
Sara Campos\textsuperscript{a}  Pedro Amaro\textsuperscript{a}  Inês Cunha\textsuperscript{a}  João Fraga\textsuperscript{b}  
Maria Augusta Cipriano\textsuperscript{b}  Luís Tomé\textsuperscript{a}  
\textsuperscript{a}Gastroenterology Department, and \textsuperscript{b}Pathology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

\textbf{Abstract}

\textbf{Introduction:} Lynch syndrome (LS), the most common hereditary colorectal cancer syndrome, is characterized by mutations in mismatch repair (MMR) genes leading to an increased cancer risk, namely colorectal cancer. \textbf{Case:} In the context of surveillance colonoscopy, a 40-mm flat lesion (0-IIa+b, Paris classification) was identified and submitted to piecemeal mucosal endoscopic resection in a 64-year-old LS patient with an MLH1 germline mutation (262delATC) and two previous segmental resections due to metachronous colorectal cancer. Pathology raised the suspicion of superficial submucosal invasive carcinoma with poor differentiation. Immunochemistry showed heterogeneous MLH1 expression and PMS2 loss. In a short-term follow-up colonoscopy, another 30-mm advanced carcinoma was identified. The patient was referred to surgery. \textbf{Conclusion:} This case raises several issues: (1) the potentially fast tumorigenesis and progression to carcinoma in LS and implications for endoscopic screening and surveillance; (2) pitfalls in the interpretation of MMR proteins immunochemistry; (3) the role of endoscopic resection in LS.

\textbf{Keywords}

Lynch syndrome · Immunochemistry · Endoscopic resection
Introduction

Lynch syndrome (LS), the most frequent hereditary colorectal cancer (CRC) accounting for 1–3% of all CRC [1–5], is an autosomal dominant disorder in which there is a deleterious germline mutation in one of a set of DNA mismatch repair (MMR) genes – \textit{MLH1}, \textit{MSH2}, \textit{MSH6}, or \textit{PMS2} – or loss of expression of the \textit{MSH2} gene due to deletion in the \textit{EPCAM} gene [6–9], with increased risk of cancer, namely CRC [1–5].

CRC in LS, comparing to sporadic CRC, is diagnosed in younger ages, more frequently localized in the right colon, with a faster sequence adenoma-carcinoma, distinct CRC histological characteristics, and a better prognosis [10].

Clinical Case

The authors describe a case of a 64-year-old male patient followed in Gastroenterology for 11 years due to LS. He had been previously submitted to colorectal surgery when he was 39 years old due to malignancy of the rectosigmoid junction. At the age of 43, he underwent a new surgery due to a metachronous malignant lesion in the ascending colon. Both lesions were treated while he was abroad, with no additional information available, namely the histopathologic features. LS diagnosis has been confirmed through genetic testing (mutation in codon 262 of MLH1 gene, 262del-ATC). Concerning his family history, he had a brother with surgically treated colon cancer at 49 years who died at 59 years due to gastroesophageal junction adenocarcinoma, a son with colon cancer at 24 years treated with curative surgery, and a daughter of 36 years without bowel lesions; all these relatives have a positive genetic testing for the culprit mutation and those still alive are under endoscopic surveillance. The mother died of uterine cancer when she was 50 years old (Fig. 1). The index patient started follow-up in our department at the age of 53 years. Since then, he had surveillance colonoscopies without sedation every 1–2 years, showing a right hemicolectomy with ileocolic anastomosis and a colorectal anastomosis at 10 cm of the anal verge. A few low-grade tubular adenomas, the largest with 12 mm, have been excised by polypectomy. In the last of these procedures, a superficial nonpolypoid 40-mm flat lesion (type 0-IIa+b, Paris classification) was identified in the proximal colonic surgical stump (Fig. 2). In the two previous endoscopic examinations, suboptimal bowel preparation (poor bowel preparation was found above the splenic flexure, Boston subscore 1) was noticed, and a shorter surveillance interval (yearly) and an optimized preparation protocol were proposed. A piecemeal endoscopic mucosal resection (EMR) was performed and complicated by a 6–7 mm perforation that was successfully closed with 3 long (clip arm length 9 mm) endoclips (EZ-Clip HX-610-090L, Olympus™; Fig. 3). EMR was finished and there were no signs of residual lesion. During this colonoscopy, additional flat/sessile lesions with smaller size were identified throughout the remaining colon, four of which, with 5 to 12 mm, were also excised. However, considering the long duration of the procedure, the perforation (even if endoscopically treated), and patient discomfort probably related to pneumoperitoneum (no \text{CO}_2\text{ insufflation available}) (Fig. 4), other few similarly small and apparently unremarkable lesions were intentionally left behind. Inpatient conservative treatment with antibiotics and analgesia was proposed and the patient was discharged 5 days later with no further complications. All piecemeal EMR specimen fragments were retrieved and showed intraepithelial flat tubular and villous adenoma with high-grade neoplasia/intramucosal carcinoma with various patterns (tubular, solid, syncytial, signet ring, and mucinous). One of those fragments exhibited a solid and syncytial...
pattern associated with chronic inflammatory cells suspicious of focal superficial submucosal invasive carcinoma (possible sm1) with no lymphovascular invasion or tumor budding; the pathology was evaluated by two pathologists in our institution and further revised in a second institution. Immunohistochemistry (IHC) showed PSM2 loss of expression, and MLH1 heterogeneous expression pattern, negative in solid areas and positive in villous lesions; MSH6 and MSH2 were conserved (Fig. 5). CK7 and CK20 were negative. The larger (12 mm) of the remaining four excised lesions was a noninvasive neoplasia that also displayed a similar solid and syncytial pattern with intense chronic inflammatory infiltrate, while the others showed tubular adenoma with low- and high-grade dysplasia.

A multidisciplinary discussion on further management was carried out and additional surgery was considered. However, the patient preferred to be submitted to further endoscopic and imaging evaluation. Thoracic abdominal CT scan showed several millimetric pulmonary lesions, but FDG-F18-PET-CT scan confirmed no distant metastasis. A revision colonoscopy took place only 5 months later, showing a regular scar with absence of neoplastic residual tissue in the surgical colonic stump; however, an endoscopically advanced 30-mm ulcerated neoplastic lesion was identified 28 cm from the anal verge, corresponding to a carcinoma composed by cellular cords and signet ring cells within mucinous pools.

The patient was then referred to surgery, and coloprotectomy with definitive ileostomy was performed. Ileorectal anastomosis could not be accomplished due to severe rectal perianastomotic adherences and fibrosis.
Pathology showed an invasive carcinoma formed by irregular glands, a major component of signet ring cells (Fig. 6) and a component of extracellular mucin less than 50% of the neoplasia. The tumor invaded up to the muscularis propria without lymphovascular/neural invasion or metastatic disease in 21 lymph nodes (TNM classification: T2N0M0). There was no sign of residual lesion from the previous EMR in the surgical stump.

**Discussion**

LS diagnosis is based on clinicopathological features comprised in the Amsterdam (I or II) criteria or the revised Bethesda guidelines and should be confirmed at molecular level with genetic testing. Direct mutation
screening, the approach followed in this case, is both
time-consuming and expensive. Research has been done
to define a more efficient workup algorithm, initially us-
ing tumor DNA microsatellite instability (MSI) and more
recently tumor MMR protein detection by IHC to justify
direct genetic testing. The latter approach has gained
preference as first line; however, MSI still has a role as an
alternative in cases of inconclusive/normal IHC and a
high clinical suspicion [11]. A missing protein suggests a
mutation in the gene that codes for that protein. This is
generally the case with loss of expression of MSH2 or
MSH6; however, when MLH1 and PMS2 are lost, a BRAF
mutation and/or MLH1 promoter hypermethylation,
which may be involved in MSI-high CRC in older pa-
tients without LS, must be excluded before proceeding to
MMR mutation testing.

In this case, tumor IHC was not mandatory because a
diagnosis was already established by the identification of
an MLH1 gene mutation; however, heterogeneous tu-
mor MLH1 expression and PMS2 absence illustrates one
pitfall of IHC that must be acknowledged. In their func-
tional state, MMR proteins form heterodimers: MSH2
dimerizes with MSH6 and MLH1 is usually attached to
PMS2 [11]. MLH1 germline mutations can be due to a
nonsense mutation in two-thirds of the cases usually de-
termining loss of MLH1 tissue expression; however, in
the other one-third, a missense mutation results in an
inactive mutant protein that is antigenically intact, pro-
ducing a false-normal staining pattern in IHC [11]. In
these cases, MLH1 antibodies are unable to detect all
MLH1 abnormalities; the same may happen by an un-
known mechanism even with protein-truncating muta-
tions and large in-frame deletions in MLH1 [11]. An-
other possible explanation comes from the second hit
that inactivates the second normal allele, which may also
result in a nonfunctional antibody-binding MLH1 pro-
tein and variable staining patterns in IHC [11]. For these
reasons, even though PMS2 mutations probably account
for only 6% of all LS, the inclusion of PMS2 antibody in
the IHC testing panel is mandatory because the absence
of PMS2 expression may increase the accuracy by detect-
ing up to 23% of MLH1-mutated tumors missed by
MLH1-IHC [12].

LS management, when it concerns CRC prevention, is
based on regular high-quality endoscopic surveillance
[13, 14]. In this case, suboptimal bowel preparations may
have hindered the detection of already existing lesions,
namely the two larger ones.

Endoscopic treatment is the first-line approach of ear-
ly lesions; total colectomy with ileorectal anastomosis is
indicated for advanced neoplasia/lesions not removable
by endoscopy. In this case, a 40-mm superficial neoplasia
was completely removed by EMR, but it was not curative
considering the poor differentiation and the suspected
superficial submucosal invasion in the context of piece-
meal resection [15]. Additional surgery was recommend-
ed in spite of the uncertainty if the criteria defining cura-
tive endoscopic resection, which were designed for spo-
radic neoplasia, should be similarly applied to LS. In fact,
the prognosis of LS is more favorable for reasons still not
clarified but eventually in relation to an intense immu-
nological reaction, as was the case. Unfortunately, an
endoscopically advanced neoplasia was detected in the
post-EMR colonoscopy, making surgery mandatory.
This lesion was missed earlier, probably due to the par-
ticular circumstances of the procedure (perforation, re-
trieval of all the fragments, long duration, patient dis-
comfort).

The authors present herein a case of LS to draw atten-
tion to this syndrome with CRC predisposition and fast
malignization of small nonpolypoid colonic lesions,
where a specific protocol of colorectal surveillance is
needed to ensure prevention against CRC. Additionally,
the case underscores the peculiar histopathologic fea-
tures of LS and the IHC pitfalls in cases with MLH1 mu-
tations. Finally, the role of endoscopic resection in LS
with large neoplastic lesions needs further evaluation
and guidance.

Acknowledgements

The authors would like to thank Prof. Dr. Fátima Carneiro, Pa-
thology Department, Centro Hospitalar de S. João, for slides re-
view and helpful discussion.

Statement of Ethics

This study did not require informed consent nor review/approval by the appropriate ethics committee.

Disclosure Statement

The authors have no conflicts of interest to declare.
References


