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Impact of Microsatellite Instability in Colon Cancer

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Abstract

Colorectal cancer (CRC) is one of the leading causes of death worldwide, thus a public health concern. Even though most of the cases have a sporadic origin, a significant 10% are affected by a genetic predisposition. An inherited impaired function in the cellular mismatch repair (MMR) system of any MMR gene diagnoses the most frequent genetic syndrome associated with CRC, Lynch Syndrome. The faulty MMR system is directly associated with the presence of microsatellite instability. In this project, the authors present a microsatellite instability retrospective analysis in CRC samples of 252 patients, performed between 2013 and 2018, in the Molecular Analysis Laboratory of the Tumor Bank of Uruguay. Data from both high risk and general population-CRC patient samples were tested for microsatellite instability. From a cohort of 252 non-selected CRC patients who underwent MSI screening, 91 CRCs with MSI-H patients were identified to be in need of a different therapeutic and follow up approach. Additional benefits on utilizing MMR status rely on: prognosis information, differential chemosensitivity, and immunotherapy applicability. Timely management, treatment, and risk-reducing strategies are vital for MMR carriers. The modification of therapeutic standards by making a more opportune risk selection, benefits the patient, their families and has a positive economic impact. The recognition of colon cancer molecular subtypes represents the present reality for personalized medicine.

Keywords: Colorectal cancer; Microsatellite instability; Lynch syndrome; Tumor bank; Genetic syndrome

Introduction

Colorectal cancer (CRC) is the fourth most common cancer and the second leading cause of cancer-related death worldwide, according to statistical analysis updated to year 2018 [1]. Ranks second in incidence in Uruguayan population, responsible of 15% annual prevalence [2,3]. Within the general population, approximately 90% of cases occur sporadically, with an accumulated lifetime incidence of 6.6% [3]. The resultant 7-10% is attributed to a hereditary genetic predisposition, mainly due to Lynch syndrome (LS), which is the most common type of hereditary CRC, accounting for 1% to 3% of all cases.

An inherited impaired function in the cellular mismatch repair (MMR) system of any MMR gene (*MSH2, MLH1, MSH6*, and *PMS2*) confirms LS diagnosis [4]. The underlying function defect leads to the accumulation of errors during DNA replication. As a result, LS patients will characteristically have MMR deficiency (dMMR), associated to the presence of microsatellite instability (MSI) or loss of MMR protein expression (detected by immunohistochemistry). The first assay detects MMR-deficient tumors by a polymerase chain reaction, using five MSI markers validated by the National Cancer Institute (NCI). The second assay detects the presence or absence of protein expression of MMR proteins. Both tests (MSI and IHC) are different and provide complementary results [5].

In the year 1991, the International Collaborative Group on hereditary non-polyposis colorectal cancer, proposed the clinical criteria for suspecting LS, named Amsterdam I criteria and posteriorly the extended Amsterdam II criteria [6]. However, these are limited because of low sensitivity, excluding 50% of LS families [7]. Consequently, the National Cancer Institute proposed the Bethesda Guidelines, and more recently the Revised Bethesda Guidelines (RBG), which still dismisses 28% of LS families [8]. MSI testing has rapidly become the cornerstone for identifying Lynch syndrome individuals (Supplementary Table 1).

Testing all incident CRC (universal screening) for defective MMR, is highly recommended by the National Comprehensive Cancer Network (NCCN) [9]. Even beyond its association to LS, MSI test is beneficial for different purposes: a) determine the need for adjuvant treatment [10]; b) predict response to chemotherapeutic agents; c) serve as a prognostic tool (improved overall and disease-free survival) [11], and d) indicate the need for immune-checkpoint blockade [12].

This article portrays the MSI status in a Uruguayan colon cancer cohort, in which pathologic and molecular features were analyzed.

Methods and Methods

This is a retrospective and descriptive study of MSI analysis in CRC samples, performed between 2013 and 2018, in the Molecular Analysis Laboratory of the Tumor Bank of Uruguay. Analyzing data for both high risk and general population-CRC patient samples, tested for MSI by a modified-PCR technique.

Inclusion criteria comprises: colon cancer diagnosis, signed informed consent, peripheral blood sample, paraffin block from tumor tissue, complete family tree with at least 3 generations, and a copy of the colon cancer pathology report. The following information was registered: positive family history (defined as: Amsterdam I, Amsterdam II or Revised Bethesda Guidelines criteria), age at diagnosis, pathological tumor staging, presence of intra tumoral lymphocytes and posterior genetic testing by multigene panel analysis.

The MSI status was assessed by polymerase chain reaction (PCR), with DNA extracted from paraffin-embedded tumoral tissue and from peripheral blood, using the DNeasy blood and tissue kit (Qiagen). The DNA of the paraffin-embedded tumor tissue samples was extracted from three 10 µm thick sections, dewaxed with xylol alcohol and

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subjected to the QIAamp® DNA FFPE Tissue kit (Qiagen). The following markers were amplified: 2 mononucleotide (BAT25 and BAT26) and 3 dinucleotide (D2S123, D5S346 and D17S250), proposed in the Bethesda panel [13]. For each marker PCR was performed as described by Watharin Loilome in 2016 with modifications [14]. The international criteria used for tumor MSI classification is as follows: tumors that exhibit a high degree of microsatellite instability are considered when at least two or more markers are unstable (MSI-H); tumors with low MSI when a single marker is unstable (MSI-L) [15] and tumors with stable MSI are those with five stable markers (MSS) [16].

All colon cancer patients meeting Amsterdam I, II or Revised Bethesda´s guidelines regardless of MSI status; or MSI-high pattern, irrespective of meeting clinical criteria, were offered genetic counselling and multigene panel testing. Germline gene testing was performed by commercial clinical laboratories, usually INVITAE or Color, next-generation sequencing using the Illumina HiSeq2500 (Illumina Inc., San Diego, Calif) [17].

Statistical analysis

Demographic variables and histopathological characteristics were obtained, as well as the degree of tumor differentiation and stage according to the classification of the American Joint Cancer Committee / Union Internationale Contre le Cancer (AJCC / UICC) 7th edition. The MSI status was analyzed. The categorical data was summarized in frequencies and percentages. Bivariate analyzes was performed according to: age group, sex, degree of differentiation and tumor location; calculating the Odds Ratios (OR) and their 95% confidence intervals. A chi-squared analysis was used when qualitative variables were involved, with a correction of Fisher's exact test if necessary, due to expected values less than 5. T-Student was applied depending on the existence or not of equality of variances and normality. The variable equality was tested with the Levene test and normality with the Komogorv Smirnof test, since samples had more than 30 patients. All the analyzes were performed in two tails and in all cases, the statistical significance was placed at a p value <0.05 using the statistical program SPSS version 23.0**.**

Results

During a study period of five years, we enrolled a total of 252 patients with CRC who met inclusion criteria. The mean age of diagnosis was 59 ± 12.53 years (IC 95%, 57.24-60.82), in a range of 18-92 years and with a male-to-female ratio of 1.21. Positive family history was confirmed in 75,4% of tested individuals. From the entire analyzed cohort, 36.1% were MSI-H (91/252) and 63.9% (161/252) were MSI-L or stable. Table 1 illustrates the gender, age, stage, tumor location and MSI status. Several aspects are highlighted regarding MSI-H tumors (91): a) 39.5% (36/91) (p=0.001) are below 50 years of age at diagnosis; b) 48/91 (52.7%) (p=0.02) were right sided colon cancer; c) 3% (3/91) of patients presented with stage IV tumors; d) 29.8% (23/77) were MSI-H and high TIL, but there was not any high TIL tumor among MSI-L or stable. Table 2 describes MSI status and: mucinous histology, poor differentiation, and Crohn's-like reaction, which had no statistical significance in any group; 86% (78/91) of MSI-H tumors were find in patients with familial history (2 or more relatives). Table 3 associate the presence of MSI status with family history. Patients meeting clinical criteria for hereditary colon cancer, or MSI-H, or both, were offered genetic counselling and multigene panel testing (45/78). Table 4 remarks the positive results of the 18 patients out of 45 tested and diagnosed with a class 5 pathogenic germline variant.

Variables	MSI-H (n)	MSI-L/S (N)	MSI-H and MSI-L/S (OR IC 95%)	P-value
Gender				
Male	44	94	1	0.29
Female	47	67	1.49 (0.89-2.51)	
Age				
≤50 years old	36	29	1	0.001
>50 years old	55	132	2.9(1.66 -5.32)	
Stage TNM				
I	8	25	1	0.021
II	55	76	0.61(0.36-1.02)	
III	25	50	1.30(0.74-2.30)	
IV	3	10	2.06 (0.55-7.69)	
Location				
Right	48	102	1	0.02
Left	43	59	0.93(0.55-1.58)	

Table 1: Gender, age, stage and tumor location stratified by MSI status.

Variables	MSI H (n= 91)	MSI L or MSS
TIL high	14	0
TIL absent, moderate or low	69	161
No TIL described	8	-

Table 2: TIL (absence, low, moderate or high) stratified by MSI status.

MSI-HIGH positive F.H.	MSI-HIGH negative F.H.	MSI-Low/Stable Positive F.H.
78/91 (86%)	13/91 (14%)	37/161 (23%)

Table 3: Presence or absence of family history stratified by MSI status.

Variables	MSI-H	MSI-L/S
Genetic Testing	55/91 (60%)	20/37 (54%)
Presence of pathogenic variants	14/55 (25%)	4/20 (20%)
Genes	*MLH1 (5), MSH2 (3), MUTYH[1] (4), APC (1) and FAN1 (1).*	*MLH1 (2) and MUTYH[1] (2).*
MUTYH[1]: Homozygous and composed heterozygous		

Table 4: Genetic testing and pathogenic variants summary stratified by MSI status.

Discussion

Microsatellite instability is present in approximately 15% of al CRC, with about 2.5% resulting from genetic inheritance and the remaining 12.5% being sporadic [18].

Differentiating the overlying etiology is key to offer best course of treatment. Distinct oncogenetic pathways could explain the dMMR: 1) LS diagnosis; 2) the CpG island methylator phenotype, associated with hypermethylation of the *MLH1* promoter; c) somatic point mutation in the *BRAF* gene; or, d) double somatic mutations or one somatic mutation and loss of heterozygosity (LOH) in MMR genes [19]. Lynch Syndrome is the first cause of hereditary colon cancer worldwide. Epidemiologic studies indicate that 1 over 35 CRC patients carries a MMR gene pathogenic variant [7]. Timely management, treatment and risk reducing strategies, are vital for MMR carriers. Diagnosis is only reachable by genetic testing. Detecting MSI-H associated with *MLH1* and *MSH2* germline mutations have the greatest sensitivity, with a lesser degree for *MSH6* and *PMS2* involvement [20]. Consequently, it is currently recommended to test CRCs by MSI PCR and/or IHQ,

according to: the Evaluation of Genomic Applications in Practice and Prevention in 2009 [21], the National Comprehensive Cancer Network in 2014 to 2019 [22], the US Multi-Society Task Force in 2014 [23], the American College of Gastroenterology [24] and the American Society of Clinical Oncology since 2015 [25].

There are specific clinical – histological characteristics related with MSI-H status: preferentially right sided CRC [26]; younger age of diagnosis (less tan 51 years of age) [27]; mucinous phenotype; presence of signet ring cells or a medullary phenotype; increased intratumoral lymphocytes, and Crohn's -like reaction with prominent lymphoid aggregates at the periphery of the tumor [28,29].

The favorable prognosis observed in CRC patients associated with LS and *MLH1* promoter hypermethylation appear to be similar [30]. Tumor infiltration of T cells in CRC patients (TIL), has also been related to good prognosis [31], specifically, a dense infiltration of CD3 and CD8 lymphocytes [32]. Interestingly, an increased lymphocyte density has previously been found in MSI-H CRCs [33]. Although they are two independent features, the combination of both provides a particularly superior prognosis [34]. Clinical studies had proved that some MSI patients do not benefit from 5-FU therapy. More recently, a 2015 meta-analysis of 14 studies concluded that there is a trend for lack of benefit of 5-FU in MSI cancers [35,36]. A newer approach regarding MSI usefulness, is to predict immunotherapeutic response in patients who have failed conventional therapy. MSI-H tumors, had significant upregulation of immune checkpoint proteins, including PD-1 and PD-L1, enabling them to survive. In MSI colorectal cancer, the PD-L1 expression appears to be located on tumor-infiltrating lymphocytes, rather than tumor cells [32].

According to our results, from a non-selected cohort of 252 CRC patients, 91 were MSI-H and 161 MSI L-S, with 86% and 23% displaying positive family history respectively. All underwent genetic counselling and for the ones with MMRd and/or positive family history (AI, AII and RBG) genetic testing was encouraged. A total of 18 non-related individuals were found to have pathogenic variants, from which all meet clinical and/or molecular criteria for germline MMRd. Nonetheless, 8 had a pathogenic variant outside a LS related gene, diagnosed due to comprehensive gene panel testing, portraying the heterogeneity of oncogenetic pathways. Because CRC patient's cohort was obtained from both high risk and standard risk kindred, the percentage of confirmed hereditary cancer in MSI-H was higher than expected: 20% vs 2,5%. The younger age at diagnosis for MSI-H, was confirmed with statistical significance. Other parameters such as: mucinous histology, poor differentiation, and Crohn's-like reaction, had no statistical significance in any group.

Best choice of care was adjusted to MSI status. The modification of therapeutic standards by making a more opportune risk selection, benefits the patient, their families and has a positive economic impact. Patients with stage II CRC MSI-H (60,4% (55/91)) did not need adjuvant chemotherapy, because of the lack of impact in global survivor and cancer specific survivor. For stage III CRC MSI-H systemic treatment with 5- FU was modified. Metastatic debut is rather uncommon (3%), but with MSI-H therapeutic modifications are in order. Immunotherapy (not first line) is best course of treatment for MSI-H or dMMR pathway. In fact, patients with MSI and a high tumor mutation score should consider receiving immunotherapy as their first treatment, while those with MSI and a low tumor mutation score (less than 37) should be considered for chemotherapy rather than immunotherapy as their first treatment option [37].

Selection bias was observed associated to the high referral of CRC-patients under 50 years old, or with positive family history. Almost all paraffin blocks had to be re analyzed for evaluation of TIL presence. Determination of TIL is not considered standard practice yet. To our knowledge, this is the first written article in Uruguay regarding MSI clinical value to date.

Conclusion

Colon cancer is a common malignancy worldwide, both in incidence and mortality. As a heterogeneous disease, its complexity should be taken into consideration when managing different treatment options. Etiologically, the majority develops sporadically, only less than 10% is associated with a hereditary genetic predisposition. The main associated genes are related to Lynch Syndrome, however genetic predisposition can rise through other molecular pathways. Molecular screening tools rapidly overcome clinical bias, and aid in the selection of highly suspicious LS CRC patients through MSI analysis. Ancillary benefits on utilizing MMR status rely on: prognosis information, differential chemosenstitivity and immunotherapy applicability. Despite fundamental concerns such as: result interpretation, best treatment management and the determination of reflex genetic testing and genetic counseling; the recognition of colon cancer molecular subtypes represents the present reality for personalized medicine.

From a cohort of 252 non-selected CRC patients who underwent MSI screening, we were able to recognize 91 CRCs with MSI-H patients who would need a different therapeutic and follow up approach. Patients meeting clinical or molecular criteria for LS (115) had genetic counselling and a total of 75 patients underwent genetic testing (MSI-H= 55, MSI-S/L= 20). Results analysis revealed: Class 5 variants found in 10 LS related genes and in 8 non-LS related genes. Intratumoral lymphocytes were considered as a pathological complement to prognosis assessment, albeit it is a not standardized feature in the pathology report. Treatment and prognosis were properly adjusted to stage and MMR proficiency/deficiency status. Microsatellite testing benefits highly overcomes the administrative, economic and technical difficulties. In the present time, MMR status aids in treatment options, follow up, prevention strategies and serves as a prognostic tool. Acknowledging deficient MMR as a crucial part of CRC heterogeneity, is key towards identifying individualized target therapies, and adequate surveillance according to risk profile, which can only improve patient's outcomes and hence impact mortality rates.

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