, and 37% of affected patients die earlier, at about 37 years<sup>(3,5)</sup>. Other clinical signals in FAP affected patients include polyps in the gastrointestinal tract (small bowel and stomach), papillary thyroid cancer, periampullary carcinoma, sarcomas and brain tumors. The hypertrophy of retinal pigment epithelium is a characteristic finding that is congenitally present in 80% of the carriers. There are variants of this syndrome such as attenuated FAP, Gardner syndrome and Turcot syndrome, representing distinct clinical syndromes caused by specific mutations in *APC* gene (5q21)<sup>(5)</sup>.

Since FAP shows well defined histopathological and clinical features, its diagnosis usually can be done based only in clinical findings and it is easily distinguishable from HNPCC.

However, there is a FAP variant with a mild phenotype, the attenuated familial adenomatous polyposis (AFAP). AFAP is characterized by few colonic adenomas (50-100), most of them located in proximal colon. Upper gastrointestinal tract lesions are common, especially duodenal adenomas and fundic glands polyps. The majority of AFAP patients develop CRC in later age ( $\approx$  55 anos) than patients with FAP ( $\approx$  39 anos). Mutations in *APC* gene associated with this syndrome are located in both 5' and 3' ends of the gene<sup>(24)</sup>. Clinically, diferential diagnosis between HNPCC and AFAP can be not so simple. Thus, molecular diagnosis becomes very important for distinction between HNPCC and this syndrome, as well as other CRC hereditary predisposition syndromes like the following:

- a) **Peutz-Jeghers syndrome (PJS)** is an autosomal dominant syndrome of cancer hereditary predisposition. PJS is characterized by muco-cutaneous melanic pigmentation and intestinal polyposis (specially in small bowell, but can also occur in stomach and large intestine). The polyps are harmartomas, with conjunctive, muscular and epithelial tissue components. Rarely extra-intestinal tumors can occur in ovaries, uterine cervix, testicles, pancreas, and breast. The responsible gene, *LKB1*, is located at the short arm of chromosome 19 (19p13.3). There is a defined tendency to malignization of the "harmartomatous" epithelial component, but the carcinogenetic steps are not well defined yet.
- b) **Cowden syndrome** is an autosomal dominant syndrome characterized by the presence of multiple harmartomas, especially muco-cutaneous and gastrointestinal. Breast and thyroid carcinomas are the two malignant neoplasias more commonly described in this syndrome. About 80% of affected families have germline mutations in *PTEN/MMAC1* gene.
- c) **Juvenil polyposis syndrome** is an autosomal dominant syndrome characterized by multiple (about 200 to 500) juvenil polyps predominantly located in the large intestine. In some cases, the *SMAD4*, located at the long arm of chromosome 18 (18q21.1), is the responsible gene. Its histopathology shows a polilobulated bizarre structure. The occurrence of CRC is described in 30%-40% of cases and gastric cancer in 10%-15% of cases of gastric location. A more severe and fatal form, with diarrhea, anemia and hypoalbuminemia can occur in childhood.

### SCREENING GUIDELINES

Screening and prevention guidelines for HNPCC patients are detailed in Figure 3.

According to the International Collaborative Group in HNPCC, patients that fulfill Amsterdam criteria or that are germline mutation carriers should initiate colonoscopy at 21 years or 5 years earlier than the earlier case in the family, and have this procedure at every 1-2 years<sup>(25,47)</sup>. It is necessary a previous very good preparation for colonoscopy, to insure a minucious exam of all colorectal mucosa. Patients should be advised that the colonoscopy is not

Cancer type	Recommendation	Interval	Evidence Level (†)	Consensus
Colon	Total colonoscopy (until cecum)	Annual or bi-annual since 20-25 years	2-3	Yes
	Prophylactic colostomy	Discuss as prophylaxis and/or polyps diagnosis	3	No
	Post-colostomy screening by rectal endoscopy (*)	Annual post-colostomy if rectum was preserved	3	Yes
Endometrium	Pelvic exam	Annual since 25-35 years	5	Yes
	Aspirate of endometrium	Annual since 25-35 years	5	Yes
	Transvaginal ultra-sound	Annual since 25-35 years	5	Yes
	Histerectomy (panhisterectomy)	Discuss as an option post-family constitution	5	No
Ovaries (**)	Transvaginal ultra-sound + CA 125 seric	Annual since 25-35 years	5	No
Urinary tract (**)	EQU + urinary citology	Annual since 25 years	5	No
Stomach (**)	High digestive tract endoscopy	Annual or bi-annual since 35 years	5	No

FIGURE 3 – Recommendations of cancer screening and prevention in HNPCC-associated gene mutation carriers<sup>(4)</sup>

(\*) Rectal endoscopic screening is mandatory in patients submitted to prophylactic colostomy with ileum-rectal anastomosis due to the high incidence of rectal metachronous tumors post-colostomy (25%-40%)  
 (\*\*\*) Only in members of HNPCC affected families and diagnosis of these tumors in at least one relative (and that can be associated to the syndrome)  
 (†) According to Physician Data Query (PDQ) Screening and Prevention Statement Levels of Evidence

a perfect screening procedure and should be informed about the possibility of prophylactic colectomy as an option. The mortality risk in prophylactic colectomy is low, but there are not enough evidences that the prophylactic surgery is more efficient than the periodic screening through colonoscopy in increasing survival. Patients with colectomy should continuously have endoscopy of the remainder rectal mucosa, because the risk of cancer development in this region is of about 1% for year<sup>(25)</sup>.

Women at-risk should do screening for endometrium and ovarian cancer, that are the more common extra-colonic neoplasias. Suggested procedures include aspiration curettage of endometrial cells for histopathologic study, pelvic transvaginal ultrasound, cervico-vaginal cytopathologic exam and pelvic examination every 1-2 years starting at 30 years of age<sup>(7, 34, 35)</sup>. In addition, some investigators suggest that all at-risk individuals, independently of sex, should have screening for other extra-colonic tumors if there is a positive family history for this type of tumors. For gastric and biliary tract tumors, procedures such as esophagogastroduodenoscopy, gastric biopsy, hepatobiliary transabdominal ultrasound, and hepatic function tests are indicated at 1-2 years intervals starting at 30 years of age. For kidneys, urethra and bladder tumors the recommended procedures include ultrasound, cystoscopy, cytology of urine also at intervals of 1-2 years<sup>(40, 47)</sup>. It is important to note that there is not a consensus about such prevention guidelines for extra-colonic tumors other than endometrium and ovarian.

In addition to clinical screening, patients can be screened for the presence of germline mutations in MMR genes, and CRC affected individuals can be tested for MSI (Figure 4).

### PREVENTION PROCEDURES

Total abdominal colectomy with ileo-rectal anastomosis should be considered as an option for HNPCC patients, which should have annual screening for rectal cancer. This procedure is justified by the high incidence of metachronic tumors (25% to 40%) in patients submitted to partial colectomy. The proctocolectomy should also be considered for cancer affected patients, depending on disease's stage at diagnosis. Patients with multiple polyps or that are certainly mutation carriers can be directed to prophylactic colectomy, although its benefits are not well defined yet<sup>(20)</sup>. For CRC affected women the prophylactic hysterectomy and the bilateral salpingo-oophorectomy should be considered at the moment of colectomy, especially if the woman has already constituted her family and if she has a positive family history for at least one of these tumors.

There are evidences that, in vitro, CRC cells that are deficient in one of the MMR genes (*hMLH1*, *hMSH2* e *hMSH6*) have reduced MSI after exposition to aspirin or sunlidac<sup>(22, 25)</sup>. Since these drugs intervene with molecular events, the chemoprevention can inhibit or revert the development of adenomas or the progression from adenoma to carcinoma<sup>(38)</sup>. Aspirin and other nonsteroidal antiinflammatory drugs (NSAID) are the more attractive and the more widely studied substances to CRC chemoprevention<sup>(44)</sup>. They inhibit the ciclo-oxygenase 1 (COX-1) and the ciclo-oxygenase 2 (COX-2), that are catalytic enzymes. COX-2 expression is increased in sporadic and hereditary colonic carcinomas and adenomas, if compared with normal tissue. However the use of COX-2 inhibitors is at moment not an ideal strategy due to the reported cardiovascular incidents.

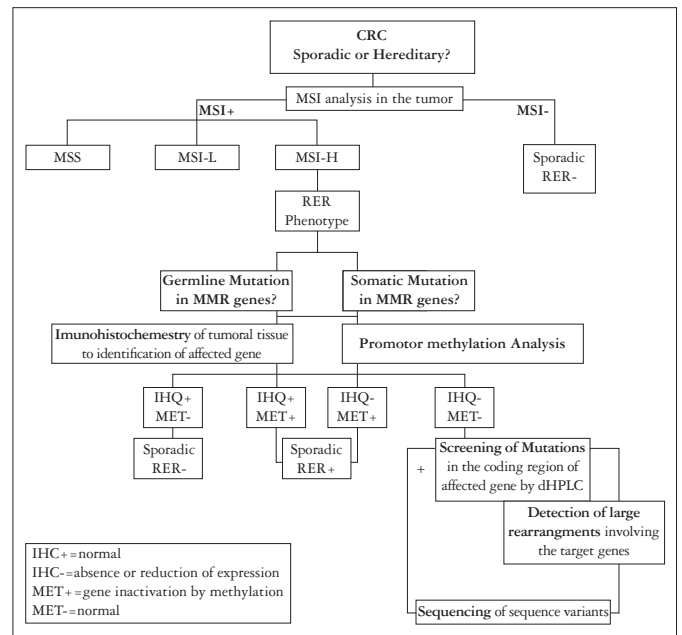


FIGURE 4 - Laboratory diagnosis flow chart

Rodents treated with sulindac, a NSAID that inhibit COX-1 and COX-2, show a reduction of more than 90% in the number of intestinal polyps and of more than 52% in the total volume of colonic tumors. However, aspirin and other NSAID can act through COX-independent pathways as through the inhibition of activation of  $\kappa$ B nuclear factor (NF- $\kappa$ B) or through the interference in the ligation of the activated receptor of peroxisome proliferator  $\delta$  to DNA<sup>(21, 37)</sup>. These interventions have been evidenced a protective effect in FAP affected patients (evidence level V), but the impact of the use of NSAID in other CRC hereditary predisposition syndromes (like HNPCC) still needs to be elucidated.

In addition to chemoprevention, a nutritional approach have also been considered in terms of sporadic CRC prevention. There are indications that some substances as folate, calcium, estrogens and antioxidants can have a protective effect against the carcinogenesis process. In the other hand, vitamins (excet folic acid) and fibers seem not to have any effect in the development of adenomas or colorectal carcinomas<sup>(20)</sup>. Additional studies should be done to evaluate the impact of these prevention strategies on the risk of cancer in individuals with hereditary predisposition.

### IMPORTANCE OF CLINICAL AND MOLECULAR DIAGNOSIS OF INDIVIDUALS AND FAMILIES WITH HNPCC

The identification of individuals at-risk for hereditary CRC is important for several reasons. First, because affected individuals show cumulative life-risk of several types of cancer, much higher than that of the general population. Second, because other relatives of an affected individual can be at increased risk of cancer (since this genetic disease have an autosomal dominant inheritance, 50% of sibilings and 50% of descendents of an affected patient can be carriers of the same mutation). Third, because intensive

screening procedures and preventive interventions are efficient to significantly reduce the risk of cancer in mutation carriers. The CRC screening effectiveness in HNPCC affected families was evaluated by JARVINEN et al.<sup>(23)</sup> in a controlled clinical trial of 15 years. This and other studies showed that screening reduces the risk of cancer to more than a half and diminishes the overall mortality in about 65%, through an early identification and remotion of hyperplasic and atypical polyps<sup>(33)</sup>.

Current technology allows to detect a genetic mutation before the appearance of the symptoms. In the case of hereditary predisposition to colon cancer, an adult disease, the pre-symptomatic and predictive diagnosis of an affected individual has an enormous potential for the reduction of cancer risk. On the other hand, the accurate identification of unaffected individuals in an at-risk family tranquilizes them, and eliminates the costs and complications of unnecessary screening and preventive interventions. Moreover, the genetic tests make possible the identification of several non-symptomatic individuals at-risk in the family. Thus, the use of

genetic tests can in such a way contribute for the reduction of the mortality as well as of the incidence of CRC and extracolonic tumors in families with HNPCC.

In this context, the referral of families and/or individuals with the diagnostic suspicion of HNPCC for genetic counseling is fundamental. During genetic counseling, the diagnostic hypothesis will be made through the discerning analysis of the family history (pedigree) and the options to confirm this clinical hypothesis by genetic test will be discussed. It is important to anticipate the meaning of the possible genetic test results and the therapeutic options, as well as to discuss its implications for the other at-risk relatives (children, siblings, etc)<sup>(47)</sup>. A discerning analysis of the of HNPCC suspicious cases and the correct pursuing of the diagnostic guidelines and of preventive handling has immense potential of reduction of the risk of cancer for these patients, resulting in better survival and quality of life very close to the normal one.

Coura RS, Ashton-Prolla P, Prolla JC. Câncer colorretal hereditário não-polipomatoso: predisposição hereditária, diagnóstico e prevenção. *Arq Gastroenterol* 2004;42(2):99-105.

**RESUMO - Racional** – O câncer colorretal é o terceiro tumor em frequência e o segundo em mortalidade nos países desenvolvidos. No Brasil, está entre as seis neoplasias malignas mais encontradas e é a terceira em mortalidade. Cerca de 20% dos tumores colorretais têm etiologia hereditária. **Objetivo** - Revisão sobre aspectos genéticos e clínicos, bem como diagnóstico, tratamento e prevenção na síndrome do câncer colorretal hereditário não-polipomatoso, que apresenta a forma mais frequente de câncer colorretal hereditário. A importância dessas abordagens se deve, principalmente, à possibilidade de manejo, prevenção e rastreamento específico para indivíduos em risco para câncer colorretal hereditário não-polipomatoso que conferem um aumento considerável na sobrevida desses pacientes e seus familiares em risco.

**DESCRITORES** – Neoplasias do cólon, genética. Neoplasias colorretais hereditárias sem polipose. Hereditariedade.

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