Case report

Assessment of the status of DNA mismatch repair proteins by immunohistochemistry. Proposal for evaluation with two antibodies

Leonardo Saul Lino-Silva[1], Ángeles Galán-Ramírez[1], Sabrina B Martínez-Villavicencio[2], Luisa Rivera-Moncada[2], César Zepeda-Najar[3], Hanna I Ortega-Martínez[2]

[1]Instituto Nacional De Cancerologia De Mèxico, Mexico City, Mexico
[2]AFINES program, Medicine Faculty, National Autonomous University of Mexico (UNAM), Coyoacan, Mexico City, Mexico
[3]Hospital Angeles Tijuana, Tijuana, Baja California Norte, Mexico

Determining the status of DNA mismatch repair (MMR) proteins is crucial for patients because they may respond differently to specific treatments and have a better prognosis. We proposed a panel with only 2 antibodies to determine the status of the MMR proteins, improving costs, workload, and delivery of results. Patients with adenocarcinoma and MMR determination were reclassified using only the evaluation of PMS2 and MSH6. The diagnostic performance of the 2-antibody test (proposed panel) and 4-antibody (traditional panel) test was compared against the polymerase chain reaction study (reference standard). A total of 202 cases were identified. The predominant histological type was adenocarcinoma not otherwise specified, the predominant histological grade was 2, and 60.9% of the cases were found in clinical stage II. When comparing the diagnostic performance of the traditional panel of 4 antibodies against a panel of 2 antibodies, no statistically significant differences were found (sensitivity 95.35% vs. 90.7%; specificity 98.74% vs. 98.11%; positive predictive value 95.35% vs. 92.86%; negative predictive value 98.74% vs. 97.50%; area under the curve 0.970 vs. 0.944; $p = 0.419$). Analysis of MMR status determination with only 2 antibodies demonstrates that it is as effective as using 4 antibodies.

Key words: microsatellite instability, mismatch repair, colon cancer, adenocarcinoma, diagnostic performance.

Introduction

Microsatellite instability (MSI) is a genetic phenomenon that involves alteration in the length of repetitive regions of DNA known as microsatellites. These microsatellites are short DNA sequences in which a nucleotide unit (such as A, T, C, or G) is repeated multiple times in tandem. Microsatellite instability can arise due to errors in the DNA replication process, error repair, and other genetic alterations. In the context of colon cancer, MSI is particularly relevant. Most colon cancers are sporadic and not associated with an inherited genetic predisposition. However, 15–20% of colon cancer cases exhibit MSI, often related to an inherited genetic predisposition or a specific gene mutation [1].

Microsatellite instability in colon cancer is due to inactivation of DNA repair systems. DNA error repair systems are responsible for correcting mutations during normal cell replication. When these

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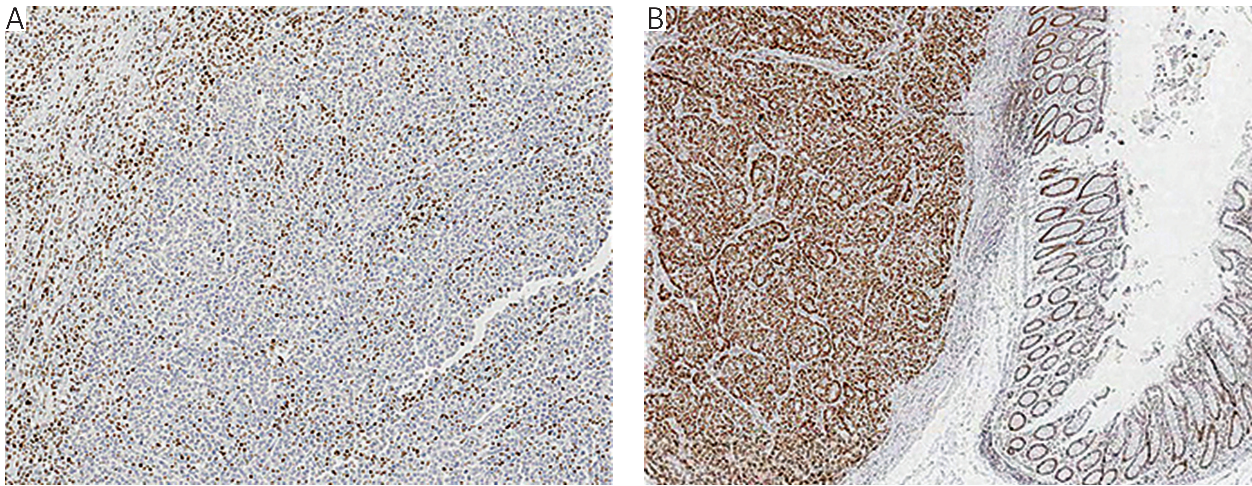

Fig. 1. Immunohistochemistry against PMS2 (left side) and MSH6 (right side). In the PMS2 reaction there is a loss of nuclear reactivity to the protein and in the MSH6 reaction, there is nuclear expression of the protein

systems do not work correctly, errors in the length of the microsatellite repeats accumulate, which can lead to the activation of oncogenes or the inactivation of tumour suppressor genes. This can contribute to the development of cancer [2].

Lynch syndrome, or hereditary nonpolyposis colorectal cancer syndrome, is an inherited condition associated with MSI in colon cancer. People with Lynch syndrome are at increased risk of developing colon cancer and other cancers due to mutations in specific DNA repair genes.

The search for biomarkers in colon cancer has been extensive, and various candidates have been studied [3, 4], such as MSI. Detection of MSI in colon cancer tumours may have important clinical implications. Tumours with MSI may respond differently to specific treatments and have a better prognosis than tumours without this feature [2].

Due to the above, the determination by immunohistochemistry (IHC) of the status of the mismatch repair (MMR) proteins is ideally performed routinely in all cases of colon and rectal cancer, using the 4 antibodies [1]. However, this use of resources is only possible worldwide due to logistics, economics, and access to the tests; in addition, just over 80% of cases are expected to show a normal test result. Due to this, it is ideal to identify an abbreviated approach that allows us to establish the status of MMR proteins, making costs, delivery times, and workload efficient. This has been demonstrated in stomach cancer and in a recent systematic review on colon cancer [5, 6].

Since the MLH1 and MSH2 proteins are essential components of the DNA repair system in detecting and correcting errors, while MSH6 and PMS2 are additional components of the DNA repair system [7], we hypothesise that a panel using only these 2 antibodies will have a diagnostic performance comparable to the panel of 4 antibodies. A reduced panel with diagnostic accuracy similar to a large panel reduces costs, workload, and delivery times, which is especially useful in laboratories/health systems with limited resources or in countries with economies in transition or developing economies.

Material and methods

Samples

The pathology files of our institution were searched for cases of colon cancer treated consecutively in the year 2017. The patients had a histopathological diagnosis of adenocarcinoma and determination of the MMR by IHC with the 4 proteins and polymerase chain reaction (PCR).

Monoclonal antibodies were used against MLH1 (clone G168-728, Cell Marque, Rocklin, California, USA), PMS2 (clone MRQ-28, Cell Marque, Rocklin, California, USA), MSH2 (clone G219-1129, Cell Marque, Rocklin, California, USA), and MSH6 (clone SP93, Cell Marque, Rocklin, California, USA) performed automatically on Benchmark Ultra II equipment (Roche Diagnostics, Rotkreuz, Switzerland) following the instructions of the supplier, and the results were classified. Cases such as pMMR or dMMR are by the guidelines of the College of American Pathologists [8] (Fig. 1). Once the status of the MMR system was determined, the cases were reclassified as dMMR or pMMR using only the evaluation of PMS2 and MSH6, considering one case as dMMR when it showed negative immunoreaction against one of the 2 markers, and classifying it as pMMR. When both were absent. The evaluation was performed by a pathologist experienced in gastrointestinal pathology or pathology oncology (> 10 years of experience, > 50 publications in the last 5 years). The pathologists reviewed the slides again to minimise the performance bias (interpretation bias). With this information, a comparison of the diagnostic accuracy of both tests was

made using the PCR test as the reference standard, which was performed using the QIAamp C.A.A mini kit (Qiagen, Valencia, CA, USA), and MMR was determined in the tumour DNA using EasyPGX® readyMSI, including mononucleotide repeats BAT25, BAT26, NR21, NR22, NR24, NR27, CAT25, and MONO27.

For all cases, the relevant clinical and pathological data were obtained from the clinical history and the pathology file, including the following data: sex, age, tumour site, tumour size, histological type, histological grade, vascular invasion, perineural invasion, depth of invasion, lymph node status, and clinical stage.

Statistics

Descriptive statistics were performed with a summary of categorical data with count and percentage, and for the numerical variables by median and interquartile range in the case of non-parametric distribution and by mean and standard deviation otherwise. Said determination was made with the Kolmogorov-Smirnov test. To calculate diagnostic accuracy and compare both trials, sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated, and the areas under the curve were compared with a χ^2 test with a receiver operating characteristic curve, as described previously [7]. SPSS version 29.0 software (IBM, Armonk, NY, USA) was used for all analyses, and statistical significance was considered for p-values less than 0.05.

Results

We identified 202 cases, of which the average age was 57.9 + 15.2 years (range 20–90 years), 119 (58.9%) were women, and 83 were men. A family history of cancer was reported in 48 (23.8%) cases and a personal history in 13 (6.4%) cases. There was a history of colonic polyps in 48 cases (23.8%).

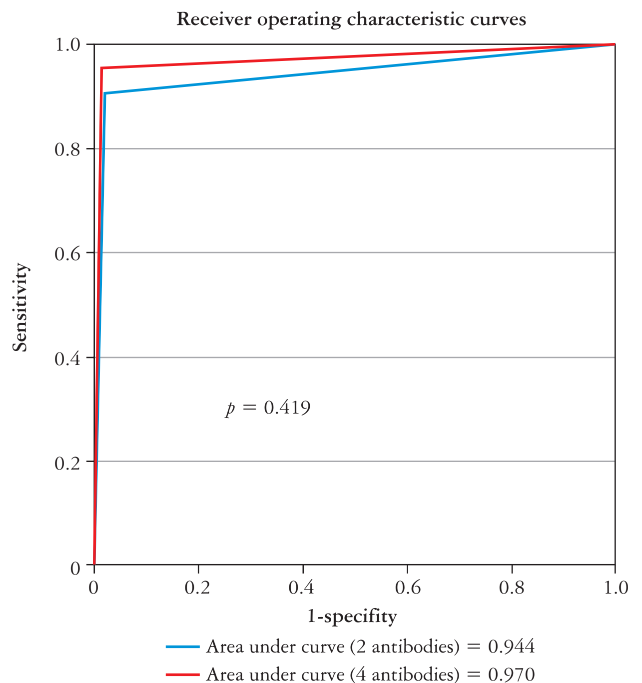

Fig. 2. Receiver operating characteristic curves of immunohistochemical classification of mismatch repair system proteins status. Comparison between 2-antibody determination against the classical 4-antibody determination

A total of 107 (53%) cases were presented in the sigmoid, followed by the descending colon in 53 (26.2) cases, the right colon in 34 (16.8%), and in the transverse colon in 8 (4%) cases. The predominant clinical stage was stage II in 60.9%, followed by stage III (19.3%), stage IV (12.4%), and stage I (7.4%). In 75.2% of the cases (n = 152), the histological type was an adenocarcinoma without other specification (NOS); 12.4% were mucinous, and the remaining 12.4% represented different histological subtypes. The predominant histological grade was G2 (59.9%), followed by G3 (34.2%) and G1 (5.9%). Lymphatic permeation was observed in 38 (18.8%) cases, venous invasion in 21 (10.4%), and perineural charge in 24 (11.9%).

Table 1. Comparison of the predictive capacity of mismatch repair protein deficiency detection by analysing the four antibodies (MLH1, PMS2, MSH2, MSH6) against a 2-antibody test (PMS2, MSH6)

Parameters	Four-antibody test (95% CI)	Two-antibody test (95% CI)
Correctly diagnosed patients	98.02 (94.58–99.36)	96.53 (92.70–98.47)
Sensibility	95.35 (82.94–99.19)	90.70 (76.95–96.98)
Specificity	98.74 (95.06–99.78)	98.11 (94.15–99.51)
Positive predictive value	95.35 (82.94–99.19)	92.86 (79.45–98.14)
Negative predictive value	98.74 (95.06–99.78)	97.50 (93.32–99.20)
Positive likelihood ratio	75.80 (19.09–300.94)	48.07 (15.61–148.06)
Negative likelihood ratio	0.05 (0.01–0.18)	0.09 (15.61–148.06)
Area under the curve*	0.970 (0.933–1.000)	0.944 (0.892–0.996)

* *The p-value of the comparison of area under the curve was 0.419*

The diagnostic accuracy of determining the MMR protein status using the analysis of the 4 antibodies was compared against the analysis using only 2 antibodies (Fig. 2, Table 1), which were very similar when comparing the areas under the curve ($p = 0.782$).

Discussion

In this study of 202 cases of colon cancer with known MSI status, we compared the determination of said status by IHC with a traditional panel of 4 antibodies against a panel of 2 antibodies without finding statistically significant differences in their diagnostic capacity (sensitivity 95.35% vs. 90.7%; specificity 98.74% vs. 98.11%; positive predictive value 95.35% vs. 92.86%; negative predictive value 98.74% vs. 97.50%; and area under the curve 0.970 vs. 0.944, respectively; $p = 0.419$).

The evaluation of MMR protein status is critical for understanding tumour biology and guiding treatment strategies, particularly in cases with suspected Lynch syndrome. Traditionally, IHC using 4 antibodies (MLH1, PMS2, MSH2, and MSH6) has been the standard approach to assess MMR protein expression. However, recent efforts to streamline diagnostic workflows have explored whether a reduced panel of 2 antibodies (PMS2 and MSH6) can achieve comparable accuracy.

Our findings revealed no statistically significant difference in diagnostic accuracy between the 2 approaches, as shown by the comparable areas under the curve ($p = 0.782$). This suggests that the use of 2 antibodies is sufficient to identify MMR-deficient cases effectively, which has several practical implications.

The use of 2 antibodies instead of 4 offers notable advantages, including reduced costs, shorter turnaround times, and decreased resource utilisation, which are particularly relevant in resource-constrained settings. Furthermore, PMS2 and MSH6 directly reflect the function of their respective heterodimeric partners (MLH1-PMS2 and MSH2-MSH6). Loss of MLH1 or MSH2 typically results in the concurrent loss of PMS2 or MSH6, respectively, making these markers reliable surrogates for the entire MMR pathway.

Detection of MSI has become an essential tool in colon cancer diagnosis and patient stratification [10]. Tumours with MSI often have distinct clinical and pathologic features, including location in the right colon, a specific histologic pattern (cribriform or medullary), and lymphocytic infiltration. Identification of MSI in tumours can help guide treatment and prognostic decisions. Colon cancer patients with MSI tend to respond better to specific treatments. The high immunogenicity of MSI tumours may lead to increased T-lymphocyte infiltration and increased sensitivity to immunotherapy, such as immune checkpoint inhibitors (e.g. PD-1/PD-L1 inhibitors) [9]. Also, some studies suggest that MSI tumours may be less resistant to certain types of chemotherapy, such as 5-fluorouracil [12, 13]. MSI is also linked to specific inherited syndromes, such as Lynch syndrome. Identifying MSI in patients with a family history of cancer can lead to early identification of individuals at risk and implementation of prevention and early detection strategies [14]. Understanding the importance of MSI in colon cancer is critical to developing more personalised therapeutic approaches and effective early detection strategies.

On the other hand, saving and making the pathology laboratory more efficient is a relevant topic in medicine and biomedical research. Pathology laboratories play a crucial role in diagnosing and treating various diseases, providing detailed information about tissue and cell samples obtained from patients [15]. However, the pathology analysis process can be costly regarding time, human resources, and finances. Therefore, finding ways to save and improve efficiencies in these laboratories can positively impact healthcare and research. Performing a diagnostic test with IHC against 2 antibodies instead of 4 reduces the labour and financial burden on pathology laboratories, patients, and health systems.

This has already been done in colon cancer. A recent systematic review [6] shows that the evaluation of a test with 2 antibodies is very similar to the traditional one with 4, specifically showing a weighted percentage of 1.1% of reported cases with non-dimeric loss that would not have been identified using a 2-antibody approach. Considering the overall low rate of cases with non-dimeric loss, ($< 0.5\%$), the implementation of the 2-antibody test algorithm seems appropriate for both screenings to identify patients at increased risk of Lynch syndrome, as well as identifying patients who may benefit from checkpoint inhibition therapy. Using this 2-antibody testing algorithm will substantially reduce costs by 50% in all MSI cases while only causing a minimal delay in MMR status assignment in MSI cases. This systematic review has limitations, most related to heterogeneity between studies.

Our study attempted to reduce bias by using a reference standard as a control, performing IHC in a standardised and automated manner, and evaluated by pathologists with experience in gastrointestinal pathology, with objective cut-off points. In addition, to reduce selection and performance biases, the cases correspond to cases consecutively treated and studied over one year.

When evaluating a diagnostic test, it is essential to consider several biases that can influence the interpretation of the results and the precision of the test. The limitations of our study are that there may be a subtle selection bias because it was performed in

a national referral hospital, and that the evaluation of IHC in cases with heterogeneous expression in large surgical specimens and years after being performed may differ from the initial evaluation.

Finally, the 4-antibody panel should be used in cases with unusual staining patterns or where initial results using 2 antibodies are ambiguous (for example, isolated loss of PMS2 or MSH6 may still necessitate additional testing to confirm the underlying defect), but the 2-antibody approach is promising for routine diagnostics.

Conclusions

The results support the potential for a 2-antibody panel as a reliable and cost-effective alternative for MMR protein assessment in most scenarios. However, judicious application of the 4-antibody panel is recommended to address atypical findings and ensure diagnostic accuracy. Future studies should evaluate the clinical and cost-effectiveness of these approaches in broader populations and settings.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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Address for correspondence

Leonardo Saul Lino-Silva, MD
Instituto Nacional de Cancerologia de Mèxico
Mexico City, Mexico
e-mail: saul.lino.sil@gmail.com