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WORD COUNT 2757
TIME SUBMITTED 23-MAR-2023 02:05PM
PAPER ID 97916315

Name of Journal: *World Journal of Clinical Cases*

Manuscript NO: 83586

Manuscript Type: CASE REPORT

Discrepancy among MSI detection methodologies in non-colorectal cancer – report of three cases

Immunohistochemistry and Microsatellite Instability

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Abstract

BACKGROUND

Microsatellite instability (MSI) is a predictive biomarker for cancer immunotherapy. The tumor-agnostic nature of MSI makes it a denominator for immunotherapy in several solid tumors. It can be assessed using next-generation sequencing (NGS), fluorescent multiplex polymerase chain reaction (PCR), and immunohistochemistry (IHC).

CASE SUMMARY

Here, we report three cases with discordant MSI results detected using different methods; a cholangiocellular carcinoma case revealed proficient MMR by IHC but MSI-H by liquid NGS, a cervix cancer case with deficient MMR (dMMR) by IHC, MSS (microsatellite stable) by PCR but MSI-H by NGS and lastly, endometrial cancer case found pMMR by IHC but MSI-H by NGS.

CONCLUSION

IHC for MMR (mismatch repair) status is the first choice due to its several advantages. However, in case of indeterminate IHC results, molecular testing by MSI-PCR is preferred. Recently, NGS-based MSI assays are being widely used to detect MSI high tumors. All three methods have high accuracy; however, the inconsistencies between them may lead to misdiagnosis.

Key Words: Discordance; Immunohistochemistry; Microsatellite instability; Next-generation sequencing

Şenocak Taşçı E, Yıldız İ, Erdamar S, Özer L. Discrepancy among MSI detection methodologies in non-colorectal cancer – report of three cases. *World J Clin Cases* 2023; In press

Core Tip: Microsatellite instability (MSI), a predictive biomarker for cancer immunotherapy can be assessed using next-generation sequencing (NGS), fluorescent multiplex polymerase chain reaction (PCR), and immunohistochemistry (IHC). Even though IHC for mismatch repair status is the first choice, in case of indeterminate IHC results, molecular testing by MSI-PCR is preferred. Recently, NGS-based MSI assays are also being widely used. Although all methods have high accuracy, they may have inconsistent results leading to misdiagnosis.

INTRODUCTION

In the era of immunotherapy, microsatellite instability (MSI) is a key biomarker of genetic alteration. It is indicated by high number of mutations within microsatellites, which are repeat sequences of 1–9 nucleotides[1]. While the DNA mismatch repair (MMR) system can correct DNA replication errors in normal tissues, the loss of function or lack of MMR genes in tumor cells causes MSI[2]. Thus, MSI is an important factor in tumor development and its incidence correlates positively to survival[3].

MSI can be distinguished into three types: high (MSI-H), low (MSI-L), and stable (MSS)[4]. Lately, MSI has been identified in several cancer types[5]. The recent American Society of Clinical Oncology provisional guidelines on somatic mutations in metastatic and locally advanced cancer recommends the evaluation of mismatch repair deficiency status, as MSI is accepted as a tumor-agnostic factor in all patients who are potential candidates for immunotherapy[6].

The most widely used methods for MSI assessment are next-generation sequencing (NGS), fluorescent multiplex polymerase chain reaction (PCR), and immunohistochemistry (IHC)[1]. IHC is the gold standard method due to its easy access, high sensitivity, and practical nature. It detects the expression of MMR proteins (MLH1, PMS2, MSH2, and MSH6) in tumor tissues[7]. NGS-based multiplex gene assay, approved for use in all solid tumors, can indirectly measure mismatch repair status using DNA extracted from formalin-fixed paraffin-embedded tissue specimens, where deficient MMR (dMMR) tumors usually have a hypermutated phenotype[8]. Finally, PCR

is a molecular approach that can be carried out on a tumor DNA, measuring the mismatch repair protein apparatus functionality[9]. In case of indeterminate MSI status with IHC, i.e. if loss of only one heterodimer unit is present. Two reference panels of PCR, Bethesda and pentaplex, were designed for colorectal cancer (CRC); and have shown poor performance in other cancer types[10]. Despite the high accuracy of these methods (94.6%, 99.9%, and 89–95% for PCR, NGS, and IHC, respectively), the inconsistency between them may result in misdiagnosis[11-13]. The specific guidance regarding preferred methodology is still lacking.

Here, we report a cholangiocellular carcinoma case revealing proficient MMR(pMMR) by IHC but MSI-H by liquid NGS, a cervix cancer case that was dMMR by IHC, MSS by PCR but MSI-H by NGS and an endometrium cancer case found to be pMMR by IHC but MSI-H by NGS.

CASE PRESENTATION – 1

Chief complaints

A 43-year-old female patient with a history of Klatskin tumor was referred to our clinic with progressive disease.

History of present illness

Progression of the present illness was found during treatment response evaluation 2 weeks ago.

History of past illness

She had presented with jaundice, epigastric pain, itching, and weakness to her doctor in 2018, and her abdominal ultrasonography revealed a mass near the liver. Magnetic resonance imaging (MRI) of the abdomen confirmed obstruction due to tumor confluence of the bile ducts. Secondary to the mechanical obstruction, there was external drainage of the bile ducts from the right anterior and posterior sections of the liver. She underwent a left hemi hepatectomy with total caudal lobectomy, cholecystectomy, and extended

lymphadenectomy. The pathology revealed moderately differentiated adenocarcinoma, $CK7^+/CK20^-/CK17^+$, consistent with cholangiocarcinoma, and thus, stage IIA disease. IHC revealed PD-L1 combined positive score of 0 and MSS disease. She received six cycles of adjuvant gemcitabine-cisplatin treatment. During follow-up, in 2020, computed tomography (CT) demonstrated recurrence of the underlying disease with predominancy of peritoneal carcinomatosis, after which she was again initiated on gemcitabine and cisplatin. After five treatment cycles, cisplatin intolerance developed, and treatment was continued with capecitabine and gemcitabine. The response evaluation CT revealed progression of the underlying disease with an increase in the size of the known lesions, ascites, and pleural effusion.

Personal and family history

Significant family or personal history was not detected.

Physical examination

Vital signs were in normal range. No abnormalities were found during systemic examination.

Laboratory examinations

Carbohdrate antigen 19-9 level was elevated (1200 U/mL). Other analyses were in normal range.

Imaging examinations

Positron emission tomography (PET/CT) was carried out for re-staging and it revealed development of new hypermetabolic lesions in the left supraclavicular region, L2 corpus, and peritoneum.

FURTHER DIAGNOSTIC WORK-UP

NGS (FoundationOne CDx, 2021) was recommended for detailed molecular analysis instead of IHC and PCR. The molecular results from the surgical specimen revealed *STK11* and *ARID1A* mutations and MSI-H disease (Figure 1).

FINAL DIAGNOSIS

The final diagnosis was stage IV Klatskin tumour.

TREATMENT

FOLFIRINOX chemotherapy was initiated with palliative radiotherapy.

OUTCOME AND FOLLOW-UP

She was lost to follow-up, months after admission to our hospital.

CASE PRESENTATION -2

Chief complaints

A 29-year-old female patient with history of locally advanced cervical cancer was referred to our clinic for a second opinion.

History of present illness

Progression of cervical cancer was found during a screening a week ago.

History of past illness

She was first diagnosed in 2019 and had received radical chemoradiotherapy. Local recurrence occurred in 2020. She received four cycles of carboplatin-paclitaxel and underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. Topotecan and bevacizumab were administered in June 2021 due to disease progression; however, a ureterovaginal fistula developed, for which she underwent surgery.

Personal and family history

Significant family or personal history was not detected.

Physical examination

Vital signs were as follows: body temperature, 36.0°C; blood pressure, 100/60 mmHg; heart rate, 90/min. She had colostomy. No other abnormalities were found during systemic examination.

Laboratory examinations

Carbohydrate antigen 19-9 level was elevated (264000 U/mL). Other analyses were in normal range.

Imaging examinations

PET/CT was carried out for optimal staging in August 2021, which revealed increased uptake in the pelvis, more prominent in the left supra/peri vesical, left paracolic, and cutaneous regions. She was referred to our clinic with the results. We performed an MRI of the abdomen, which confirmed a recurrent mass, 60 × 81 mm in size, near the sigmoid colon.

FURTHER DIAGNOSTIC WORK-UP

A biopsy was performed for molecular analysis and concluded as metastases of cervix cancer. IHC for MMR proteins showed loss of *MLH-1 and PMS-2* expression, leading to a conclusion of MSI-H disease (Figure 2A). NGS (FoundationOneCDx, 2021) results from the pelvic mass revealed *AKT1, ATR, CREBBP,* and *MLH1* mutations, as well as a tumor mutation burden (TMB) of 6 Mb (Figure 2B). Her MSI status could not be determined. PCR was performed to confirm the MSI status, and MSS disease was noted (Figure 2C).

FINAL DIAGNOSIS

The patient was diagnosed as metastatic cervical cancer.

TREATMENT

Pembrolizumab treatment was initiated with gemcitabine–carboplatin and showed 50% metabolic regression after four treatment cycles. Secondary to the bladder and rectum invasion, pelvic sepsis developed, and pelvic exenteration was performed. The pathology revealed moderately differentiated squamous cell carcinoma infiltrating the bladder and rectum and, thus, a pT3bN0 tumor. IHC findings of the surgical specimen again showed loss of MLH-1 and PMS-2 expression.

OUTCOME AND FOLLOW-UP

Since the patient was tumor-free, pembrolizumab monotherapy was planned. After 3 months of immunotherapy, a restaging PET/CT demonstrated marked disease progression with multiple abdominopelvic hypermetabolic lesions. She was initiated on XELOX chemotherapy but could not tolerate the treatment. Her situation deteriorated and she was lost after 3 months of palliative treatment.

CASE PRESENTATION – 3

Chief complaints

A 62-year-old female patient with a history of endometrial cancer presented with acute, intermittent mid-back pain for the past 3 months.

History of present illness

Pain had worsened for the past 2-3 weeks.

History of past illness

She had undergone a surgery in 2010 for endometrioid adenocarcinoma with squamous differentiation. The pathology results revealed pT1bN0 with 60% estrogen receptor, 90% progesterone receptor, and 50% Ki-67 expression, and thus was classified as stage I disease. She did not receive any adjuvant treatment.

Personal and family history

Significant family or personal history was not detected.

Physical examination

On physical examination, the vital signs were as follows: body temperature, 36.5°C; blood pressure, 110/68 mmHg; heart rate, 80/min. Systemic examination did not reveal any pathology.

Laboratory examinations

Levels of serum tumor markers were elevated (carbohydrate antigen 125, 51U/mL; carbohydrate antigen 19-9, 175 U/mL).

Imaging examinations

MRI of the lumbar spine done because of backpain showed a 5 cm soft tissue mass near the left renal vein. She referred us with MRI results and staging PET/CT confirmed the mass lesion without distant metastases.

FURTHER DIAGNOSTIC WORK-UP

Renal mass and lymph node biopsy confirmed an adenocarcinoma, $PAX8^{+}/CK7^{+}$, consistent with primary endometrium cancer, and thus, stage IV endometrial cancer. IHC revealed no staining for human epidermal growth factor receptor 2, and did not show any losses for mismatch repair proteins. NGS was recommended for detailed molecular

analysis. However, NGS results from the metastases revealed MSI-H disease with a TMB of 54 mutations/Mb (Figure 3).

FINAL DIAGNOSIS

Recurrence of endometrial carcinoma.

TREATMENT

Due to the recurrence of endometrial carcinoma, she underwent surgery for tumor removal, and the pathology results are pending.

OUTCOME AND FOLLOW-UP

She was lost to follow-up.

DISCUSSION

The incidence of MSI differs across solid tumors. Most of the studies in this field focus on CRC, which is closely related to MSI. Our case series included three different solid tumors with discordant MSI results, which, to our knowledge, is the first in the literature. According to recent reports, the frequency of MSI is 0–2.1%, 12%, and 25% for cholangiocarcinoma, cervical, and endometrial cancers, respectively[14-15]. The optimal method for detection of MSI remains unclear. In addition to sensitivity, easily accessible and cost-effective methods are required in daily practice; therefore, IHC is most frequently used. There are limited data on the concordance analysis of MSI status between IHC and NGS for CRC and gynecologic cancers, and lack of data for other solid tumors.

The decision to screen for DNA MMR gene mutations using IHC and/or PCR and/or NGS for MSI involves several considerations. IHC, as a gold standard, has several advantages such as its high specificity, accuracy (96.1% and 99.2%, respectively) and sensitivity[16]. In addition, it is inexpensive and easy to use. Moreover, it can be performed on small biopsy samples, and can clearly suggest the affected gene (*MLH1, MSH2, MSH6,*

or PMS2). However, there are some limitations, such as quality of tissue preparation interfering with results, an experienced pathologist need, and non-immunoreactivity due to missing missense or frameshift/truncation mutations[17]. Studies comparing different methodologies in MSI analysis concluded that some MSI-H cases may be missed if IHC is used alone, with the incidence ranging between 11.8% and 32.9[18-20]. In addition, IHC results were prone to change after neoadjuvant and radiation therapy, which may have changed the preferred screening in some cases[21]. An alternative method, PCR, mostly covers the inadequacies of IHC, especially since it is not limited to protein expression. However, it has its disadvantages, such as the need for a specialized genetic facility, longer turnaround time, normal tissue requirement, and the fact that pre-analytic issues such as fixation, may interfere with the PCR reaction[22]. The most important limitation is the *MSH6* mutation, which may cause non-diagnostic MSI test by PCR, secondary to functional redundancy, leading to misdiagnosis as MSS. In conclusion, although close to 100% sensitivity/accuracy, neither of the methods help identify all tumors with defective MMR genes. The likelihood of misdiagnosis can be overcome using the both methods; however, still, there may be discordant results. Beradibelli *et al*. [16] evaluated MSI with IHC and PCR and reported eight discordant results in a total of 996 patients with CRC. Thus, for these cases, they proposed the addition of a new marker as complementary analysis and suggested the use of PCR over IHC. Several other studies including CRC found discordances between IHC and molecular analysis ranging from 1% to 10[10]. The cause of the discordance was mostly related to factors like low tumor cell proportion, pre-analytical difficulties, non-expert physician, neoadjuvant treatment, tumor heterogeneity, and discordance of tumor biopsy[10]. It was also mentioned that molecular panels used during PCR analysis were principally recommended for CRC; however, they were used in all types of solid tumors and may show poor performance in other types of cancer[9].

False positive results are important to overcome since recent reports suggest that primary resistance to immune checkpoint inhibitors may be related to the misinterpretation of MMR tests[10]. The development of NGS led to the emergence of a

new technique to improve MSI detection. NGS can simultaneously detect MSI and screen for MMR mutations. Although it has 100% sensitivity and specificity, the high cost limits its use[23]. In addition, the panels used in NGS show better performance in non-colorectal cancer[9]. A study evaluating the concordance analysis of MSI between PCR and NGS for solid tumors reported a concordance of 98.8%[24]. Another study investigating discrepant MMR IHC and MSI PCR test results in gynecologic cancers, reported 6 out of 328 discordant results using NGS and demonstrated that NGS could help resolve discrepant MMR and MSI results[25]. The usefulness of NGS in the determination of MSI, with a sensitivity of 95.8%, specificity of 99.4%, positive predictive value of 94.5%, and negative predictive value of 99.2% in 26 cancer types, was supported by several other studies with a concordance of 99.4% compared with PCR-based testing[26].

At our clinic, we prefer screening MSI using IHC due to its fast turnaround time and use NGS as an additional method to investigate a large variety of gene alterations at once. The discordant results were interpreted as MSI-H. MSI-H status is supported by high TMB results, a finding apparent in our third case. This finding has also been conclusively reported by other studies[27]. However, the reliability of the IHC results remains uncertain when NGS shows MSS tumor. Our second case with cervical cancer showed rapid progression after the surgery. Although, seeding during the exenteration procedure may explain the recurrence, another reason may be the loss of tumor antigenicity after the surgery, restricting the trigger in host cell immune response since the patient was tumor-free. These facts may also explain resistance to immunotherapy rather than the discordance. More trials comparing the IHC and NGS results are needed for better assessment.

There may be two limitations to our study. First is different pathologists performing the histological analysis. Although international guidelines exist in terms of evaluation, the experience of the pathologist may interfere with the results. Secondly, as seen in other studies, different samples may cause discrepancy between the results. However, it is not always easy to access the surgical/biopsy specimens when the time interval between the diagnosis and metastases is long.

CONCLUSION

The rare non-colorectal MSI cases in the literature and the lack of investigation into IHC-NGS discordance highlights the uniqueness of our cases. Today, the gold standard of MSI analysis is IHC. However, considering the defined 100% and 98.7% positive and negative predictive values, respectively[24], with reduced costs and turnaround time, NGS may be the preferred first-line option for MSI analysis to reduce the incidence of misdiagnoses in the future.

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