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Review

High microsatellite instability in digestive and gynecological cancers. Review article

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Abstract. Microsatellites are repetitive DNA sequences that indirectly reflect either mutations or epigenetic alterations in mismatch repair (MMR) genes. When the aforementioned genes are altered, microsatellites present a higher number of indels of repetitive units, event termed microsatellite instability (MSI). Because MSI indirectly shows a state of genomic instability, which can lead to the formation of neoantigens, several groups have tried to use MSI as a marker for checkpoint inhibition sensitivity and for prognostic assessment. This has been applied in several digestive and gynecological malignancies that will be further discussed in this review. This review synthesizes the diagnostic, incidence and clinical implication of high microsatellite instability (MSI-H) in digestive and gynecological malignancies.

Key Words: MSI-H, immunotherapy, cancer

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Introduction

Microsatellites are simple sequence repeats, consisting of 1-6 nucleotides, that are widely distributed throughout the genome. These have been shown to influence both DNA replication and gene expression through its action on the reorganization of chromatin structure (Guang et al 2019).

Microsatellite instability (MSI) is caused by indels affecting the repetitive units of microsatellites, leading to the formation of new alleles.

As genomic instability is considered one of the hallmarks of cancer, it is of no surprise that several studies have shown the importance of MSI in oncogenesis and in influencing prognosis and response to therapy.

Depending on the number of alterations, microsatellite stability is categorized in three different subtypes, represented by MSI high (MSI-H), MSI low (MSI-L), and microsatellite stability (MSS) (Boland et al 1998).

MSI-H or mismatch repair deficiency (dMMR) leads to an increase in gene mutations which both initiates oncogenesis, but also leads to a therapeutic sensitivity in these cases. This genomic instability creates a high number of neoantigens that makes the malignancy to be better detected by the host's immune system. Because of this, therapies that enhance immune response, as in the case of checkpoint inhibitors have seen important results in clinical trials (Bever et al 2018).

An example that reflects the high therapeutic impact that MSI has shown is represented by the US Food and Drug Administration (FDA) approval for pembrolizumab, an anti PD-1 antibody, to be used as a therapeutic option for any advanced solid tumor that presents either MSI-H or dMMR (Prasad et al 2018). This has been the first instance of FDA approving an oncologic therapy that can be used regardless of the primary origin of the malignant cell.

Because of the important advances in this field, coined with the 2019 Nobel Prize for Physiology or Medicine for the development of checkpoint inhibition, the current review will offer an overview on the importance that MSI has posed for different digestive and gynecological malignancies.

Detection of microsatellite instability

There are four procedures used to assess MSI status which will be further discussed.

Polymerase Chain Reaction (PCR) and Capillary electrophoresis (CE)

This is considered the golden standard for MSI evaluation as it has been repeatedly shown that this method generates reliable results. This method assesses the alterations in: BAT-25, BAT-26, D2S123, D5S346 and D17S250 in the malignant cells compared to the matched normal tissue. Nonetheless, the large-scale application of this method is hindered by the facts that it is relatively expensive and that it requires a matched normal tissue, which is not always easy to obtain (Yuji et al 2020, Kai et al 2020).

Immunohistochemistry (IHC)
An indirect alternative to the previous method of detecting MSI is represented by IHC for proteins implicated in MMR. These are generally represented by MLH1, MSH2, MSH6 and PMS2. It has been shown that if one or more of these proteins does not present expression on the tumor cells, but present expression on matched tissue normal cells, it can be considered that the analyzed malignancy has the status of MSI-H (Umar et al 2004). This is a cheaper method compared to the previous one, but still has the drawback of necessitating the use of matched normal tissue. Additionally, as in the case of all tests, IHC does not perfectly match the golden standard for the detection of MSI, but it is considered reliable enough for clinical application (Kai et al 2020).

Next-generation sequencing (NGS)
NGS is a more recently implemented method compared with the previous two, that evaluates the mutational status of several genes to assess the MSI status in tumor tissues. This method is sensitive (97%) and specific (95%) when compared with corresponding PCR and IHC assessments of the same tissue samples. Additionally, the method requires a small tissue sample, doesn't need a matched normal sample and has the additional advantage of offering information regarding the tumor mutation burden (TMB) (Sally et al 2019).

Single-molecule molecular inversion probes (smMIPs)
This is even a more recent method that uses single molecule reverse probe capture and high-throughput sequencing to assess the pan cancer MSI. The accuracy is high (95%) and does not require matched normal tissue. Unfortunately, it can be used actual only to identify MSI status in colorectal, prostate and endometrial cancer (Waalkes et al 2018).

Incidence and clinical importance of MSI-High

Colorectal cancer
Colorectal cancer (CRC) is one of the most frequent malignancies in both men and women, with an important impact in the morbidity and mortality of the affected patients. An important pathway of CRC pathogenesis is represented by the initial loss of the APC gene, which leads to genomic instability and second mutations that further initiate the oncogenic process. Similar genomic alteration events can be also observed in MSI-H tumors, which are initiated by the loss of MLH1, MSH2, MSH6 or PMS2, which have an important role in repairing single base pair alterations (Markowitz et al 2009). A special case is represented by Lynch syndrome, which is caused by the germline loss of APC, leading to an increased occurrence of CRC with age, showing the importance of genomic instability in the development of CRC (Shibata et al 1994)
The incidence of MSI-H in CRC is between 16.6 and 19% depending of localization, more frequent on the right sided colon cancer (13.5-27%) compared to left-sided colon cancer (2.0-2.2%) and rectal cancer (2.2-9.2%) (Yuji et al 2020)
MSI-H tumors are considered to have a better outcome compared to the MSS ones. This effect is most probably linked to the fact that MSI-H tumors are infiltrated with dense cytotoxic T-cells, which can lead a better tumor control (Kai et al 2020) These tumors also have a high expression of PD-L1 and a strong activation of immune evasion pathways, leading to a potential target for immune checkpoints inhibitors therapy (ICI) (Yuji et al 2020).
Bandalamenti et al (2018) has shown that one of the most important cells that respond to MSI-H CRC are represented by CD8+ T cells, which lead to an inhibition of tumor progression. This came as an important explanation to the study of Samowitz et al 2015, in which it was shown that MSI-H CRC presents reduced invasive potential. In accordance to these results, Mohan et al 2016 has shown that stage I/II MSI-H CRC has a lower risk of developing metastasis.
The data from phase 2 KEYNOTE -016 trial (Lee et al 2018) investigating the efficacity of pembrolizumab in CRC patients with dMMR versus proficient MMR (pMMR) showed a 50% vs 0% ORR and 89% vs 16% DCR. The phase 2 KEYNOTE-164 trial (Coelho et al 2017) showed a 32% ORR after pembrolizumab therapy in 63 patients with metastatic previously treated MSI-H CRC. A similar trial, CheckMate-142 has also shown the important effect of nivolumab and low-dose ipilimumab combination therapy in the treatment of MSI-H CRC.

Gastric cancer
MSI is considered to be related to carcinogenesis in gastric cancer. Li et al 2015 and Sugimoto et al 2016 reported that MSI appears in precancerous lesions and it's frequency increases as the lesion progresses to gastric cancer.
The incidence of MSI-H in gastric adenocarcinoma ranges between 7.5 and 21.9% (Yuji et al 2020) It appears to be more frequent in women and distal stomach (Marelli et al 2016). For the gastroesophageal junction the incidence is 4-8 % and 0% for gastrointestinal stromal tumors.
Choi et al 2014 and Zhu et al 2015 showed that the MSI-high patients with gastric cancer have better prognosis than MSS and MSI-L patients.
Conversely, Polom et al 2017 showed that the prognosis of gastric cancer is more impacted by age than by MSI. An et al 2012 indicated that DFS shows no significant differences between MSI-H and non MSI-H patients at any stage.
Miceli et al showed a 9% incidence for the MSI-High tumors, with prevalence of older age, antral localization, T3-T4 stage and intestinal type. Also, there was a better prognosis for these patients as compared to the non MSI-H patients.

Pancreatic cancer
MSI-High can be detected in a few number of patients with ductal adenocarcinoma (Kai et al 2020) inactivation of MLH1 and MSH2 was detected in pancreatic cancer patients.
Nakata et al 2002 showed a 17.4% MSI-H positivity on 46 patients who underwent pancreatic resection for cancer, while Yamamoto et al 2001 identified 13 MSI-H patients of 100 patients assessed.
For the patients wits pancreatic cancer and MSI-H /dMMR Yamamoto showed a significantly better prognosis with checkpoint inhibition (Kai et al 2020) The patients with MSI-H tumors had a significantly prolonged survival times compared to patients with MSI-L and MSS tumors (Macherla et al 2018).

The advances on the application of MSI in pancreatic cancer has been coined by the 2019 NCCN guidelines, which recommend MSI testing for any pancreatic ductal adenocarcinomas with more than an in situ presentation. Moreover, the same guidelines suggest the treatment of these tumors with pembrolizumab if MSI-H is observed (Yuji et al 2020).

Hepatocellular carcinoma (HCC)

Because of the high capacity for intra end extrahepatic recurrence and metastasis, the prognosis of HCC remains poor. Considering the inflammatory phenotype associated with the presence of cirrhosis, it was no surprise when it has been shown that HCC developed on cirrhotic liver presents a higher incidence of MSI-H compared to the one developed on non-cirrhotic liver (Yuji et al 2020). Nonetheless, this finding has to be interpreted in context, as the cause of HCC development and the populations in which it appears differ between cirrhotic and non-cirrhotic patients.

The KEYNOTE-224 and KEYNOTE-240 trials showed no statistical significances after treatment with ICI in compare with tyrosine kinase inhibitors (TKI) in advance HCC patients. Because of this, a promising therapy for a subset of HCC patients can be represented by the combination of ICI with TKIs (Nishida et al 2018).

Breast cancer

One of the most important genes observed to have an impact in prognosis, therapeutic choice and germline predisposition to breast cancer is represented by BRCA1. This gene is implicated in the principal DNA repair pathway that occurs in breast cells, with it's loss-of-function having been shown to increase the incidence of MSI-H, leading to an important pathogenic queue (Kai et al 2020). Mutations in BRCA1 have been shown to also affect the normal replication of these cells, leading to further abnormalities (Zhu et al 2011). Clinically, it has been shown that MSI-H breast cancer patients present a worse prognosis, but this can be due to it's association with BRCA1 mutations (Ozer et all 2002).

Cervical cancer

In June 2018, pembrolizumab was approved for the treatment of patients with recurrent or metastatic cervical cancer with disease progression after chemotherapy and PD-L1 expression more than 1% (Xiamo et al 2019)

Tamura et al in 2019, investigating the effect of Nivolumab in Japanese patients with advanced/recurrent uterine cervical cancer, obtained an overall response rate (ORR) of 20% but no MSI-H identified in the study group. The same study showed that the ORR was higher in patients with PD-L1-positive tumors (33%) than in those with PD-L1 – negative tumors (0%)

The KEYNOTE-158 trial obtained an ORR of 14.3% with 2.6% complete response and 11.7% partial response among patients treated with pembrolizumab.

Endometrial cancer

Endometrial cancer (EC) is the fourth most common malignancy in women and the most common gynecologic cancer, most of them being sporadic tumors. In 2%-5% of cases there are familial cases due to specific germline mutations in the mismatch repair genes. The MSI-H, due to alteration of MLH1, MSH2, MSH6, PMS2 was shown in both, sporadic and hereditary endometrial cancer patients (Di Tucci et al 2019).

EC cells overexpress PD-L1 (75% cases) and PD-L2 (25-100% cases) which can bind PD-1 receptors, expressed on tumor – infiltrating CD4 and CD8 Tcells and inactivate them. Targeting PD -1 with Pembrolizumab, was proven to be efficient in MSI-H/dMMR tumors (Di Tucci et al 2019).

The KEYNOTE-028 trial in 2017, investigating the effect of Pembrolizumab in advanced EC patients, showed an ORR of 13%, a six months progression-free survival (PFS) and overall survival (OS) of 19%, respectively 68.8% (Ott et al 2017).

Makker et al in 2017 obtained an ORR of 48% with Pembrolizumab therapy in advanced/metastatic EC patients, while Flemming et al 2017 shown an 13% ORR with Atezolimumab therapy on 15 patients with advanced endometrial cancer.

Ovarian cancer

In ovarian tumors, the percentage of tumors positive for MSI has been reported between 6 and 37 %(Bo-Sung et al 2008).

MSI is infrequent in borderline and malignant ovarian tumors. MSI-H/dMMR was predominantly seen in endometrioid cancers (23%), in serous carcinoma (0.3%) and absent in mucinous carcinomas, clear cell carcinomas and carcinosarcomas according to Fraune et al 2020 study on o group of 582 ovarian cancers. Sood et al 2001 failed to find a clinical significance of MSI in ovarian cancers.

Conclusion

Microsatelite instability is closely related to tumor. For the detection of the MSI status, IHC and PCR are widely used, with good sensitivity, but for improving the accuracy the new NGS seems to be a promising alternative.

Early detection of the MSI status has a great role in taking preventing measures in some cancers such Lynch syndrome and can predict and orientate a future immunotherapy with ICI.

The clinical importance of MSI status was underlined by the recent FDA approval of pembrolizumab for MSI-H/dMMR tumors, the first time when a cancer treatment was driven using a biomarker without importance of tumor localization.

This benefit depends from tumor site and the time of therapy. For example, the ICI had shown the best results in advanced metastatic colorectal cancer. The use of immune checkpoint inhibitors has shown durable clinical responses in a subset of malignancies. MSI has a clinical importance especially for colorectal, gastric and endometrial cancers. The response to the therapy with ICI in many cancers seems to be due to expression of neoantigens that confer susceptibility to clearance by T cells. MSI-H tumors have a higher likelihood of neoantigen generation because of the increased overall mutational burden induced by MMR deficiency.

Due to the complexity of immuno-regulatory mechanisms and the heterogeneity of tumors, combination therapies represent the next generation treatment that can lead to a better prognosis and overcome the limitations associated with single-agent therapy. It was shown that MSI-H can be used as a biomarker for patients who are more likely to respond well to immune checkpoint inhibitor therapy. Further studies are needed to explore the best biomarkers that can predict a good response to immunotherapy.

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