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Left-sided early onset colorectal carcinomas: A sporadic neoplasm with aggressive behavior

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ABSTRACT

Background: Early onset (≤50y) colorectal carcinomas (EO-CRCs) are increasing in incidence according to epidemiological data. We investigated clinical-pathological, molecular features and outcomes of 62 left sided EO-CRCs (EOLS-CRCs) and compared them to a group of late onset (≥65) LS-CRCs (LOLS-CRCs).
Materials and methods: Samples were evaluated for pathological features and microsatellite instability (MSI). Overall survival (OS), disease free survival (DFS) and disease specific survival were evaluated in both groups.
Results: Five out 62 (8%) EOLS-CRCs showed MSI phenotype. Interestingly these cases were aged 26–39y. Most EOLS-CRCs present at advanced stage and this was statistically significant when compared to LOLS-CRCs. OS was better in EOLS-CRCs whilst DFS showed a worst profile in EOLS-CRCs either in low and high stages even though young patients were treated more often with adjuvant chemotherapy compared to older ones at the same disease stage.
Conclusions: Most EOLS-CRCs are sporadic non Lynch, microsatellite stable (MSS) CRCs. Our data show that when compared with LOLS-CRCs the early group represents an aggressive disease with worst outcome underlining a possible different carcinogenic pathway.

1. Introduction

Colorectal carcinomas (CRCs) represent, in the majority of cases, a neoplasm of older patients even though epidemiologic evidences have been showing increasing incidence rate in patients ≤50 years (so-called early onset colorectal carcinoma, EO-CRCs) especially in left colon.[1,2] EO-CRCs defined only by age encompass a heterogeneous group that may underline cancer with different carcinogenic mechanisms due to site of origin (right or left colon), hereditary predisposition (Lynch Syndrome-LS and familial adenomatous polyposis-FAP) and inflammatory bowel diseases (IBD).[3,4] FAP and cancer IBD associated show specific clinical features that allow a straight identification. LS CRCs show a more heterogeneous phenotype, usually with a proximal location and very often harbor high-microsatellite instability (H-MSI) due to germinal defects in mismatch repair (MMR) genes.[5] Data from either single institutions or large multicentre studies show that sporadic EO-CRCs mainly affect left-colon, are likely to present at higher stage compared to late onset cases, and that, despite early age of presentation, do not develop in the context of hereditary syndrome or cancer risk factors.[6–8]

Attempt of identifying specific molecular alterations in the group of EO-CRCs compared to LO-CRCs have been conducted. Most of them took into consideration colonic neoplasm regardless the site of involvement in colon. However, if in the larger group of EO-CRCs left sided CRCs are extrapolated, most cases showed microsatellite stability/CpG islands methylator phenotype negative (MSS/CIMP-) phenotype, BRAF wild-type, KRAS mutation in 35–40%.[9–11]

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When compared to LO-LSCRCs some molecular differences emerge. EOCRCs MSS/CIMP-have been reported to show high deregulation of beta-catenin,[9] different pattern of DNA methylation,[10] higher index of Genomic Instability,[11] allelic imbalance on chromosome.[12–14] These data suggest that LSEO-CRCs may differ in molecular progression from LSLO-CRCs and this could represent an important implication for tailored therapy and screening.

In this study we focused specifically on left-sided EO-CRCs (EOLS-CRCs). We collected all patients (aged ≤50) that underwent surgical resection in our hospital for left-sided CRCs between 2003 and 2014. Patients were not filtered by a genetic counseling and information about Amsterdam criteria II or Bethesda guidelines were not available. The aim was to evaluate in EOLS-CRCs clinical and pathological features, prevalence of Lynch Syndrome (LS) by investigating MSI phenotype; moreover we compare overall survival and progression free survival of the EOLS-CRCs group with a group of LOLS-CRCs (aged ≥65).

2. Material and methods

2.1. Study design

Ninety two patients ≤50 years with a left-sided CRCs (EOLS-CRCs) who underwent surgical resection and histological examination at the Sant'Andrea Hospital of Rome from 2003 to 2014 were consecutively collected and retrospectively analyzed. For each patient we retrieved demographics data (sex and age at surgery), surgical procedure, tumor location and pathological features. Cancers were categorized as rectal (within 15 cm from the anal verge by endoscopy), sigmoid tumors or neoplasm of the descending colon (distal to the splenic flexure). Adjuvant treatments performed after surgical resections were also recorded. Pathological data included the T and N stage of colorectal cancer, Grading, the number of nodes harvested in the surgical field (LNH) and the lymph node ratio (LRN). Moreover, clinical staging was recorded for all cases and considered for survival analysis.

Patients undergone neo-adjuvant chemo-radiotherapy (20 patients) or those who presented an association with inflammatory bowel diseases or polyposis (10 patients) were excluded.

Of note, since a detailed oncological family history and genetic counseling were not available for most of the selected cases and anamnestic data collection at the time of hospital admission was not aimed to familial syndromes, patients were enrolled in the present study independently of the Amsterdam criteria or Bethesda guidelines.

Part of the EOLS-CRCs group (22 patients) belonged to a series of EO-CRCs previously described.[10]

Furthermore, EOLS-CRCs were compared with a group of MicroSatellite Stable (MSS) late onset left-sided colorectal carcinomas (LOLS-CRCs) for statistical purposes. In particular, from a series of 119 colorectal cancers previous described (enrolled by our group between 2000 and 2004) patients were selected if ≥65years, with a neoplasm located in the descending colon-sigma or rectum and MSS phenotype.[13]

A signed consent for the treatment, research purpose and evaluation of data was obtained from all patients before the surgical procedures.

2.2. DNA extraction and microsatellite instability evaluation

A fragment of cancer tissue was collected for each selected patient. For each sample of paraffin embedded tissue, 3 sections of 7 μm were cut and put on slides, de-waxed, rehydrated and stained with haematoxylin. A pathologist performed micro-dissection of the normal mucosa and neoplastic tissue under microscope using a needle. DNA extraction was carried-out with 50 μl of lysis buffer containing 10 mM of proteinase K, overnight digestion at 37 °C followed by incubation at 95 °C for 10 min. After centrifugation, supernatant was stored at −20 °C.

At molecular level all samples were evaluated for microsatellite instability (MSI) through PCR amplification of quasimonomorphic mononucleotide loci as previously described.[10] Presence in both loci of extra alleles in neoplastic tissue was defined as high microsatellite instability (H-MSI).

2.3. Immunohistochemistry

All EOLS-CRC cases selected were stained for hMSH6 (hMSH6, clone 44, Diagnostic BioSystems) and visualized by Envision-Flex (Dako) in a Dako Autostainer instrument. Immunohistochemistry was performed on 4 μm-thick sections. Nuclear protein expression in neoplastic cells was assessed as retained (normal) or lost. Inflammatory cells in the samples were used as positive internal control. Samples that showed H-MSI were also stained with the following antibodies for mismatch repair proteins: hMLH1 (BD Pharmingen, clone G168-15); hMSH2 (BD Pharmingen, clone G219-1129); PMS2 (Biocare Medical, Clone A16-4) and visualized by Envision-Flex (Dako) in a Dako Autostainer instrument. Nuclear protein expression in neoplastic cells was assessed as retained (normal) or lost. Inflammatory cells in the samples were used as positive internal control.

2.4. BRAF exon 15 mutational analysis

BRAF (exon 15) mutational analysis was performed in H-MSI positive cases using direct sequencing of PCR amplified products. BRAF-FW 5′-TCATAATGCTTGCTCTGATAGGA-3'; BRAF-REV 5′-GGCCAAAATTTAATCAGTGGA-3'. All PCR products were sequenced bidirectionally using the Big Dye Terminator chemistry and Life Technology Automated 3100 DNA Analyzer (Biotechnology Resource Center, Cornell University, Ithaca, NY).

2.5. Follow-up

Follow-up of the patients has been updated yearly by the surgical team with the end-points of overall survival (OS, any cause of death), disease free survival (DFS, first recurrence after colorectal resection) and disease specific survival (DSS, death related to colon cancer).

2.6. Statistical analysis

Continuous variables were analyzed using means and standard deviations, whereas categorical variables were analyzed using frequencies and percents. EOLS-CRCs were compared to LOLS-CRCs for the clinical/pathological features and outcomes. Sub-groups were compared using the T-test (for continuous variable) and Chi-square test or Fisher test (for categorical variables). All tests were performed two-tailed and a p value < 0.05 was considered as statistically significant. Logistic regression using the stepwise model (enter variable if $p < 0.05$, remove variable if $p > 0.1$) was conducted with the endpoint of LNH>12 and the following co-variates: date of surgical procedure (before 01.01.2005 = 1; later = 0), age (<50 years = 1; >65 years = 0), Grading (G3 = 1; G1-2 = 0), sex (male = 1; female = 0) and T Stage (T3-4 = 1; T1-2 = 0). Survival analysis has been conducted using the Kaplan-Meier method-log-rank test. All statistical analyses were obtained using MedCalc for Windows, version 10.2.0.0 (MedCalc Software, MariaKerke, Belgium).