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The utility of immunohistochemical testing for mismatch repair proteins in fine needle aspiration specimens of pancreatic adenocarcinoma

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ABSTRACT

Introduction: Microsatellite instability (MSI) testing is recommended for all colonic and endometrial carcinomas to screen for Lynch syndrome. The role of MSI testing in pancreatic adenocarcinoma has not been well-established. Screening can be done via immunohistochemical (IHC) staining for mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, PMS2). We report our experience and the clinical utility of MMR IHC on pancreatic adenocarcinomas in fine-needle aspiration (FNA) specimens.

Materials and methods: We performed a retrospective review to identify all patients diagnosed with pancreatic adenocarcinoma by FNA at our institution between December 2017 and September 2019. For cases with sufficient tumor cells for testing, the MMR results and morphology were summarized, as well as corresponding clinical information, including age, clinical stage, treatment, and concurrent other cancers.

Results: From December 2017 to September 2019, there were a total of 184 pancreatic FNAs with a diagnosis of adenocarcinoma. Of these 184 FNAs, 65 (35%) contained sufficient material in the cell block to perform IHC for MMR. The cell block material was collected in either RPMI or CytoLyt. Poor technical quality precluded interpretation of PMS2 in 4 cases and MSH6 in 2 cases. All other cases showed intact expression of all four proteins.

Conclusions: IHC for MMR proteins can be done on specimens collected in RPMI or CytoLyt, but RPMI appears to be more reliable. None of the pancreatic adenocarcinomas in this study showed loss of MMR protein expression. Routine testing of MMR loss may not be indicated in pancreatic adenocarcinomas in the general patient population.

1. Introduction

In 2017, the Food and Drug Administration (FDA) approved pembrolizumab for the treatment of unresectable or metastatic solid tumors that are microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR), and have progressed following prior treatment [1]. This FDA approval, which is the first in which a cancer treatment indication is independent of the tumor primary site of origin, has resulted in increased and widespread MSI testing, especially for cancers where there is a relatively poor prognosis even with current treatments [1].

Such is the case for pancreatic cancer. With a 5-year survival rate of 8%, pancreatic cancer has a worse prognosis than most malignancies [2]. While pancreatic cancer is the ninth most common cancer in women and tenth most common in men, it is currently the fourth leading cause of cancer-related death and by 2030 is expected to be the second leading cause of cancer-related death [3]. Unfortunately, MSI does not appear to be involved in the etiology of many pancreatic adenocarcinomas.

MSI, which refers to a hypermutable condition resulting from defects in DNA mismatch repair, is well known for its association with Lynch syndrome. Lynch syndrome is an inherited cancer syndrome that is seen in 1 in 300 people [4]. It is associated with an increased risk for a number of cancers, chiefly colorectal and endometrial carcinomas, but the syndrome does carry a nine-fold increased risk for pancreatic adenocarcinoma [5,6]. While pancreatic adenocarcinoma is seen in 3.7% of people with Lynch syndrome, < 1% of pancreatic cancers are seen in people with Lynch syndrome [5,6]. Furthermore, and in keeping with the fact that MSI in cancers is most often sporadic, an even smaller

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Table 1
Summary of cancer type and occurrence rate associated with MSI and Lynch syndrome.

	Colorectal cancer	Endometrial cancer	Pancreatic cancer
% with MSI	15% [15]	30% [17]	< 1% [7]
% with Lynch	2–3% [15]	5% [18]	Unknown
% of Lynch with the corresponding cancer	50% in women, 80% in men [16]	50% [15]	3.7% [5]

proportion – significantly < 1% – of pancreatic adenocarcinomas show evidence of MSI (Table 1) [7].

There are two commonly used ways of testing for MSI. One can simply use immunohistochemistry to stain tumor tissue for the four main mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, PMS2). Alternatively, one could perform a PCR-based assay to compare a series of microsatellites between lesional and non-lesional tissue to look for differences that would be indicative of microsatellite instability in the tumor. There are other less commonly used methods, but these two methods are sufficiently sensitive and specific for screening purposes. The immunohistochemical method is cheaper and less technical so it is used most often even though the PCR-based method is more sensitive. Mutations that impair the function of without altering the expression of the mismatch repair proteins may result in false negatives by IHC that could be detected by PCR. Since most MSI is acquired and due to somatic hypermethylation of the *MLH1* gene promoter, as opposed to the germline mutations of mismatch repair protein genes seen in Lynch syndrome, IHC is generally considered a sufficient screening method for MSI. However, some have reported that in pancreatic cancer, unlike colorectal cancer, most MSI is due to germline mutations in the MMR genes [8].

Despite the rarity of MSI in pancreatic adenocarcinoma, a distinct phenotype has been recognized. Compared to pancreatic adenocarcinomas in general, MSI-H pancreatic adenocarcinoma is more commonly mucinous, poorly-differentiated, or frankly medullary; has increased tumor-infiltrating lymphocytes; more commonly has wildtype KRAS; and is associated with intraductal papillary mucinous neoplasms (IPMNs), localized disease at presentation, and a better prognosis [8-12]. Even so, the majority of MSI-H pancreatic adenocarcinomas are neither medullary nor show specific morphologic features [8].

At the University of Kansas Health System we perform IHC for MLH1, MSH2, MSH6, and PMS2 proteins on all pancreatic adenocarcinomas at the request of our oncologists in response to the aforementioned FDA approval. There is no current testing guideline on pancreatic adenocarcinomas. In this study we reviewed our institution's experience in testing MSI by IHC on pancreatic adenocarcinoma in FNA specimens and discuss its utility.

2. Material and methods

2.1. Case selection

This study was approved by the Institutional Research Board (IRB) at the University of Kansas Medical Center. We retrospectively reviewed our medical record system to identify all pancreatic FNA specimens with a diagnosis of adenocarcinoma at our institution between December 2017 and September 2019. Pursuant to our quality assurance program, slides from each case had been reviewed by two pathologists who independently agreed on the diagnosis before the original report had been issued. We then refined our sample to cases that had sufficient material for MMR IHC. There is no established criterion on minimum number of tumor cells required for testing; we empirically chose 50 tumor cells as the cut-off for testing. IHC stains were not ordered on cellblocks that contained fewer than 50 tumor cells.

2.2. Clinical and pathologic data collection

Additional information was collected on all cases in which there had been a FNA diagnosis of adenocarcinoma and which also had MMR IHC results. From the medical record system, the following information was collected and recorded for each case: results of MMR IHC, the fixative in which the FNA sample was collected, patient age, patient sex, patient cancer history, staging information for the pancreatic cancer, treatment history for the pancreatic cancer, any morphologic details of the pancreatic cancer, and whether any work-up for MSI other than IHC had been done.

2.3. Cell block preparation

Material from which the cell blocks were to be made was collected in either RPMI (43 cases) or CytoLyt (22 cases). The specimen was transported to the lab and centrifuged for 5 min at 2200 rpm. The supernatant was carefully removed with a disposable transfer pipette so as minimize the loss of sediment. The sediment was resuspended in the small amount of fluid that remained by brief vortexing. Four drops of plasma and four drops of reconstituted thrombin were added, and the solution was agitated. This step was repeated as many times as necessary for clot formation. The clot was carefully poured into a mesh biopsy bag which was transferred to a cassette that was labeled with two patient identifiers. The cassette was placed in formalin and transported to the histopathology laboratory where it underwent routine processing.

2.4. MMR IHC

Immunohistochemical stains for MMR were performed on 10% neutral buffered formalin-fixed, paraffin-embedded tissue. The slides were antigen retrieved in specific Target Retrieval Solutions from Agilent/DAKO in the DAKO PT Link. EnVision FLEX Target Retrieval Solution, High pH (pH 8) is used for the primary antibodies MLH-1 (1:50 titer), MSH-6 (1:50 titer), and PMS2 (pre-dilute). EnVision FLEX Target Retrieval Solution, Low pH (pH 6) is used for the primary antibody MSH-2 (1:50). All primary antibodies were supplied by Biocare Medical, LLC., Concord, CA. Slides were loaded on the DAKO Autostainer Link 48 with the corresponding program selected. EnVision FLEX + Detection System (K8002) was used and consists of EnVision FLEX Peroxidase-Blocking Reagent, EnVision FLEX HRP, EnVision FLEX Mouse Linker, and Envision FLEX DAB+ Chromogen Substrate.

The scoring criteria for MMR IHC endorsed by College of American Pathologists are used [13] although various criteria exist in the evaluation of histologic samples [14]. Any positive staining in the nuclei of tumor cells is considered as intact expression. The positive staining can be focal or patchy. Loss of expression requires a complete absence of labeling in the nuclei of neoplastic cells with intact labeling of internal controls such as nonneoplastic epithelium, stromal cells, or lymphocytes.

3. Results

There were 184 pancreatic FNAs with the diagnosis of adenocarcinoma at our institution between December 2017 and September 2019. Of these, 65 (35%) contained sufficient cell block material to perform MMR IHC. Sixty of the 65 cases showed intact expression of all four mismatch repair proteins (Fig. 1). Five cases showed absence of labeling for one or two of the mismatch repair proteins in the nuclei of neoplastic cells; however, the results were not valid due to lack of staining in internal nonneoplastic cells (Fig. 2). It was PMS2 in four cases and MSH6 in two cases. Of note, all five cases were collected in CytoLyt and all five cases had marked necrosis and cellular degeneration.

The clinical and pathologic features of the studied cases are summarized in Table 2. The distribution of ages, sex, and clinical stage were

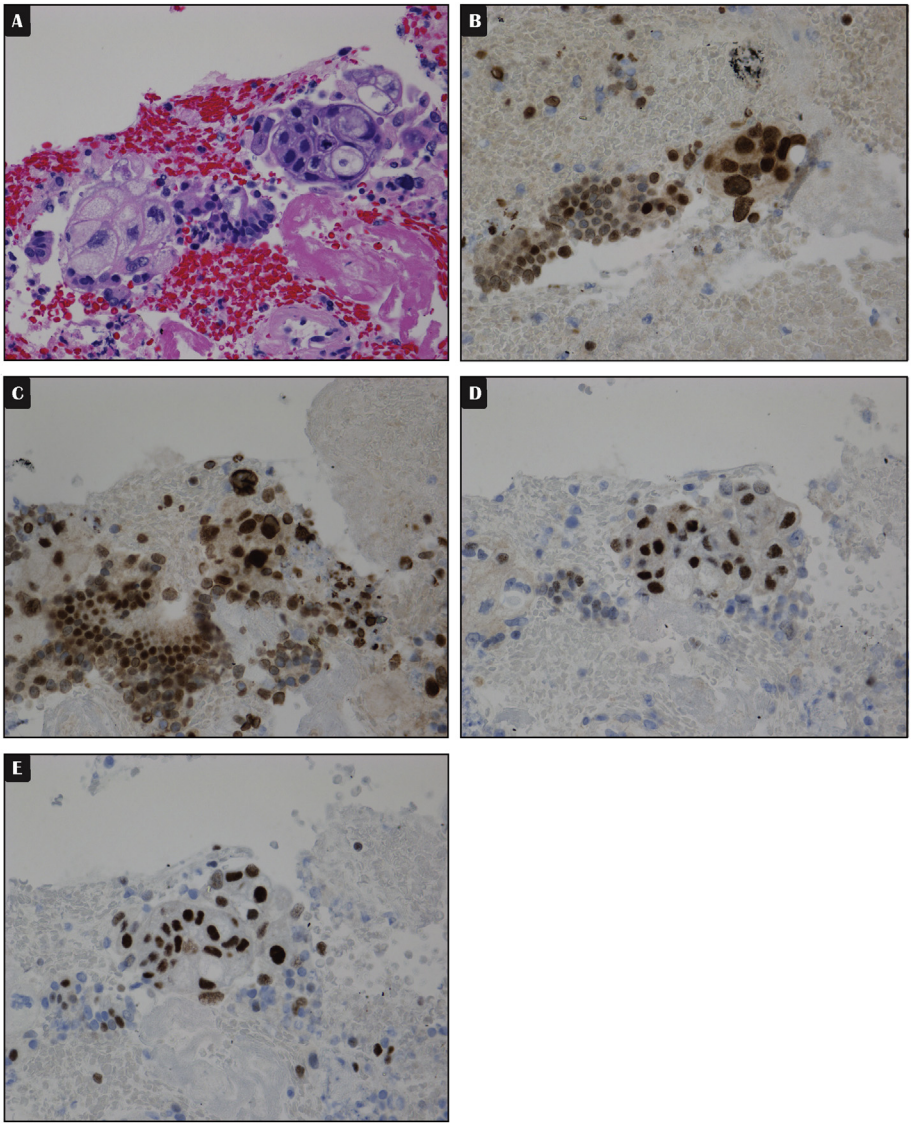

Fig. 1. MMR IHC testing in a case of pancreatic adenocarcinoma. A. Tumor cells and adjacent normal gastrointestinal epithelial cells in cell block (H&E stain, x400); B. Intact MLH1 expression (IHC stain, x400); C. IntactMSH2 expression (IHC stain, x400); D. IntactMSH6 expression (IHC stain, x400); E. IntactPMS2 expression (IHC stain, x400). Note the internal control cells are positive in all four stains.

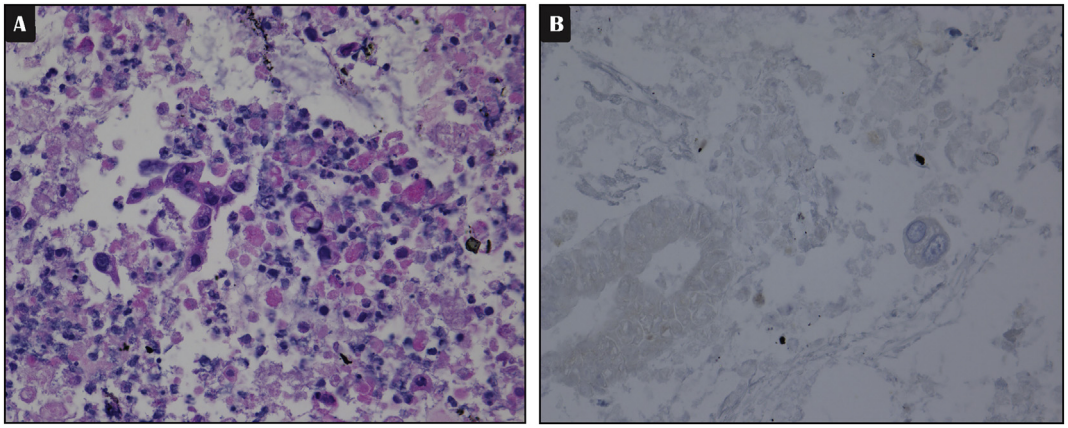

Fig. 2. MMR IHC testing in a case of pancreatic adenocarcinoma. A. Tumor cells and adjacent normal gastrointestinal epithelial cells in cell block (H&E stain, x400); B. Loss of PMS2 expression (IHC stain, x400). However, the result is invalid due to absence of positive staining in adjacent internal control cells. Note the background is necrotic and the cells have a degenerated appearance.

Table 2
Clinical and pathologic features of the 65 pancreatic adenocarcinoma cases with IHC MMR testing.

Features	Results	Comments
Age (years)	34–93 (Mean: 69; Median: 71)	
Sex (M:F)	35:30	
Clinical stage		
Not clinically staged:	26	
Stage IA:	1	
Stage IB:	10	
Stage IIA:	2	
Stage IIB:	1	
Stage III:	2	
Stage IV:	23	
Other cancers (n = 11)		
Prostatic adenocarcinoma:	4	
Urothelial carcinoma:	1	
Colonic adenocarcinoma:	1	
Lung adenocarcinoma:	1	
Endometrioid adenocarcinoma:	1	
Multiple myeloma:	1	
Urothelial and basal cell carcinoma:	1	
Urothelial and squamous cell carcinoma:	1	
Morphologic features on cytology		
Adenosquamous:	2	
Mucinous:	3	
With squamous features:	1	
Osteoclast-like giant cells:	1	
No special features:	62	
Subsequent surgery	10 (1 well-differentiated, 2 poorly differentiated, 7 moderately differentiated)	Repeat testing on surgical specimens yielded concordant results
Additional testing	None	

all consistent with what has been well-established for pancreatic adenocarcinoma. The patients ranged from 34 to 93 years of age with a mean of 69 years of age and median of 71 years of age. There were 35 males and 30 females. Of the 65 pancreatic adenocarcinomas, 26 were not clinically staged. Of the 39 that were clinically staged, one was stage IA; ten were stage IB; two were stage IIA; one was stage IIB; two were stage III; and twenty-three were stage IV.

None of the patients were known to have had Lynch syndrome and none had MSI testing other than the MMR IHC done on their pancreatic FNAs. Eleven of 65 patients had at least one other malignancy including one with colon cancer and the other with endometrial cancer. These are the only two patients who met the Bethesda criteria that is used in MSI screening of colorectal cancers.

In terms of morphologic features, the ductal adenocarcinomas were predominantly of no special type. On FNA, two cases showed adenosquamous features; three showed mucinous features; one was poorly-differentiated with squamous features; and one showed numerous osteoclast-like giant cells. Ten patients had subsequent surgery. Repeat MMR IHC (for the purpose of this study) on the resected tumor showed concordant negative results. Most patients received conventional chemotherapy, and none received pembrolizumab.

4. Discussion

To our knowledge, this is the first study to investigate the utility of MMR IHC testing in the FNA specimens of pancreatic adenocarcinomas. Our study demonstrates that testing on FNA cell blocks can be successful and reliable and that RPMI is preferred over CytoLyt as a collecting medium for such testing. The utility of cytologic material for molecular testing has been discussed in the literature [19-21]. All cytologic preparations including smears and cellblocks are considered suitable for testing. CytoLyt fixation alone has been found to alter antigenicity [20]. In our study, the 22 cases collected in CytoLyt had all been fixed in formalin after the cellblock was made. Among the 10 cases which we had the perfect concordance of MMR IHC staining between cytology samples and the subsequent surgical specimens, three were collected in CytoLyt. We also used the presence of positive internal controls to assess the validity of the results. It is noted that the five cases that lacked proper internal controls were all collected in CytoLyt and all five cases also had marked necrosis and cellular degeneration. Therefore, it is important to monitor the quality and validity of IHC testing in cytology specimens and follow the proper guideline [21]. Another important and practical question is the adequacy of the specimen required for testing. For context, a minimum of 100–200 tumor cells are required for next generation sequencing (NGS) assays; 100 is used as the cutoff number of tumor cells required for PD-L1 IHC assays in non-small cell lung cancer; and 50 tumor cells are required for ALK translocation assessment by Fluorescent in-situ hybridization [22,23]. In this study, we arbitrarily used the number of 50 as minimal tumor cells required for MMR IHC testing. Our results showed concordant results between the FNA sample and the subsequent surgical specimens, therefore we propose 50 tumor cells as a reliable minimum number required for future MMR IHC testing on cytology samples.

Currently there is no guideline on MSI testing in pancreatic adenocarcinoma. All 65 cases tested in our study showed intact MMR expression in tumor nuclei indicating low probability of microsatellite instability-high (MSI-H) status. This is consistent with the literature that reports MSI-H to be present in < 1% of cases [7,8]. Due to the rarity of MSI-H in pancreatic adenocarcinomas and from the results of this study, we believe it is not cost-effective to routinely perform IHC MMR in all pancreatic adenocarcinoma specimens. Additional studies are needed to establish criteria to selectively test pancreatic adenocarcinomas for MSI. For example, it may be reasonable to order testing in cases that show mucinous features, poor differentiation, or numerous tumor-infiltrating lymphocytes. We will expand our study to test these selective morphologic criteria in performing IHC MMR in pancreatic adenocarcinoma on FNA specimens. It is important to point out that with the frequent emergence of new tests, pathologists should serve as “gate keepers” to ensure evidence-based guidelines dictate practice and resource utilization. Pathologists, as physicians with uniquely extensive and specialized training in laboratory science and practice, should not simply acquiesce to oncologists' requests. For these reasons, it is imperative that we prioritize remaining up-to-date on the literature and evolving treatment strategies, particularly in the fields of targeted and immune therapies, so as to cement our place in the clinical management team. We should also collect our own data and educate our physicians about appropriate evidence-based testing. Pathologists should be strong advocates on test-utilization in all aspects of clinical testing.

This study is limited by small case numbers in a single institution, no corresponding confirmatory germline testing results for MMR, inconsistent specimen handling, and low rate of adequate material for testing in collected specimens (35%). Further research is needed to establish whether MSI in pancreatic adenocarcinoma is primarily due to germline mutations of the MMR protein genes or somatic hypermethylation of the *MLH1* gene promoter. If it is primarily due to germline mutations, risk assessment for Lynch syndrome would be useful, especially since Lynch syndrome is de novo in < 3% of cases (Table 1) [24].

In conclusion, MMR IHC can be performed on pancreatic adenocarcinoma FNA cellblocks. Fifty tumor cells appear to be an adequate minimal tumor cell quantity for MMR IHC testing. Routine testing of MMR loss may not be indicated in pancreatic adenocarcinomas in the general patient population. Further study is necessary to refine the selection criteria for testing. Evidence-based guidelines should be followed in all ancillary testing of pathology specimens for predictive and

prognostic purposes, and potentially genetic counseling.

Author contributions

Daniel Mettman and Erin Haer- data curation, formal analysis, writing-original draft.

Mojtaba Olyaee and Amit Rastogi- Conceptualization, investigation, methodology.

Rashna madan, Maura O'Neil, Sarah Kelting, Katie Dennis- formal analysis, validation, writing-review and editing.

Fang Fan- Conceptualization, formal analysis, investigation, project administration, supervision, validation, writing- review and editing.

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