DAHL-CHASE Focus

October 18, 2010

TECHNICAL BULLETIN: MICROSATELLITE INSTABILITY ANALYSIS

Technical Brief

Screening for Lynch Syndrome by Microsatellite Instability

Background Information

Microsatellite instability (MSI) results from defective function of mismatch repair (MMR) proteins (MLH1, MSH2, MHS6, or PMS2). MSI is a key feature of Lynch syndrome, the most common inherited cause of colorectal cancer, due to mutation of one of the MMR genes. Close to 90% of Lynch syndrome-associated colorectal carcinomas display high degree of MSI (MSI-H) and show loss of expression of one (or more) of the MMR genes.[1] Identification of Lynch Syndrome patients is critical, given their high risk for additional carcinomas, as well as the risk of cancer affecting their family members. Screening to detect Lynch syndrome in individuals with newly diagnosed colorectal carcinoma was proposed in 2009 as a strategy to reduce morbidity and mortality in their relatives by the EGAPP (Evaluation of Genomic Applications in Practice and Prevention).[2]

MSI status has also become increasingly important in sporadic colorectal carcinomas, approximately 15% of which also show MSI-H and loss of MMR protein expression (typically MLH1 and PMS2).[3] MSI-H in sporadic carcinomas is most commonly due to methylation of the MLH1 gene promoter. While it does not indicate the presence of Lynch syndrome in these cases, there is evidence that sporadic carcinomas with MSI-H have a better prognosis than those without.[4] In addition, patients with MSI-H carcinomas do not appear to benefit from adjuvant fluorouracil-based chemotherapy.[5,6]

There is high concordance between PCR-based MSI status testing and loss of MMR protein expression as detected by immunohistochemistry (IHC). [7] MSI testing can occasionally detect tumors with defective MMR that show no expression loss by IHC, as some mutations may not affect protein detection by IHC. Conversely, IHC testing can occasionally detect cases that show only low levels (or even an absence) of MSI (e.g. tumors with MSH6 mutations). Based on the EGAPP review, overall sensitivities of MSI PCR and IHC are 89% (in cases with MSH6 defect 77%) and 83%, respectively, with specificity of both techniques being is comparable at about 90%.[2] IHC helps to focus further analysis on the MMR gene likely causing MSI. This can increase cost efficiency of the subsequent testing by eliminating the need of expensive and laborious DNA sequencing of all 4 major MMR genes. Therefore, MSI PCR testing and MMR IHC can be viewed as complementary.

Clinical Indications and testing algorithm

Our laboratory performs PCR to assess the MSI status on most newly diagnosed colorectal carcinomas. MSI-H tumors are reflexed to MMR IHC testing and BRAF V600E mutation. Since this mutation only rarely occurs in Lynch syndrome, its presence helps to identify sporadic (non-germline) MSI-H (see separate technical brief for more information about BRAF mutation testing).

Methodology

The MSI assay performs optimally in formalin-fixed tissue. Testing is performed by PCR analysis on genomic DNA extracted from patient tumor and normal tissue, using PCR amplification of seven loci (five mononucleotide repeats BAT-25, BAT-26, NR-21, NR-24 and MONO-27 and two pentanucleotide repeats (PentaC and PentaD). Fluorescently labeled PCR products are subjected to capillary electrophoresis. Allele sizing data are compared for normal and tumor samples and the presence of MSI is determined by the appearance of new alleles in the tumor sample. The pentanucleotide repeats serve only to verify specimen identity and are not used to determine MSI status.

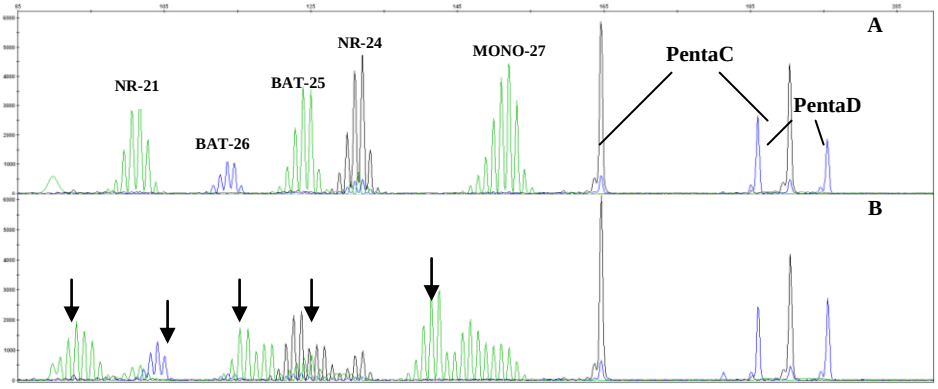

Fig. 1. – Capillary electropherogram showing a downward shift of 5 of the 5 analyzed microsatellite loci (arrows) in the cancer (B) compared to normal DNA (A), indicating high microsatellite instability (MSI-H). The identity markers PentaC and PentaD match between normal and tumor specimens.

Test Overview

Test Name	Microsatellite Instability Analysis
Individuals Suitable for Testing	Patients with newly diagnosed colorectal carcinoma, Lynch syndrome screening
Limitations	Pathologist's evaluation of the tissue section used for DNA extraction is required to ensure that tumor cells are present in adequate quantity/concentration. The MSI assay will detect mononucleotide repeat shifts as long as they constitute at least 10% of the DNA sample mix.
Reference Range	Microsatellite Stable (MSS): none of the 5 microsatellite markers unstable High microsatellite instability (MSI-H): ≥2 of 5 microsatellite markers unstable Low microsatellite instability (MSI-L): 1 of 5 microsatellite markers unstable
Specimen Requirements	Formalin-fixed tissue containing a sufficient amount of tumor (generally at least several mm of tumor tissue in the tissue block). Normal tissue sample for allelic comparison
Turn-around time	Assay is started every Monday and typically takes 3 days to complete, with results reported on Thursday
CPT Codes	**88381, 81301, G0452**

References

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Our current molecular diagnostics test menu also includes:

- UroVysion bladder cancer FISH assay
- *HER2* gene amplification FISH assay
- KRAS codon 12 & 13 Mutational Analysis
- BRAF V600E Mutation Analysis

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