

Phenotypic Heterogeneity by Germline Mismatch Repair Gene Defect in Lynch Syndrome Patients

Heterogeneidade Fenotípica por Mutação Germinativa nos Genes Mismatch Repair em Doentes com Síndrome de Lynch



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ABSTRACT

Introduction: Lynch syndrome is the most common form of hereditary colorectal cancer, being also responsible for endometrial and other types of cancers. It is associated with germline mutations in DNA mismatch repair genes and microsatellite instability. *MLH1* and *MSH2* mutations have a “classical” Lynch syndrome phenotype, with *MSH2* having a higher association with extracolonic cancer. *MSH6* and *PMS2* mutations have an atypical phenotype. Clinical expression is heterogeneous, with correlation between mismatch repair mutated gene and phenotypic patterns.

Material and Methods: We retrospectively analyzed data from patients fulfilling Amsterdam criteria or having mismatch repair gene mutations, between September 2012 and October 2015.

Results: We identified 28 patients. Seventeen had colorectal cancer with right colon predominance. Five developed endometrial cancer (median age of diagnosis – 53), with no *MSH6* mutations. Five developed other cancers. All mutated mismatch repair cases studied had microsatellite instability.

Discussion: Most cases had *MSH2* mutations despite *MLH1* being described in the literature as the most frequently mutated. Interestingly, colorectal cancer patients showed no tendency for high inflammatory infiltrate. Despite the high incidence of synchronous and metachronous tumours, most patients underwent a partial colectomy. Prophylactic hysterectomy and adnexectomy was performed in menopausal/perimenopausal patients.

Conclusion: A standardized registration of patient’s data may lead to better management and knowledge about Lynch syndrome. Use of Bethesda Guidelines might identify new cases non-identified by Amsterdam criteria. Microsatellite instability analysis must be performed in a much larger scale. The genotypic/phenotypic correlation described in the literature was not verified in our study with statistical significance, perhaps due to small data sample and insufficient clinical registration.

Keywords: Colorectal Neoplasms, Hereditary Nonpolyposis; DNA Mismatch Repair; DNA Repair-Deficiency Disorders; Endometrial Neoplasms; Neoplastic Syndromes, Hereditary.

RESUMO

Introdução: A síndrome de Lynch é a forma hereditária mais comum de cancro colo-rectal, sendo também responsável por cancro do endométrio e de outros tipos. Associa-se a mutações germinativas nos genes de mismatch repair do ADN e a instabilidade de microssatélites. As mutações *MLH1* e *MSH2* têm um fenótipo de síndrome de Lynch ‘clássico’, sendo o *MSH2* mais associado a cancro extra-cólico. Mutações do *MSH6* e *PMS2* têm um fenótipo atípico. A expressão clínica é heterogénea, existindo uma correlação entre o gene *mismatch repair* mutado e o padrão fenotípico.

Material e Métodos: Análise retrospectiva dos dados clínicos de doentes que cumpriam os critérios de Amsterdão ou que tinha mutações nos genes *mismatch repair*, entre setembro de 2012 e outubro de 2015.

Resultados: Identificámos 28 doentes. Dezassete tinham cancro colo-rectal sendo a localização no cólon direito predominante. Cinco tiveram cancro do endométrio (mediana da idade de diagnóstico – 53), sem qualquer mutação no *MSH6*. Cinco desenvolveram outros cancros. Todos os casos com mutações *mismatch repair* estudados tinham instabilidade de microssatélites.

Discussão: Na maioria dos casos foi encontrada mutação no *MSH2* apesar de o *MLH1* ser descrito na literatura como o gene mais frequentemente mutado. Interessa dizer que os doentes com cancro colo-rectal não evidenciam uma tendência para ter muito infiltrado inflamatório. Na maioria dos casos foi realizada colectomia parcial apesar da incidência elevada de lesões síncronas e metácrónas associadas. Histerectomia e anexectomia profilática foi realizada em doentes em menopausa/perimenopausa.

Conclusão: O registo standardizado dos dados dos doentes poderá levar a um melhor acompanhamento e conhecimento desta síndrome. O uso das Guidelines de Bethesda poderá identificar novos casos que escapam aos critérios de Amsterdão. A pesquisa de instabilidade de microssatélites deve ser feita em muito maior número. Embora seja descrita na literatura uma correlação genótipo/fenótipo, o nosso estudo não verificou esta correlação de forma estatisticamente significativa, talvez por a amostra ser pequena e os registos clínicos insuficientes.

Palavras-chave: Neoplasias Colorretais Hereditárias sem Polipose; Neoplasias do Endométrio; Reparação de Incompatibilidade de ADN; Síndromes Neoplásicas Hereditárias; Transtornos por Deficiências na Reparação de ADN.

INTRODUCTION

Colorectal cancer (CRC) is a very common type of cancer. It has an incidence of 1,200,000 cases annually on a global scale. The mortality rate is of approximately 50%.¹

Among these, approximately 20% account for familial forms of CRC.²

Lynch syndrome (LS) - also known as hereditary non-

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polyposis colorectal cancer - is the most commonly seen hereditary form of CRC. It is responsible for 3% - 5% of CRCs³ and approximately 3% of endometrial cancers (EC).⁴ This incidence refers to the general population worldwide.⁵ A linkage is also shown between LS and other types of cancers.⁶⁻⁸

Therefore, 36,000 – 60,000 of CRCs worldwide are associated with LS,⁹ with an average age of onset of 45 years.^{10,11}

This is a dominantly inherited disorder with 85% of penetrance,¹² featuring germline mutations in at least one of the DNA mismatch repair (MMR) genes.^{1,2} Those mutations are found in more than 80% of patients with LS.¹³

DNA MMR 'complex' is responsible for proofreading and edition posteriorly to the DNA replication phase, ensuring the integrity of the genome.¹⁴

Manifesting the mutations in the MMR genes, these patients tend to have a fast process of carcinogenesis¹⁵ with cancers showing microsatellite instability (MSI).¹⁶

The knowledge of the impaired activity of the MMR genes in cancers associated with LS led to a common determination of the MMR genes activity by immunohistochemistry (IHC),¹⁷ not only as a marker of a possible cancer correlated to LS, but also to discover the gene (or genes) that probably underlies the germline mutation.¹⁴

Therefore, when we identify a germline mutation in the MMR genes, we have the *sine qua non* factor that provides the definitive diagnosis of LS.¹⁴

Despite that, we should not test every CRC case for these mutations,¹⁸ having family history a crucial importance in that decision¹⁹ and in the diagnostic evaluation.¹ Amsterdam I²⁰ and II²¹ criteria have been useful for that purpose. Bethesda Guidelines for testing CRC for MSI may also be of utility.^{22,23} Some patients fulfill Amsterdam criteria but have no MMR mutation, which may hamper the diagnostic process and the correct management of those patients at high-risk of developing LS-related tumours.¹¹

In general, it was shown that the lifetime risk of having a cancer in families that are identified with a MMR mutation are the following: 28% - 75% in men and 24% - 52% women for CRC; 27% - 71% for EC; 3% - 13% for ovarian cancer; 2% - 13% for gastric cancer; 1% - 12% for urothelial cancer; 4% - 7% for small-bowel cancer; 1% - 4% for CNS cancer and 2% for bile duct or gallbladder cancer.^{18,24,25} The wide range of the intervals (specifically for colorectal and endometrial cancers) may be due to the documented phenotypic variation related to germline mutations in different MMR genes.^{11,12}

Those are the *MLH1* (mutL homologue 1, located in chromosome 3p21), *MSH2* (mutS homologue 2, located in chromosome 2p16), *MSH6* (mutS homologue 6, located in chromosome 2p15) and *PMS2* (postmeiotic segregation increased 2, located in chromosome 7p22).^{12,14}

Data from the International Society for Gastrointestinal and Hereditary Tumours (InSiGHT) relative to the year of 2012 registered a frequency of LS-associated mutations of 42% for *MLH1*, 33% for *MSH2*, 18% for *MSH6* and 8%

for *PMS2*.²⁶ Previous data suggested a frequency of 32%, 38%, 14% and 15%, respectively.²⁷

Despite the typical course of LS (CRC of early onset),¹⁴ it is well noticed that there can be several cases of LS with different phenotypic characteristics (like later onset of cancer, 'rare' kinds of tumours).^{6-8,14}

With the large-scale implementation of MSI and genetic testing, the specific clinical phenotype of each LS patient is now better understood, according to the MMR mutation.²⁸ Some recent studies give particular emphasis on the possible relevance of epimutations.²⁹

Starting with *MLH1* and *MSH2* mutations, those tend to have LS of a 'classical' phenotype (fulfilling the Amsterdam I criteria)^{24,30} and cancer displaying high MSI.³¹

Male patients with *MLH1* and/or *MSH2* have 27% - 74% of risk for developing CRC; as for female patients that risk is 22% - 61%.³² The mean age for the onset is of 43 - 46 years.^{24,30}

MLH1 patients seem to have high predominance of CRC.¹¹ *MSH2* patients have higher probability of developing extracolonic cancer when compared to *MLH1* and are thought to have a higher risk of developing any cancer throughout life.³³

Some studies also reported that mutated *MSH2* patients are shown to present greater risk of urothelial,^{34,35} gastric^{6,36} and ovarian cancer³⁷ when compared to patients with *MLH1* mutations.

Also, *MSH2* patients have been linked to augmented risk of extracolonic cancers specific of the Muir-Torre syndrome tumours-spectrum.^{38,39}

On the other hand, germline mutations in *MSH6* and *PMS2* lead to an atypical phenotype.^{40,41} Those are patients expected to have less risk of developing CRC and older age of onset.^{40,42}

Considering particularly *MSH6* patients, the penetrance of their mutation is thought to be lower¹² and also they do not show an ubiquitous MSI profile.⁴³

For these patients, the incidence of CRC is lower⁴² (particularly in women: 10% - 30% risk of CRC; in men it is higher: 22% - 69% risk of CRC) and the age of onset for CRC is higher (most frequently between 50 - 63³² and with a mean age of diagnosis of 54 years old)¹² than that of patients with the previous enounced mutations.

MSH6-mutated phenotypes are also considered to cause an atypical display of tumours.¹⁶

These are the patients with highest risk of developing EC.^{41,44} That is considered to be the principal clinical manifestation for female patients with this mutation.^{42,44} They are usually diagnosed in patients over 50 years of age^{41,44} and it was observed that at the age of 70, 71% of female patients with this mutation will have EC.⁴⁴

Along with *MSH2*, *MSH6* mutation is more related to EC than mutations in *MLH1* or *PMS2*.⁵

Finally, *PMS2* mutations are associated with a special phenotype of LS with large number of colonic polyps and small incidence of CRC. When associated with CRC, this cancer will present MSI. However, it usually develops at an

older age and has an intriguing lack of family history.⁴⁰

These patients show a risk for developing CRC of 20% for male patients and 15% for female patients, being the diagnosis usually held at the age of 47 - 66 years.³²

As accurate as this theoretical LS phenotype according to the MMR gene defect may be, variations can be found even in patients carrying the same germline mutation, giving us a clue that maybe there are other factors correlated to clinical manifestations of LS.^{30,45,46}

According to all of this, we can realize that the clinical expression and manifestations of LS is widely heterogeneous, with particular phenotypic patterns being highly correlated with the MMR gene mutated.

Therefore, we hold as main objective of this study the correlation between different MMR genes affected with mutation and the postulated phenotypic characteristics among them.

With that purpose in mind, we conducted a retrospective study at the Department of General Surgery – Centro Hospitalar de São João (CHSJ), reporting a three year period of time, between September 2012 and October 2015.

MATERIAL AND METHODS

This retrospective study was approved by the Ethics Committee for Health of CHSJ/Faculdade de Medicina da Universidade do Porto (FMUP). There was no conflict of interest reported in the making of this study.

We considered a three year period, between September 2012 and October 2015. According to this period of time, we retrospectively identified 36 patients that were suspected of having LS, as a result of clinical evaluation by a group of physicians from CHSJ and that underwent MMR gene mutation studies. The data was obtained from the Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP) database.

Inclusion criteria were patients with mutations in one or more MMR genes according to the database of IPATIMUP or patients that fulfill the Amsterdam I or II criteria according to a clinical and family history analysis.

Exclusion criteria consisted of incomplete clinical records and patients that have no mutation in MMR genes neither fulfill Amsterdam I or II criteria.

The existing data from every patient were reviewed and additional data was collected from their clinical files.

We collected data on the age and gender of the patients as well as MMR genes tested and those that were mutated. Presence of other mutations was assessed. Fulfilling of Amsterdam I and II criteria was also analyzed. In addition, age at CRC diagnosis – if existent – was collected.

As for those with CRC, location of tumor, degree of differentiation, macroscopic characteristics, inflammatory infiltrate, mucinous characteristics, lymph nodes involvement, vascular or perineural invasion, as well as the TNM staging were assessed. MSI status data was also collected.

The surgical treatment and decision following consultation in the Colorectal Oncology Group of CHSJ

were also analyzed.

Further collection of data was performed regarding the existence of EC among women, as well as the age of diagnosis and treatment performed.

We also collected data of other extra-colic cancers, the age of diagnosis and treatment.

Finally, we assessed data regarding the global patient's follow-up for all included cases.

We mainly sorted the patients by presence of MMR gene mutation (and of these, by different mutated gene) and by fulfillment of Amsterdam I or II criteria.

Then we performed statistical analysis using IBM SPSS® (Statistical Package for Social Sciences) version 23 and conducted a descriptive analysis of all variables. The distribution of continuous variable was performed using the Kolmogorov-Smirnov test that showed that age of patients was normally distributed.

Inferential analysis was performed to evaluate possible associations between categorical variables, using the Chi-square and Fisher's exact tests.

The evaluation of categorical and continuous variables association was performed by the Independent-samples *t*-test.

For evaluating if a relationship existed between variables, the statistical significance was investigated using Chi-square and Fisher's exact test and we used $p = 0.05$ as reference.

RESULTS

After a first analysis of the 36 identified patients, eight patients were eliminated for not having inclusion criteria. The subject of our analysis was the remaining 28 patients.

We started with the analysis of the demographic data of our patients regarding to gender, the fulfilling of Amsterdam I or II criteria, diagnosis of CRC, EC or other cancers of LS spectrum and presence of MSI (Table 1).

We noticed that from the 22 (78.6%) patients fulfilling Amsterdam I or II criteria, only seven (31.8%) had MMR gene mutations. On the other hand, all the patients that did not fulfill Amsterdam I or II criteria – six (21.4%) – had at least one MMR gene mutated.

We also described the CRC, endometrial (Table 2) and other cancers of LS spectrum cases.

We then sorted the CRC cases by their location (right colon, left colon and rectum).

Median age of diagnosis was of 46 years (min = 36; max = 68) for right colon patients and of 34 years (min = 23; max = 68) for left colon cancers. The only case of cancer of rectum was diagnosed at age of 43 years.

Regarding the CRC tumor characteristics, histologic grading was low in most cases. Also, all cases of CRC in the left colon had mucinous characteristics and so did most of the studied cases in the right colon.

Regarding CRC staging according to AJCC,⁴⁷ most patients were stage II or III and only one case (in the right colon) was stage IV.

Considering the Colorectal Oncology Group decision,

all patients presented at the group consultation with AJCC Stage III or IV were proposed for adjuvant chemotherapy. The rectum case did chemotherapy + radiotherapy. The chosen chemotherapy scheme was FOLFOX (folinic acid, 5-FU and oxaliplatin).⁴⁸

Our data showed that the median age of EC diagnosis was 53 years (min = 31; max = 65). None of these cases had CRC. It is important to note that four female patients with CRC performed prophylactic hysterectomy and adnexectomy.

We obtained follow-up data (Table 3) of 24 patients. From these, 19 (79.2%) are free of disease. At the time of last follow-up, one (4.2%) had a metachronous rectal cancer and four (16.7%) patients died. Of those, only one case died of CRC, the other three from endometrial, gastric and CNS cancers.

Concerning MMR genes, 22 patients (78.6%) were tested for *MLH1* mutations, 27 (96.4%) for *MSH2*, 14 (50.0%) for *MSH6* and 14 (50.0%) for *PMS2*.

Finally, we sorted patients by mutated MMR gene (Table 4). We accounted for 13 total cases with mutations and 17 individual mutations among them (with four patients having two concomitant mutations), accounting *MLH1* mutations for 17.7% of these individual mutations, *MSH2* for 52.9%, *MSH6* for 23.5% and *PMS2* for 5.9.

The median age of CRC diagnosis was 49 years (min = 37; max = 42) for *MLH1*, 43 years (min = 30; max = 57) for *MSH2* and 36 years (min = 30; max = 47) for *MSH6*. No case of CRC was reported as having *PMS2* mutation.

For EC diagnosis, only one case was described for *MLH1*, *MSH2* and *PMS2* and age of diagnosis was, respectively, of 65, 39 and 65 years. No case was reported for *MSH6*.

Only few patients presented other cancers of the LS spectrum and MMR mutations, being those of *MSH2* and *MSH6* genes.

When regarding the mutated germline MMR gene, at last follow-up, all patients with *MSH2* and *MSH6* mutations were free of disease. From the three cases of *MLH1* mutations, two (66.7%) resulted in death and one (33.3%) had a metachronous CRC. The *PMS2* mutation case also resulted in death. It is of notice that this last patient also had a mutation in *MLH1*.

As for mutations other than those of the MMR genes, 25 (89.3%) patients did not show any mutation. However, one (3.6%) patient had a mutation in p53 gene and two (7.1%) patients had a mutation in *KRAS*.

Inferential statistics were performed comparing several variables. However, none of the attempted correlations presented statistical significance.

DISCUSSION

The main objective of this study was to establish a correlation between MMR gene mutation and phenotypic characteristics.

Only seven patients out of 22 that fulfilled the Amsterdam I or II criteria had at least one MMR gene mutation, which could mean that some of these patients may have a MMR gene mutation yet they might not be spotted as it is known that there are mutations still to be discovered in all four of the MMR germline genes.⁴⁹ We also verified that all patients not fulfilling Amsterdam I or II criteria had at least one mutated MMR gene, meaning that they could be a first familiar case. All these patients (both having mutation or fulfilling the Amsterdam I or II criteria) were managed as LS patients.

Table 1 - Descriptive demographic table

			Total*
Gender	Male [n (%)]	12 (42.9)	28
	Female [n (%)]	16 (57.1)	
Amsterdam criteria	Yes [n (%)]	22 (78.6)	28
	No [n (%)]	6 (21.4)	
MMR gene mutation	Yes [n (%)]	13 (46.4)	28
	No [n (%)]	15 (53.6)	
CRC diagnosis	Yes [n (%)]	17 (60.7)	28
	No [n (%)]	9 (32.1)	
Endometrial cancer diagnosis	Yes [n (%)]	5 (31.3)	16
	No [n (%)]	11 (68.7)	
Other cancers diagnosis	Yes [n (%)]	5 (17.9)	28
	No [n (%)]	23 (82.1)	
MSI	Yes [n (%)]	9 (81.8)	11
	No [n (%)]	2 (18.2)	

* Total number of available data per variable

We realized that CRC is in fact the most frequent type of cancer in our patients, followed by EC and other cancers (two central nervous system – CNS - cancer cases, one case with both gastric and urothelial cancer, one case of gastric cancer and 1 case of lymphoma and another case of breast cancer). It is of capital importance to realize that of the five cases with EC, only one patient had CRC, being of extreme relevance to manage carefully those patients as we know that LS patients may present EC as a first manifestation. Also, the only case with breast cancer was diagnosed nine years before CRC. The remaining four cases of other

cancers should be carefully assessed because they may be also preceding other cancers.

As for MSI, despite being present in most LS patients and being one of the first auxiliary test in LS suspected cases under 50 years (even if they do not fulfill Amsterdam criteria),¹⁸ only 11 of our patients underwent this test. This information may also have repercussions in terms of therapy therefore large scale implementation should be achieved. It is also of notice that Bethesda Guidelines for testing CRC for MSI were only assessed and registered in the history of 1 patient, since they might be of utility as being described

Table 2 - Descriptive CRC and EC Table

	Colorectal cancer							Endometrial cancer	
	Right colon		Left colon		Rectum		Total *	Cases	Total *
	Cases	Sub-total	Cases	Sub-total	Cases	Sub-total			
Number of cases [n (%)]	10 (58.8)		6 (35.3)		1 (5.9)		17	5 (31.3)	16
Age of diagnosis (years) [Mean ± SD]†	48.10 (10.75)	10	38.33 (16.28)	6	43 (0)	1	17	48.80 (13.64)	5
Amsterdam criteria [n (%)]	6 (60.0)	10	6 (100)	6	0 (0)	1	17	5 (100)	5
MMR gene mutated									
<i>MLH1</i> [n (%)]	2 (28.6)	7	0 (0)	6	0 (0)	1	14	1 (25.0)	4
<i>MSH2</i> [n (%)]	5 (50.0)	10	1 (16.7)	6	1 (100)	1	17	1 (25.0)	4
<i>MSH6</i> [n (%)]	2 (33.3)	6	1 (25.0)	4	0 (0)	1	11	0 (0)	2
<i>PMS2</i> [n (%)]	0 (0)	6	0 (0)	3	0 (0)	1	10	1 (33.3)	3
MSI [n (%)]	4 (100)	4	1 (50.0)	2	1 (100)	1	7	1 (100)	1
Surgical treatment									
Right hemicolectomy [n (%)]	7 (77.8)		0 (0)		0 (0)			NA	NA
Total colectomy [n (%)]	1 (11.1)		2 (40.0)		0 (0)			NA	NA
Anterior rectal resection [n (%)]	0 (0)	9	0 (0)	5	1 (100)	1	15	NA	NA
Sigmoidectomy [n (%)]	0 (0)		1 (20.0)		0 (0)			NA	NA
Endoscopic treatment [n (%)]	0 (0)		2 (40.0)		0 (0)			NA	NA
Others [n (%)]	1 (11.1)		0 (0)		0 (0)			NA	NA
Histologic grade									
G0 [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
G1 [n (%)]	0 (0)	7	0 (0)	3	0 (0)	0	10	NA	NA
G2 [n (%)]	6 (85.7)		3 (100)		0 (0)			NA	NA
G3 [n (%)]	1 (14.3)		0 (0)		0 (0)			NA	NA
Mucinous characteristics [n (%)]	5 (62.5)	8	4 (100)	4	0 (0)	0	12	NA	NA
Macroscopy									
Polyp [n (%)]	1 (12.5)		2 (33.3)		0 (0)			NA	NA
Ulcerovetant [n (%)]	3 (37.5)		2 (33.3)		0 (0)			NA	NA
Vegetant [n (%)]	2 (25.0)	8	0 (0)	6	0 (0)	0	14	NA	NA
Ulceroinfiltrative [n (%)]	2 (25.0)		1 (16.7)		0 (0)			NA	NA
Tubulovillous [n (%)]	0 (0)		1 (16.7)		0 (0)			NA	NA

(table continues on the next page)

Table 2 - Descriptive CRC and EC Table (remaining section)

	Colorectal cancer							Endometrial cancer	
	Right colon		Left colon		Rectum		Total *	Cases	Total *
	Cases	Sub-total	Cases	Sub-total	Cases	Sub-total			
Inflammatory Infiltration									
None/Mild [n (%)]	4 (57.1)	7	2 (66.7)	3	0 (0)	0	10	NA	NA
Moderate/High [n (%)]	3 (42.9)		1 (33.3)		0 (0)	0		NA	NA
Lymphatic invasion [n (%)]	4 (50.0)	8	0 (0)	5	0 (0)	0	13	NA	NA
Venous invasion [n (%)]	4 (50.0)	8	0 (0)	5	0 (0)	0	13	NA	NA
Perineural invasion [n (%)]	2 (25.0)	8	0 (0)	5	0 (0)	0	13	NA	NA
Lymph nodes involvement [n (%)]	4 (50.0)	8	0 (0)	5	1 (100)	1	13	NA	NA
T Staging									
Tis [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
T0 [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
T1 [n (%)]	0 (0)	7	0 (0)	3	0 (0)	0	10	NA	NA
T2 [n (%)]	2 (28.6)		1 (33.3)		0 (0)			NA	NA
T3 [n (%)]	3 (42.9)		2 (66.7)		0 (0)			NA	NA
T4 [n (%)]	2 (28.6)		0 (0)		0 (0)			NA	NA
N Staging									
Nx [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
N0 [n (%)]	3 (42.9)	7	3 (100)	3	0 (0)	0	10	NA	NA
N1 [n (%)]	3 (42.9)		0 (0)		0 (0)			NA	NA
N2 [n (%)]	1 (14.3)		0 (0)		0 (0)			NA	NA
M Staging									
Mx [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
M0 [n (%)]	6 (85.7)	7	3 (100)	3	0 (0)	0	10	NA	NA
M1 [n (%)]	1 (14.3)		0 (0)		0 (0)			NA	NA
R Staging									
Rx [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
R0 [n (%)]	7 (100)	7	3 (100)	3	0 (0)	0	10	NA	NA
R1 [n (%)]	0(0)		0 (0)		0 (0)			NA	NA
R2 [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
Colorectal Oncology Group decision									
Surveillance [n (%)]	4 (44.4)		5 (100)		0 (0)			NA	NA
Chemotherapy [n (%)]	5 (55.6)	9	0 (0)	5	0 (0)	1	15	NA	NA
RT [n (%)]	0 (0)		0 (0)		0 (0)			NA	NA
Chemotherapy + RT [n (%)]	0 (0)		0 (0)		1 (100)			NA	NA
Gynecologic treatment									
Hysterectomy + Anectomy [n (%)]	NA	NA	NA	NA	NA	NA	NA	1 (100)	1
Hysterectomy [n (%)]	NA	NA	NA	NA	NA	NA	NA	0 (0)	

* Total number of available data per variable; † Standard deviation.

Table 3 - Descriptive follow-up table

		Total *
Follow-up	Free of disease [n (%)]	19 (79.2)
	New CRC [n (%)]	1 (4.2)
	New non-CRC [n (%)]	0 (0)
	Death [n (%)]	4 (16.7)
		24

* Total number of available data per variable

as more sensitive for including more cases that might not be identified only by Amsterdam I or II criteria.^{22,23}

The mean age found in our study for onset of diagnosis of LS does meet the mean age described in the literature.^{10,11}

Analyzing CRC data, we noticed that most cases of CRC were reported to the right colon, which is congruent with what is described for LS.¹¹ Despite that, the cases reported to the left colon all were studied mainly because of their young age of onset and fulfilling Amsterdam I or II criteria. Otherwise they might have been considered as sporadic CRC. It is also interesting that one of the cases of CRC is present in the rectum, a localization not usually associated with LS.

When considering the mutated MMR gene, we interestingly realize that most cases of CRC had mutations in *MSH2*. *MLH1* is usually described as being the most frequently mutated gene in CRC associated with LS.²⁶

Referring to the surgical treatment performed in our patients, and despite the knowledge that LS patients have high incidence of metachronous or synchronous colorectal tumours,¹¹ most patients underwent segmental colectomy and only three performed a total colectomy, the surgical treatment preconized in LS patients.⁵⁰

Discussing the CRC tumor characteristics, most of our patients show a low histological grade of differentiation, despite LS being associated with an unfavorable histological grade.¹¹ Also, all tumours showed mucinous characteristics, which is congruent with what is described for LS.¹¹ Regarding

macroscopic characteristics, no characteristic was found to be much more frequent than others.

Despite LS cases being mainly described as having high prevalence of inflammatory infiltrate, representing the host-to-tumor response that might give these patients conditions for the described better prognosis when compared to sporadic CRC with no MSI,^{51,52} our patients did not have a tendency for the expected moderate/high inflammatory infiltrate.

Now analyzing the decision of the Colorectal Oncology Group of giving the FOLFOX scheme as adjuvant chemotherapy to all AJCC Stage III or IV patients, we have to refer the fact that patients with MSI and context of LS are reported to have a worst response to 5-FU.⁵³

It is interesting to realize that LS first was noticed and diagnosed by onset of EC in five out of 16 female patients. It is not surprising, however, as it may be the first manifestation of LS. It is intriguing that the only gene showing no mutation in these cases was *MSH6*, a gene usually described as associated with LS with EC phenotype.^{41,44} It is also of notice that four female patients with CRC underwent prophylactic hysterectomy and adnexectomy, a valid option for LS female patients in menopause or perimenopausal, after informed consent.¹⁸

Analyzing the other cancers of LS spectrum we realized that two patients presented disease related death, accounting for 50.0% of all patients that died. It is an intriguing fact to find out whether those cancers also have a better prognosis than their sporadic equivalent for the same stage (as CRC) or not.

It is of notice that *MSH2* was the MMR gene most often studied in our patients, since in our center it is preconized a sequential study, starting with both *MLH1* and *MSH2* and if both negative, move to analysis of *MSH6* and, if negative, proceed to analysis of *PMS2*.

We realized that most patients have mutations in *MSH2*, which is of notice since *MLH1* is the gene described as being the most frequently mutated. *MSH6* and *PMS2*

Table 4 - Descriptive table by MMR gene

	<i>MLH1</i>		<i>MSH2</i>		<i>MSH6</i>		<i>PMS2</i>		Total*	
	Cases	Sub-total	Cases	Sub-total	Cases	Sub-total	Cases	Sub-total		
Number of cases [n (%)]	3 (37.5)	8	9 (69.2)	13	4 (57.1)	7	1 (12.5)	8	13	
Age of CRC diagnosis (years) [Mean ± SD]†	39.5 (3.53)	2	42.4 (7.97)	9	37.67 (8.62)	3	0 (0)	0	14	
Age of EC diagnosis (years) [Mean ± SD]	65.0 (0)	1	39.0 (0)	1	0 (0)	0	65.0 (0)	1	3	
Gender	Male [n (%)]	2 (66.7)	3	2 (22.2)	9	2 (50.0)	4	0 (0)	1	17
	Female [n (%)]	1 (33.3)	3	7 (77.8)	9	2 (50.0)	4	1 (100)	1	17
MSI [n (%)]	2 (100)	2	6 (100)	6	1 (100)	1	1 (100)	1	10	
Other cancers [n (%)]	0 (0)	3	1 (11.1)	9	1 (25.0)	4	0 (0)	1	17	
Follow-up	Free of disease [n (%)]	0 (0)	3	9 (100)	9	4 (100)	4	0 (0)	1	17
	New CRC [n (%)]	1 (33.3)	3	0 (0)	9	0 (0)	4	0 (0)	1	17
	Death [n (%)]	2 (66.7)	3	0 (0)	9	0 (0)	4	1 (100)	1	17

* Total number of available data per variable; † Standard deviation.

frequencies are similar to described.²⁶ However, those results may be biased since each gene was not tested with equal frequencies.

Despite the described association of *PMS2* mutations with LS cases without suggestive family history,⁴⁰ our case with *PMS2* mutation (also mutated for *MLH1*) fulfilled Amsterdam I or II criteria.

However, as described, the high predominance of CRC in patients with *MLH1* or *MSH2* mutations and lower with *MSH6* and *PMS2* mutations were also noted in our patients. As the mean ages of onset of CRC are similar to the described for *MLH1* and *MSH2*, the same does not happen to the *MSH6*, described to have an older age of onset. This also may be due to our small data sample.

No patient with EC had mutations in *MSH6*, contrary to what is usually described. However, literature suggests that 71% of females with mutation in *MSH6* will have EC at age 70,¹¹ therefore emphasizing the previously described role of prophylactic hysterectomy and adnexectomy.

It is of notice that all patients studied for MSI with MMR germline mutations were positive, even the case mutated for *MSH6*, a gene that is described to be most often associated to MSS status than the others.⁴³

As for other cancers relation to MMR gene mutation, the *MSH6* case had CNS cancer (oligodendroglioma) and the *MSH2* case in this situation had breast cancer. The last may possibly be a Muir-Torre syndrome. Unfortunately, specific data of skin lesions (keratoacanthoma, sebaceous glands tumor) were not registered in our patients.

Besides all of this, it is widely seen that there are phenotypic variations even between patients with the same mutated MMR gene, which points to other factors that can also influence the phenotype other than the MMR gene defect.

It is not surprising that only one case had mutation in p53 as LS associates with a low frequency of this mutation.⁵⁴ This may be a factor that explains why CRC from LS have a better prognosis than sporadic CRC in the same stage.

Finally, regarding inferential statistics performed in order to obtain a relationship between several variables, they were not statistically significant, probably due to the very small data sample. However, some tendencies in the descriptive analysis were of notice, as reported in this discussion.

CONCLUSION

The small size of our data sample along with the insufficient and non-standardized registration of patient's information may be the main reason for not achieving a statistically significant correlation between variables.

Therefore, a multicenter, extended study, perhaps at a national level, might be able to recognize these correlations. Also, the creation of a standardized protocol of evaluation and registration of data for 'at risk' patients may be of capital importance for better patient care and management and for better LS understanding.

Family history assessment is of capital importance as

well as considering LS as a potential differential diagnosis in younger patients. Also, the standardized use of Bethesda Guidelines for evaluation of patients might identify new cases that do not fit Amsterdam I or II criteria. As noticed, all our patients not fulfilling Amsterdam criteria had LS diagnostic and at least one mutated MMR gene, which could mean they are a first familial case.

In fact, descriptive analysis of our data shows some tendencies. Globally, most patients have MSI status. However, this should be performed on a much larger scale than it was, since it may influence prognosis and treatment choice. As for CRC, most cases have favorable staging.

The information that most female LS patients may have EC in their lifetime is of extreme importance, especially regarding the option of performing prophylactic hysterectomy and adnexectomy in menopause or perimenopausal patients, after informed consent.

Also, further study should be done regarding the outcome of other cancers of LS spectrum when compared to their sporadic equivalents.

Standardized collection of cutaneous tumours information should be done, since that information may be of relevance, especially in patients with *MSH2* mutations. Also, a protocol for the request of MMR gene testing should be performed.

In fact, a large number of mutations in these MMR genes are not yet known or identified, which may result in misdiagnosis and mismanagement. Also, it is suggested in literature that other facts may influence LS phenotypes other than the MMR gene mutation and so further studies should evaluate these factors.

When found, these newly recognized mutations and factors should be spread through best practice guidelines so that clinicians can take advantage of that knowledge in pursuing the best patient care.

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PROTECTION OF HUMANS AND ANIMALS

This retrospective study was approved by the Ethics Committee for Health of CHSJ/Faculdade de Medicina da Universidade do Porto (FMUP).

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

CONFLICTS OF INTEREST

There was no conflict of interest reported in the making of this study.

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