Human Pathology (2015) 46, 1705–1711

ELSEVIER

Human PATHOLOGY

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Original contribution

Immunohistochemistry staining for mismatch repair proteins: the endoscopic biopsy material provides useful and coherent results☆,☆☆

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Received 21 January 2015; revised 7 July 2015; accepted 15 July 2015

Keywords:
Immunohistochemical staining;
Lynch;
HNPCC;
Reliability;
Mismatch;
Repair;
Proteins

Summary Immunohistochemistry (IHC) testing for mismatch repair proteins (MMRP) in patients with colorectal cancer can be performed on endoscopic biopsy material or the surgical resection material. Data are continuing to accumulate regarding the deleterious effect of neoadjuvant chemoradiation on MMRP expression. However, despite continuing rise in the use of endoscopic biopsies for IHC, most pathology departments still use mainly the surgical materials for IHC testing. In this study we compared the quality of stains among 96 colon cancer subjects with paired endoscopic and surgical material available for MLH1, MSH2, MSH6, and PMS2 stains (96 × 4, yielding 384 paired stains). Each slide received both a quantitative score (immunoreactivity [0-3] × percent positivity [0-4]) and a qualitative score (absent; weak and focal; strong). The quantitative scores of all MMRP were significantly higher among the endoscopic material ($P < .001$ for all). In 358 pairs (93.2%), both the endoscopic and operative material stained either strong (322, 83.9%) or absent (36, 9.4%). In 26 pairs (6.8%), the

☆ Author contribution: Vilkin Alex, design and data collection; Ya'ara Leibovici-Weissman, drafting; Marisa Halpren, Sara Morgenstern, and Eli Brazovski, pathology staining and interpretation; Rachel Gingold-Belfer and Orly Sne Arbib, data collection and review of manuscript; Baruch Brenner, data collection; Yaron Niv, financial support and help in drafting; Zohar Levi, design and final drafting.

☆☆ Conflicts of Interest and Source of Funding: None of the authors have any conflict of interests. The study was funded by a research grant from Rabin Medical Center.

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http://dx.doi.org/10.1016/j.humpath.2015.07.009

endoscopic material stained strong, whereas the operative material stained focal and weak. No endoscopic biopsy materials stained focal and weak. Our findings indicate that the biopsy material may provide more coherent results. Although these results may indicate that biopsy material provides coherent and useful results, it is yet to be determined if the demonstrated differences pose a real clinical problem in interpreting final results of IHC staining of such kind. Hence, we suggest that when available, the endoscopic material rather than the operative one should serve as the primary substrate for IHC staining.

1. Introduction

Lynch syndrome (LS), an autosomal dominant condition, is the most common cause of hereditary colorectal cancer (CRC), accounting for about 3% of newly diagnosed cases of colorectal malignancy [1]. Identifying LS cases pre-operatively may have bearing on the extent of surgery performed and the surveillance protocols thereafter [2,3]. Moreover, it is now known that patients with stage II CRC that presents high microsatellite instability (MSI), will not benefit from 5-FU–based treatments [4]. Hence, current guidelines recommend testing tumor material for mismatch repair proteins (MMRP) deficiency in all CRC patients aged 70 years or less at the time of diagnosis, and in individuals older than 70 years who have a family history suggestive of LS [3,5]. Nevertheless, one should keep in mind that the loss of MLH1 function can be a result of either a germline mutation or a somatic silencing of the *MLH1* gene [3,6]. This analysis can be performed by using immunohistochemistry (IHC) testing for the MLH1, MSH2, MSH6, and PMS2 proteins, or by testing the tumor material for microsatellite instability (MSI) [3,5]. IHC testing can be performed on either the endoscopic biopsy material or the surgical resection material [2,3,5]. It is now generally accepted that in subjects who did not undergo neoadjuvant treatment, the yield of IHC testing performed on material obtained by endoscopic biopsy is as good as that performed on surgical material [7–9]. Data are continuing to accumulate regarding the deleterious effect of neoadjuvant chemoradiation on MMRP expression. Despite the continuing rise in the use of endoscopic biopsies for IHC staining [7–10], in most cases pathology departments, including our own, still use the surgical materials for IHC testing [7,8]. This study aims to provide further evidence regarding the reliability of IHC staining performed on endoscopic biopsies of subjects with CRC, who did not undergo neoadjuvant treatment.

2. Materials and methods

2.1. Patients

Patients were identified from the surgical database of the Rabin Medical Center, Israel, which serves as a cancer referral center for the "Clalit" Health Services. Patients who underwent a colectomy without neoadjuvant treatment were included. Clinical data, including the patient's age, gender, ethnicity (Jewish or Arabic); and procedure information, were obtained from the patients' files. The study was approved by the "Clalit" Institutional Review Board.

2.2. Histologic analysis

Hematoxylin and eosin–stained sections of both the endoscopic biopsy and surgical resection specimens, as well as IHC stains, were reviewed by 2 gastrointestinal pathologists.

2.3. Immunohistochemical analysis

IHC staining was performed on formalin-fixed, paraffin-embedded tissues from endoscopic biopsies and surgical specimens. Five-micrometer-thick tissue sections were mounted on positively charged slides and were oven dried at 60°C for 2 hours. Tissue sections were deparaffinized with xylene and hydrated with water. The sections were subsequently immersed in a Nuclear Decloaker Buffer (pH 9.5) (Biocare Medical, CA) and heated in a pressure cooker for ten minutes, for nonenzymatic antigen retrieval. Hydrogen peroxide 3% was applied to tissue sections for 10 minutes to block the endogenous peroxidase activity. The primary antibodies were the following proteins: mouse monoclonal antibody against MLH1 (clone G168-15, dilution 1:100), MSH2 (clone FE11, dilution 1:100), MSH6 (clone BC/144, dilution 1:100), and PMS2 (clone A16-4, ready to use). All antibodies were purchased from Biocare Medical, Concord, CA. The sections were incubated for 90 minutes at room temperature, followed by incubation with Poly HRP Conjugate (Invitrogen, Carlsbad, CA) for 15 minutes. Staining was developed by reaction with diaminobenzide chromogen for 5 to 10 minutes and then counterstained for 2 minutes with hematoxylin. Technical adequacy of staining was validated by normal colonic mucosa and stroma as internal controls and external known positive and negative controls. An experienced laboratory technician prepared the tissue sections and performed all staining in order to optimize technical quality. In this study, all IHC stains were performed at the same time using the same techniques.