Microsatellite Instability Is Insufficiently Used as a Biomarker for Lynch Syndrome Testing in Clinical Practice

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ABSTRACT

PURPOSE The pan-cancer presence of microsatellite instability (MSI)–positive tumors demonstrates its clinical utility as an agnostic biomarker for identifying immunotherapy-eligible patients. Additionally, MSI is a hallmark of Lynch syndrome (LS), the most prevalent cancer susceptibility syndrome among patients with colorectal and endometrial cancer. Therefore, MSI-high results should inform germline genetic testing for cancer-predisposing genes. However, in clinical practice, such analysis is frequently disregarded.

METHODS A next-generation sequencing (NGS)–based technique was used for MSI analysis in 4,553 patients with various tumor types. Upon request, somatic *BRAF* gene analysis was conducted. In addition, hereditary testing of cancer-associated genes was performed in MSI-high cases using a capture-based NGS protocol. *MLH1* promoter methylation analysis was conducted retrospectively in patients with colorectal and endometrial cancer to further investigate the origin of MSI at the tumor level.

RESULTS The MSI positivity rate for the entire cohort was 5.27%. Endometrial, gastric, colorectal, urinary tract, and prostate cancers showed the highest proportion of MSI-high cases (15.69%, 8.54%, 7.40%, 4.55%, and 3.19%, respectively). A minority of 45 patients (22.73%) among the MSI-high cases underwent germline testing to determine whether the mismatch repair pathway deficiency was inherited. 24.44% of those who performed the genetic test carried a pathogenic variant in an LS-associated gene. Three MSI-high individuals had non-LS gene alterations, including *BRCA1*, *BRCA2*, and *CDKN2A* pathogenic variants, indicating the presence of non–LS-associated gene alterations among MSI-high patients.

CONCLUSION Although MSI analysis is routinely performed in clinical practice, as many as 77% of MSI-high patients do not undergo LS genetic testing, despite international guidelines strongly recommending it. *BRAF* and *MLH1* methylation analysis could shed light on the somatic origin of MSI in 42.50% of the MSI-high patients; however, *MLH1* analysis is barely ever requested in clinical practice.

ACCOMPANYING CONTENT

Data Supplement

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INTRODUCTION

The mismatch repair pathway (MMR) is one of the primary DNA repair mechanisms, responsible for maintaining genomic stability and correcting DNA mismatches. MMR-deficient cells are susceptible to mismatch errors during replication in the microsatellite regions.[1] Tumors exhibiting microsatellite instability (MSI) are characterized as MSI-high. The MSI-high phenotype has been observed in a wide variety of tumor types, with higher prevalence in colorectal cancer (CRC) and endometrial carcinoma (EC).[2,3]

Lynch syndrome (LS), the most prevalent inherited CRC syndrome, with an autosomal dominant inheritance pattern, has been linked to MMR deficiency and occurs in 3% of patients with CRC and 6% of patients with EC, but it is also associated with other tumor types.[1,4-6] It is caused by inactivating germline variants in one of the primary MMR genes (*MSH2*, *MLH1*, *MSH6*, *PMS2*, and *EPCAM*).[1,6] MMR

CONTEXT

Key Objective

The objective of this study is to examine the prevalence of microsatellite instability (MSI) in Greek patients with cancer and determine the proportion of MSI-high patients referred for germline testing as indicated by international guidelines.

Knowledge Generated

MSI analysis has pan-cancer applicability, as evidenced by the prevalence of positive cases in a variety of tumor types. In addition, the clinical value of MSI testing as a screening tool to identify patients with a higher likelihood of a germline etiology of tumor development is not well recognized. Consequently, a significant percentage of individuals with Lynch syndrome remain undiagnosed.

Relevance

MSI-positive patients constitute a high-risk group for hereditary cancer; therefore, it is critical to raise awareness of the value of genetic testing in this population immediately.

deficiency is observed in 90% of individuals with LS but it can also be detected in sporadic malignancies.[7-10]

Additionally, in dMMR/MSI-H CRC, the identification of a *BRAF V600E* gene mutation or *MLH1* gene promoter hypermethylation reveals the sporadic nature of the MMR deficiency, whereas LS should be suspected in the case of a negative result for both tests.[11,12]

The clinical utility of MSI analysis has increased since the association between MSI-high malignancies and anti–PD-L1 treatment response was established.[13-15] The prevalence of MSI varies among tumor types, with the rates ranging from 10% to 15% in CRC and EC to 0% in other tumor histologic types, including lung cancer, thereby limiting its impact on clinical decision making for certain malignancies.[2,3,16] Significantly, tumor MSI analysis could inform patient selection for inherited cancer susceptibility testing.[11,17,18]

This study aims to determine the prevalence of MSI among Greek patients with cancer. Additionally, we will examine the proportion of MSI-high patients referred for germline testing as indicated by international guidelines. Finally, the influence of additional factors, such as the patient's age and tumor histology, on the clinicians' decision for germline testing requests will be evaluated.

METHODS

Patients' Selection

In this study, 4,553 patients with metastatic cancer were referred by their treating oncologist for MSI analysis from January 2020 to April 2023. All the patients who participated in the study provided written informed consent. This study was approved by the Ethics Committee of the General Hospital of Volos. All treatment physicians were informed about the utility of *BRAF* analysis in patients with CRC and hereditary cancer predisposition testing in MSI-high patients, and the tests were performed upon request. The detailed study design can be seen in the Data Supplement (Fig S1).

Tumor Biomarkers Analysis

The Ion AmpliSeq Microsatellite Instability Panel (Thermo Fisher Scientific, Waltham, MA) was used for next-generation sequencing (NGS)–based MSI analysis in DNA extracted from formalin-fixed paraffin-embedded tumor biopsies as described previously.[19] *BRAF* analysis was conducted using a custom 23-gene Ion AmpliSeq panel (Thermo Fisher Scientific).[20] Sequencing was performed on an Ion GeneStudio S5 Prime System NGS platform (Thermo Fisher Scientific). The methylation pattern in the promoter of the *MLH1* gene was determined by methylation-specific polymerase chain reaction as previously described.[21]

NGS-Based Germline Genetic Testing

DNA extracted from peripheral blood was analyzed using a capture-based approach with custom-designed probes (KAPA HyperExplore Max 3 Mb T1, NimbleGen, Roche) targeting all coding exons and 20 bp of flanking intronic regions of 52 genes involved in hereditary predisposition to cancer. Sample preparation was performed according to the SeqCap EZ HyperCap Workflow (Roche NimbleGen, Pleasanton, CA) as previously described.[19] Sequencing was performed on the DNBSEQ-G400 NGS platform (MGI Tech, Beishan Industrial Zone, Shenzhen, PR China).

Statistical Analysis

SPSS (version 20, IBM SPSS Statistics, Armonk, NY) was used to compare the age of cancer diagnosis and the implementation of germline testing as well as the age and detection of a germline mutation using the *t*-test for independent means. A *P* value of <.05 was considered

statistically significant. The Plotly.js charting library was used to generate box plots.

RESULTS

A total of 4,553 patients were referred by their treating physician for tumor MSI analysis. Approximately half of the cases (2,289) consisted of CRCs. The remaining tumor types included those with known MSI occurrences, such as gastric, endometrial, breast, and prostate cancers, as well as tumors where MSI is not typically detected, such as pancreatic, lung, ovarian, breast, and brain tumors (Fig 1, Data Supplement).

The increased awareness of the value of MSI testing as a predictive marker for immune-checkpoint Inhibitors (ICI) has led to an increase in the number of patients undergoing such analysis, from 1,016 in 2020 to 1,370 in 2021 and 1,571 in 2022, with 571 patients already tested in the first 4 months of 2023.

Two hundred forty (5.27%) patients exhibited MSI, with an average age of cancer diagnosis of 67 years. MSI-high patients comprised 57.92% female and 42.08% male. The occurrence varied among different histologic types. MSI was detected in 7.40% of patients with CRC, and in 15.69% of endometrial cancer tumors; the percentage was also high for gastric cancer, tumors of the urinary tract, prostate cancer, and tumors of unknown origin. Brain, lung, and biliary tract tumors, as well as sarcomas, did not exhibit MSI (Fig 2). The pan-cancer presence of MSI-positive tumors supports its use as an agnostic biomarker, suggesting the utility of this analysis in immunotherapy-considering patients.

MSI should also serve as an indicator of a possible hereditary cancer syndrome. In 42 patients with CRC, a *BRAF* V600E mutation was also present, which indicates that the MMR deficiency has a somatic cause. This variant was identified in 24.85% of the MSI-high patients with CRC. No BRAF mutation was detected in the patients with EC tested.

Excluding these cases, 198 MSI-high patients were eligible for genetic testing with a hereditary cancer panel. Nevertheless, such a test was requested for only 45 of the eligible patients, accounting for 22.73% of the MSI-high cases. Patients with a germline test tended to have a lower median age at disease diagnosis of 59 years compared with 66 years for those without germline genetic analysis (Fig 3). Age and test implementation were highly correlated, indicating that the age of disease onset was a main criterion for genetic analysis requisition from the treating physician (t = 3.12112, P = .001037). Patients with colorectal (28), endometrial (nine), gastric (four), ovarian (two), breast (one), and

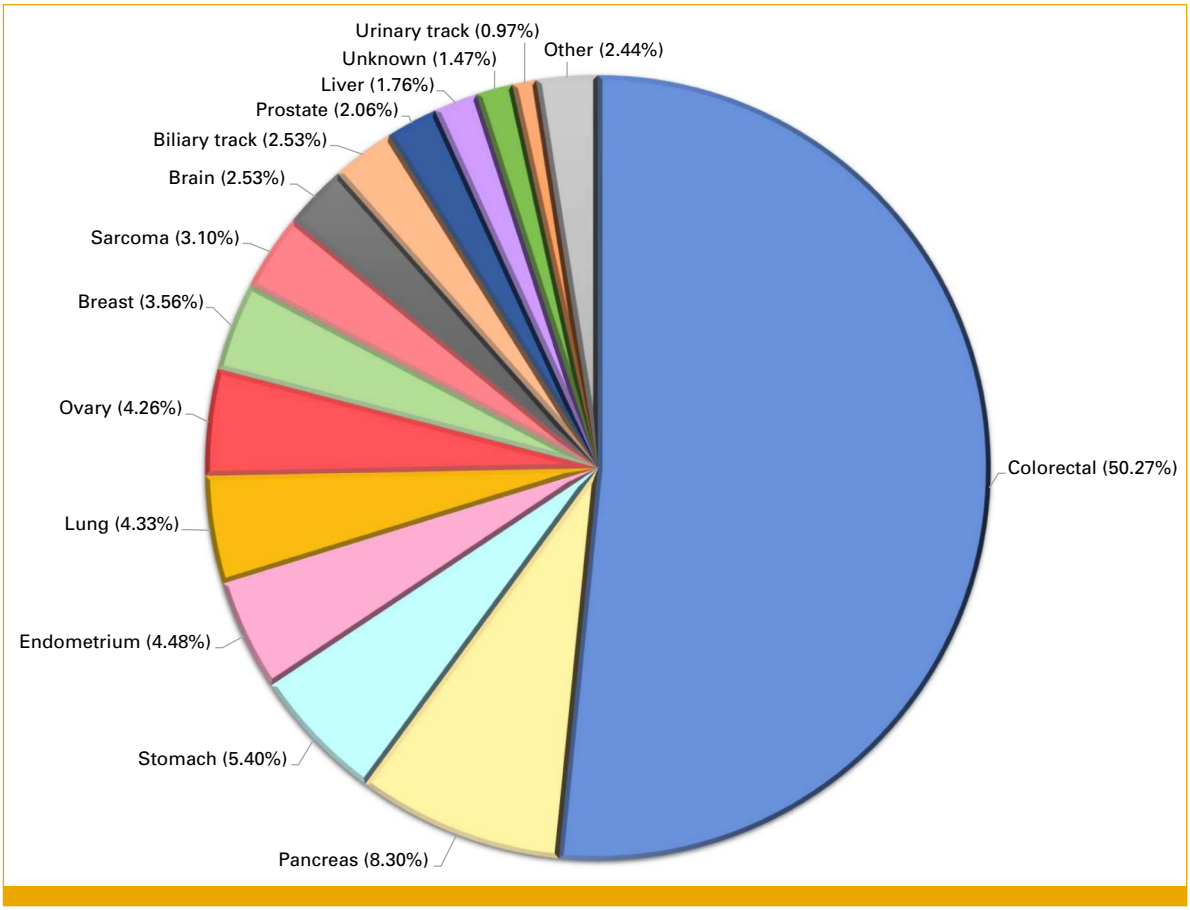

FIG 1. Tumor types of patients who were referred for microsatellite instability analysis by their respective treating physicians.

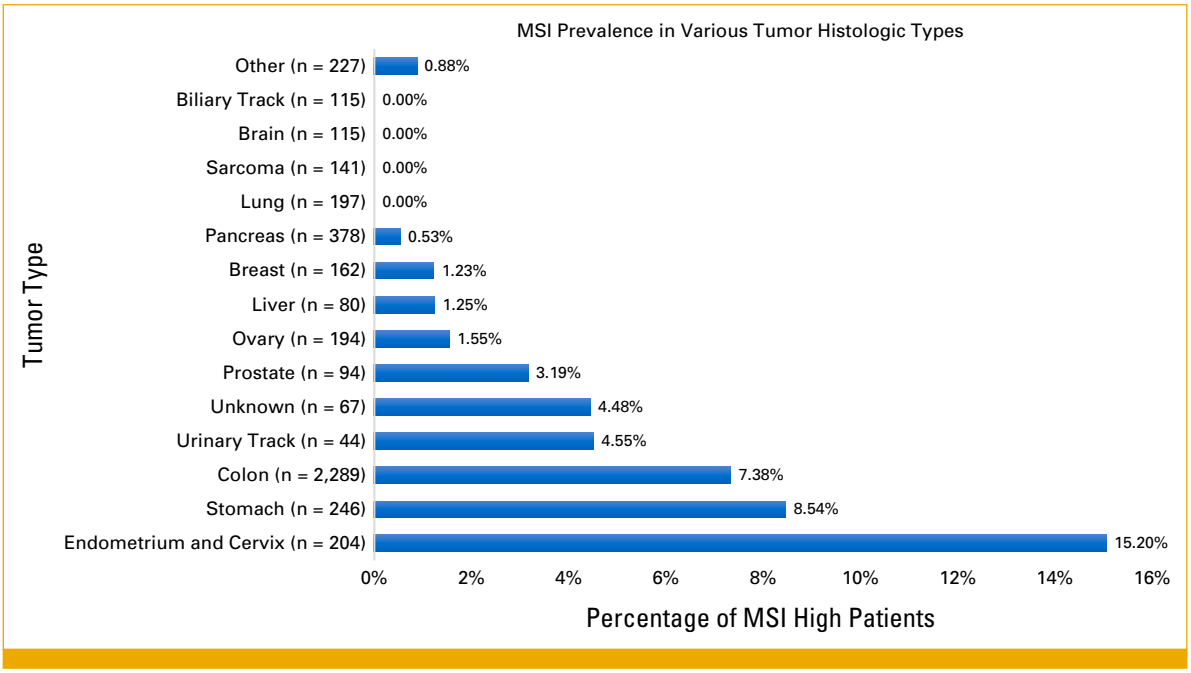

FIG 2. MSI prevalence in various tumor histologic types in the cohort of 4,553 patients with solid tumors. MSI, microsatellite instability.

gallbladder (one) malignancies underwent germline analysis. Requests for testing differed according to the tumor's histologic type. A discrete percentage of 60% (3/5) of MSI-high patients with breast and ovarian cancer underwent the analysis, predominantly because of the elevated germline analysis surveillance in these tumor types. Among other histologies, a smaller proportion of MSI-high patients underwent germline analysis, including 28.13% (9/32) of MSI-high patients with endometrial cancer, 22.05% (28/127) of MSI-high CRC patients with *BRAF* wild-type, and 19.05% (4/21) of MSI-high patients with gastric cancer. Only 2.94% of MSI-high patients with other tumor types received a germline analysis.

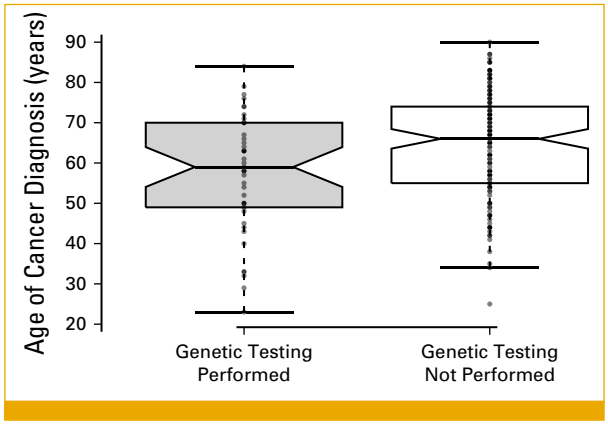

FIG 3. Boxplots displaying the age distribution and median age of disease diagnosis of microsatellite instability-high patients who requested genetic testing with an inherited cancer gene panel versus those who did not.

Eleven patients diagnosed with colorectal or endometrial cancer (24.44%) had a positive germline MMR gene result. In addition, an endometrial cancer patient with both *MSH2* and *BRCA2* variants and a CRC patient with both *MLH1* and *CDKN2A* variants harbored double mutations. MSI was also observed in one patient with breast cancer carrying a germline variant of the *BRCA1* gene. Additionally, a variant of uncertain significance was detected in 16/45 (35.55%) of the patients (Fig 4). Individuals with a pathogenic or likely pathogenic variant had a median cancer diagnosis of 48.5 years, whereas those without a clinically significant finding had a median age of 60 years. Nonetheless, 41.67% (5/12) of the patients with a pathogenic germline variant identified had a disease onset age of over 50 years. The presence of germline variants in older MSI-high individuals indicates that the analysis is also informative in this age group, as indicated by international guidelines.

Since *MLH1* methylation is an established origin of somatically driven MSI instability in CRC and EC, we conducted a retrospective analysis of the MLH1 status in BRAF wild-type patients with these tumor types who either did not undergo germline analysis or were MMR germline-negative. *MLH1* hypermethylation was present in 58 (58.58%) of the 99 patients with CRC and 20 (86.96%) of the 23 patients with EC who did not perform germline testing. Among the 26 germline MMR-negative cases, *MLH1* methylation was present in all seven EC but only in 11/19 (57.89%) patients with CRC. These results indicate that although MLH1 methylation appears to be the most prevalent mechanism of somatic MMR dysregulation in EC, alternative mechanisms,

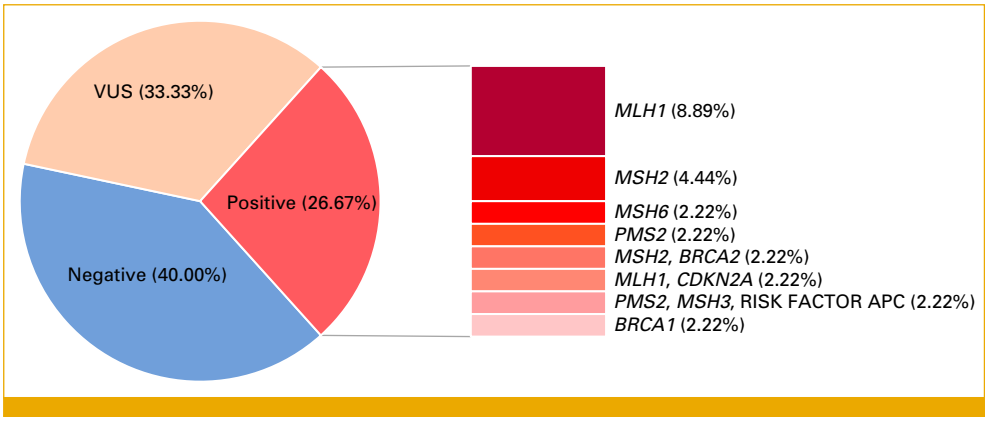

FIG 4. Germline testing outcomes and distribution of the pathogenic variants in the 45 microsatellite instability-high individuals who underwent a germline analysis. VUS, variant of uncertain significance.

such as somatic MMR or other cancer gene alterations, are also implicated in CRC.[22]

DISCUSSION

The utility of MSI analysis as a biomarker for the selection of patients eligible for ICI treatment has been well documented, thereby enhancing its implementation in clinical practice.[15,17,23-27] Consequently, the number of patients evaluated for MSI continues to rise, providing a greater understanding of its prevalence in various tumor types. Since its approval in 2017 as an ICI predictive biomarker, the number of patients undergoing such a test has doubled in our laboratory, from <450 patients per year in 2016 to over 1,500 patients per year in 2022.

MSI instability was identified in 5.19% of the cases within a cohort of 4,553 patients with cancer of Greek origin. Consistent with previous reports, the highest prevalence in MSI-high patients was detected in endometrial cancer, gastric cancer, and CRC, accounting for 15.69%, 8.54%, and 7.40% of cases, respectively.[2]

In addition, several other tumor types showed modest but substantial percentages of positivity. These findings suggest that MSI analysis of various malignancies is warranted, particularly for neoplasms affecting the urinary tract, prostate, and those with an uncertain origin. The cohort under investigation did not exhibit MSI positivity in brain tumors, cholangiocarcinoma, lung cancer, and sarcomas. Nonetheless, other studies have shown that although uncommon, MSI can also be detected in these tumors, so testing is advised.[2,3]

The utility of MSI analysis is also essential for identifying patients with a cancer-related pathogenic germline variant.[10,23] Germline testing for LS was performed in only 45 of the 240 MSI-high patients. In 42 cases, exclusion from germline testing was justifiable because of the presence of a *BRAF* mutation associated with somatic MMR inactivation in CRC. Therefore, only 45 of the 198 patients (22,73%) eligible for germline analysis were referred by the treating physician for the test. Contrary to the current guidelines, which recommend germline testing for patients with colorectal, endometrial, ovarian, high-grade prostate, and pancreatic adenocarcinomas, this has not been the case.

MLH1 methylation analysis is another method to evaluate the necessity of germline testing since its presence indicates a somatic etiology of the MSI, but it is almost never requested in clinical practice. Retrospective evaluation of the *MLH1* methylation could exclude the germline origin of MSI in an additional 96 patients with CRC and EC diagnosis. Hence, 102 of the 240 (42.50%) patients had a confirmed somatic origin of the MSI, while 57.50% (138/240) of the patients would be eligible for germline testing, considering both *BRAF* and *MLH1* methylation analysis. This is in accordance with previous studies indicating the necessity of *BRAF* and *MLH1* methylation analysis in patients with CRC and of *MLH1* methylation in those with EC.[12,28]

Moreover, current evidence supports germline genetic testing for LS in patients with MSI-high/MMR-deficient tumors, independently of the cancer type or family history.[29] According to a recent report, germline testing for cancer susceptibility genes should be implemented universally as nearly equivalent percentages of inherited alterations are detected in patients with cancer who adhere to genetic testing guidelines and those who do not (7.4% and 8.1%, respectively).[30] In addition, it is well known that patients with CRC may be carriers of non–LS-inherited variants.[5] However, as many as 6.3% of those with an MMR gene–inherited alteration could be missed using tumor MMR analysis only.[31] On the basis of these and other studies, evidence is accumulating in favor of germline testing in all patients with solid tumors.[32]

In our cohort, however, 77% of MSI-high patients did not undertake a test for LS-associated inherited mutations. In addition, unlike breast cancer, inherited CRC is underdiagnosed

as the number of patients who undergo germline testing appears to be substantially lower. The surveillance of hereditary breast and ovarian cancer syndrome has been intensified in Greece, as evidenced by the referral of over 5,000 individuals with a personal and family history of breast cancer for germline analysis in our laboratory over the past 3 years. By contrast, the number of referrals for individuals diagnosed with CRC in the past 3 years was limited to 120, although it is the third most prevalent malignancy. During the same time frame, 4,553 MSI tests were requested, indicating that somatic testing surveillance is considerably more extensive. Therefore, it appears that <3% (1 in every 37 cases) of patients with CRC undergo testing for germline alterations. In accordance with a recent study indicating suboptimal rates of MSI/IHC screening and germline genetic testing adoption in patients diagnosed with CRC, despite universal eligibility, our findings support such a hypothesis.[33]

Moreover, it has been demonstrated that a familial or personal CRC history is not predictive of CRC patients with an inherited cancer susceptibility syndrome including LS.[5] In addition, a study involving more than 15,000 patients with over 50 categories of cancer revealed that LS is detectable in 16% of MSI-H patients. Half of the LS patients with MSI-high tumors had histologic types other than CRC or EC. Moreover, on the basis of personal/family history, 45% of these patients with non-CRC/EC tumors did not meet LS genetic testing criteria. Therefore, it is recommended that all patients with MSI-high phenotype, independently of cancer type, should undergo laboratory genetic testing for LS.[29]

Despite these data, it appears that age at disease diagnosis was the primary criterion for selecting patients to undergo germline analysis. In our study, the mean age of disease diagnosis for patients who requested germline testing was 59 years, compared with 66 years for those who did not undergo germline testing. This criterion will undoubtedly result in a higher germline variant positivity rate, yet it is also stringent enough to exclude many patients with inherited mutations. In our cohort, 41.67% of patients with germline pathogenic variants were older than 60 years (range, 61-77 years). The analysis provides information regarding the patient's current condition and the likelihood of developing other LS-associated cancers, allowing the implementation of prophylactic surveillance to prevent their occurrence.[6] Moreover, the multigene analysis permits the identification of pathogenic variants in non–LS-associated genes. For example, a *BRCA1*-positive woman was identified despite her breast cancer diagnosis at age 66 years and the absence of a family history of the disease. Beyond that, the presence of double gene alterations in two patients may enhance surveillance for a variety of other tumor types in addition to the primary tumor diagnosis. The *CDKN2A*/*MLH1*-positive patient with CRC should also undergo preventive screening tests for pancreatic cancer and melanoma, while the *BRCA1*/*MSH2*-positive patient with endometrial cancer should also be screened for tumors associated with the hereditary breast and ovarian cancer syndrome.[34-36] Tumor analysis of double heterozygotes revealed a biallelic inactivation of the MMR gene with germline alteration in both samples (as defined by a variant allele frequency of >85%), whereas the other alteration was monoallelic. This finding suggests that the presence of MSI in these patients was driven by double somatic hits in the MMR genes.

Furthermore, it is essential to consider the clinical utility of the genetic analysis for the patient's relatives, as genetic counseling may provide them with crucial information regarding their own susceptibility to cancer and the necessary preventive measures.[31] Therefore, if a pathogenic variant at a high-risk gene for cancer predisposition is identified, cascade analysis of at-risk relatives for the variant identified in the proband should be offered. A positive outcome could result in appropriate monitoring and management. Conversely, unnecessary concern can be avoided in case the tested relative does not carry the pathogenic variant detected in the proband.

The dissemination of information on the importance of germline analysis, particularly in patients with high microsatellite instability (MSI-high), has been a longstanding focus of our laboratory. To achieve this aim, conferences and educational sections for physicians on LS screening have been organized. Additionally, physicians are always made aware of the need for such analysis in the event of a high MSI result. In most cases, however, they respond that the patient's priority is to identify the most effective treatment and that he or she is unwilling to undergo a germline analysis because of the worry of receiving a positive result and the high analysis cost. However, lowering the test's price had little impact on the quantity of tests requested. Therefore, the annual number of MSI-high patients undertaking germline testing has increased marginally from eight patients in 2020 to 13 in 2021 and 16 in 2022.

Therefore, it appears that patients remain unaware of the benefits of hereditary analysis for most tumor types, except for breast cancer, where the Angelina Jolie effect has led to an increase in genetic testing.[37] Therefore, it is essential to enhance the public's awareness about the necessity of preventive measures against hereditary cancer and the value of genetic analysis. To achieve this objective, implementing of a comprehensive national strategy is vital, requiring the participation of authorities within the public health system and medical organizations. These entities will be responsible for effectively disseminating information to the general population about the potential hazards associated with undiagnosed inherited cancer susceptibility. In Greece, for example, the national health system reimburses the cost of germline BRCA1/2 testing for all patients with ovarian cancer, patients with breast cancer younger than 45 years, and their relatives. Germline testing reimbursement should also be extended to MSI-high patients. Doing so will render such analysis accessible to patients who cannot afford it. Additionally, it will emphasize its significance to those in a dilemma.

This research has a number of limitations. We do not have data on the proportion of patients with metastatic cancer who undertake MSI or LS analysis; we can only report the decreased proportion of MSI-high patients who undergo germline analysis for LS. Similarly, we cannot exclude the possibility that patients who did not perform germline analysis in our laboratory will pursue such testing at an alternative facility or in the future. Actually, the median time between MSI analysis result release and germline analysis request varied from 0 to 748 days (median, 45.42 days). Another limitation is the lack of somatic analysis data from patients with MSI-high tumors to ascertain other potential somatic origins of the MSI presence.

In conclusion, our study indicates that the implementation of MSI analysis has broad applicability in the context of diverse malignancies, as demonstrated by the presence of positive cases in a wide range of tumor types. In addition, it designates that, in clinical practice, MSI testing is used primarily as a predictive biomarker for ICI response evaluation. Underappreciated, however, is its potential as a screening tool for identifying patients with a higher likelihood of a germline origin of tumor manifestation. More than half of the MSI-high cases without a known somatic etiology of MSI, and therefore eligible for germline testing, do not undergo such testing, resulting in misdiagnosis of LS and other inherited cancer syndromes.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Pećina-Šlaus N, Kafka A, Salamon I, et al: Mismatch repair pathway, genome stability and cancer. Front Mol Biosci 7:122, 2020
2. Bonneville R, Krook MA, Kautto EA, et al: Landscape of microsatellite instability across 39 cancer types. JCO Precis Oncol 10.1200/PO.17.00073
3. Hause RJ, Pritchard CC, Shendure J, et al: Classification and characterization of microsatellite instability across 18 cancer types. Nat Med 22:1342-1350, 2016
4. Ring KL, Bruegl AS, Allen BA, et al: Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort. Mod Pathol 29:1381-1389, 2016
5. Yurgelun MB, Kulke MH, Fuchs CS, et al: Cancer susceptibility gene mutations in individuals with colorectal cancer. J Clin Oncol 35:1086-1095, 2017
6. Møller P, Seppälä TT, Bernstein I, et al: Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: A report from the Prospective Lynch Syndrome Database. Gut 67: 1306-1316, 2018
7. Peltomäki P: Deficient DNA mismatch repair: A common etiologic factor for colon cancer. Hum Mol Genet 10:735-740, 2001
8. Li X, Liu G, Wu W: Recent advances in Lynch syndrome. Exp Hematol Oncol 10:37, 2021
9. Kang SY, Kim DG, Ahn S, et al: Comparative analysis of microsatellite instability by next-generation sequencing, MSI PCR and MMR immunohistochemistry in 1942 solid cancers. Pathol Res Pract 233:153874, 2022
10. Peltomäki P, Nyström M, Mecklin J-P, et al: Lynch syndrome genetics and clinical implications. Gastroenterology 164:783-799, 2023
11. Gupta S, Weiss JM, Burke CA, et al: NCCN Guidelines Version 1.2023 Genetic/Familial High-Risk Assessment: Colorectal Continue. 2023. https://www.nccn.org
12. Adar T, Rodgers LH, Shannon KM, et al: A tailored approach to BRAF and MLH1 methylation testing in a universal screening program for Lynch syndrome. Mod Pathol 30:440-447, 2017
13. Luchini C, Bibeau F, Ligtenberg MJL, et al: ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: A systematic review-based approach. Ann Oncol 30:1232-1243, 2019
14. Vikas P, Messersmith H, Compton C, et al: Mismatch repair and microsatellite instability testing for immune checkpoint inhibitor therapy: ASCO endorsement of College of American Pathologists guideline. J Clin Oncol 41:1943-1948, 2023
15. Diaz LA, Le D, Maio M, et al: Pembrolizumab in microsatellite instability high cancers: Updated analysis of the phase II KEYNOTE-164 and KEYNOTE-158 studies. Ann Oncol 30:v475, 2019
16. Le DT, Durham JN, Smith KN, et al: Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 357:409-413, 2017
17. Benson AB, Al-Hawary MM, Azad N, et al: NCCN Guidelines Version 2.2023 Colon Cancer Continue NCCN Guidelines Panel Disclosures. 2023. https://www.nccn.org
18. Monahan KJ, Bradshaw N, Dolwani S, et al: Guidelines for the management of hereditary colorectal cancer from the British Society of Gastroenterology (BSG)/Association of Coloproctology of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG). Gut 69:411-444, 2020
19. Tsaousis GN, Papadopoulou E, Apessos A, et al: Analysis of hereditary cancer syndromes by using a panel of genes: Novel and multiple pathogenic mutations. BMC Cancer 19:535, 2019
20. Tsoulos N, Papadopoulou E, Metaxa-Mariatou V, et al: Tumor molecular profiling of NSCLC patients using next generation sequencing. Oncol Rep 38:3419-3429, 2017
21. Hinrichsen I, Kemp M, Peveling-Oberhag J, et al: Promoter methylation of MLH1, PMS2, MSH2 and p16 is a phenomenon of advanced-stage HCCs. PLoS One 9:e84453, 2014
22. Lefol C, Sohier E, Baudet C, et al: Acquired somatic MMR deficiency is a major cause of MSI tumor in patients suspected for "Lynch-like syndrome" including young patients. Eur J Hum Genet 29: 482-488, 2021
23. Colle R, Lonardi S, Cachanado M, et al: *BRAF* V600E/*RAS* mutations and Lynch syndrome in patients with MSI-H/dMMR metastatic colorectal cancer treated with immune checkpoint inhibitors. Oncologist 28:771-779, 2023
24. Fabrizio DA, George TJ, Dunne RF, et al: Beyond microsatellite testing: Assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. J Gastrointest Oncol 9:610-617, 2018
25. Quintanilha JCF, Graf RP, Fisher VA, et al: Comparative effectiveness of immune checkpoint inhibitors vs chemotherapy in patients with metastatic colorectal cancer with measures of microsatellite instability, mismatch repair, or tumor mutational burden. JAMA Netw Open 6:e2252244, 2023
26. Le DT, Kim TW, Van Cutsem E, et al: Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. J Clin Oncol 38:11-19, 2020
27. Huang RSP, Haberberger J, Severson E, et al: A pan-cancer analysis of PD-L1 immunohistochemistry and gene amplification, tumor mutation burden and microsatellite instability in 48,782 cases. Mod Pathol 34:252-263, 2021
28. Buchanan DD, Tan YY, Walsh MD, et al: Tumor mismatch repair immunohistochemistry and DNA *MLH1* methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. J Clin Oncol 32:90-100, 2014
29. Latham A Srinivasan P, Kemel Y, et al: Microsatellite instability is associated with the presence of Lynch syndrome pan-cancer. J Clin Oncol 37:286-295, 2019
30. Esplin ED, Nielsen SM, Bristow SL, et al: Universal germline genetic testing for hereditary cancer syndromes in patients with solid tumor cancer. JCO Precis Oncol 10.1200/PO.21.00516
31. Pearlman R, Frankel WL, Swanson BJ, et al: Prospective statewide study of universal screening for hereditary colorectal cancer: The Ohio Colorectal Cancer Prevention Initiative. JCO Precis Oncol 10.1200/PO.20.00525
32. Subbiah V, Kurzrock R: Universal germline and tumor genomic testing needed to win the war against cancer: *Genomics Is the diagnosis*. J Clin Oncol 41:3100-3103, 2023
33. Moretz C, Byfield SD, Hatchell KE, et al: Comparison of germline genetic testing before and after a medical policy covering universal testing among patients with colorectal cancer. JAMA Netw Open 5:E2238167, 2022
34. Pauley K, Khan A, Kohlmann W, et al: Considerations for germline testing in melanoma: Updates in behavioral change and pancreatic surveillance for carriers of CDKN2A pathogenic variants. Front Oncol 12:837057, 2022
35. Ulanja MB, Moody AE, Beutler BD, et al: Early-onset pancreatic cancer: A review of molecular mechanisms, management, and survival. Oncotarget 13:828-841, 2022
36. Dwyer M: NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic. 2023. https://www.nccn.org
37. Liede A, Cai M, Crouter TF, et al: Risk-reducing mastectomy rates in the US: A closer examination of the Angelina Jolie effect. Breast Cancer Res Treat 171:435-442, 2018