MMR-MSI testing in practice: a single tertiary centre study

Joy Staniforth; Yuanxue Huang, PhD; Hongxiang Liu, PhD; Olivier Giger, MD, PhD

Introduction & Methods

Lynch syndrome (LS) is a hereditary condition associated with a high risk for colorectal cancer (CRC)

- LS is caused by germline mutations in DNA mismatch repair (MMR) genes. Incidence 2-4% all CRC

Deficient DNA MMR can be seen in Lynch syndrome, however can be also seen in sporadic CRC

- The *BRAF* V600E mutation is associated with *MHL1* promoter hypermethylation
- Sequential *BRAF* V600E and *MLH1* promoter hypermethylation testing differentiates sporadic and LS-associated CRC

The two screening tests used in the prediction of LS are MMR protein expression assessed by immunohistochemistry (IHC) and DNA microsatellite instability (MSI) analysis

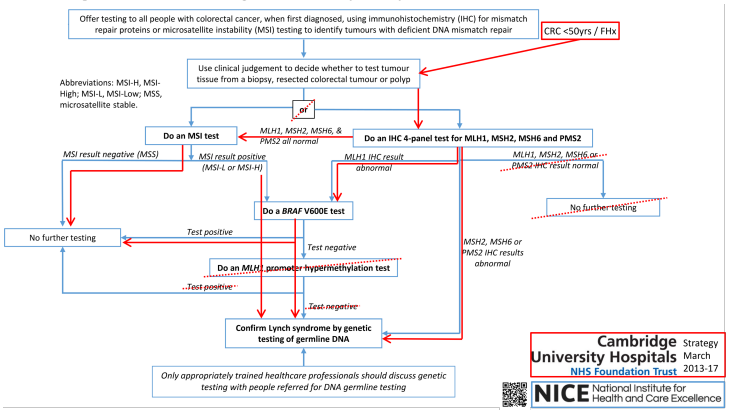
- Previously accepted knowledge had been that MSI was superior to IHC in predicting a germline mutation
- However, later studies showed that, whilst both methods had their limitations, MSI was more sensitive than IHC but slightly less specific

To ascertain the presence and level of discrepancy between the two methods, we compared our IHC and MSI results retrospectively

Patient selection:

- Preselected CRC cases (<50 years or histomorphological / family history criteria suggestive of LS)
- Where MMR IHC testing was performed between March 2013 and March 2017

Techniques: MSI: Promega MSI Analysis System; BRAF: V600E mutation



Conclusions

Discrepancies between MMR and MSI are rare (2/379 (0.5%))

- Performing either test alone may result in patients warranting further investigation for germline mutations being missed
- This has additional importance in view of recent FDA approval of pembrolizumab for MSI-H/MMR-deficient solid tumours
- Is there a need for MMR IHC guidance for pathologists in view of the difficulties of interpretation?

Testing on aged FFPE samples can be problematic

- Limitations in PCR success related to poor DNA quality
- Though aged specimens often show retained MMR IHC, IHC performance can also be affected by prolonged storage
- Failure of successful MSI testing especially on aged specimens should therefore result in routine genetic discussion regardless of MMR IHC status.

Results: Summary Table

Total cases: **516**	MMR proficient: **419** (81%)	MSI not done: **65** (15% of MMR proficient;13% of total cases)		
		MSI done: 354 (84% of MMR proficient; 68% of total cases)	**MS stable: 333/354 (94%)**	
			Likely / suboptimal MSS: 8/354 (2.3%)	
			MSI-high: 3/354 (0.8%)	1 2 3
			MSI-indeterminate: 2/354 (0.6%) *One case indeterminate due to technical failure*	
			Sample unsuitable: 8/354 (2.3%) *6 specimens were aged 11-29yrs*	
	MMR deficient: **97** (19%)	MSI not done: **72** (74% of MMR deficient;14% of total cases) **MLH1 and or PMS2 abnormal: 60/72 (83%)** • ***BRAF*** **V600E mutation 29/50 (58%)**		
		MSI done: 25 (26% of MMR deficient; 5% of total cases)	**MS stable: 4/25 (16%)** (4% of MMR deficient; 0.8% total) **MLH1 and or PMS2 abnormal: 4/4 (100%)** • ***BRAF*** **V600E mutation 0/1 (0%)**	4 5 6 7
			Likely MSI-high: 1/25 (4%) *23yrs of storage caused suboptimal DNA quality*	
			MSI-high: 20/25 (80%) **MLH1 and or PMS2 abnormal: 14/20 (70%)** • ***BRAF*** **V600E mutation 3/8 (38%)**	

Results: Identified MMR-MSI Discrepancies

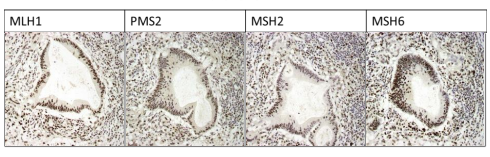
Case	MLH1	PMS2	MSH2	MSH6	Comment
1 *	+	+	+	+	No germline mutation in any of the four genes
2	+	-	+	+	All IHC originally reported as positive. Germline mutation in MLH1
3	+	+	+	-/-	All IHC originally reported as positive. Germline mutation in MSH6

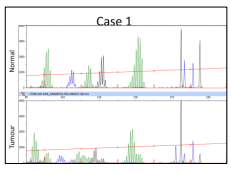
* true mismatch

Case	MLH1	PMS2	MSH2	MSH6	Comment
4	+ (weak)	+ (weak)	+/+	+	PMS2 originally reported as negative, no MLH1 germline or loss of heterozygosity (LOH)
5 *	-/+/-	-/-/-	+/+/+	-/-/-	No MLH1 germline or LOH
6	+/+	+/+	+	+	Original report states "Interpretation of MLH1 and PMS2 is difficult with focal weak nuclear positivity". BRAF mutation negative
7	+	+	+	+	MLH1/PMS2 originally reported as weak. BRAF not done

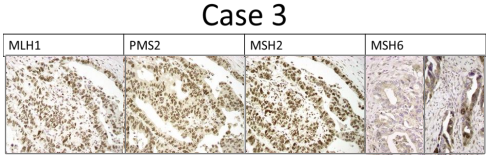
* true mismatch

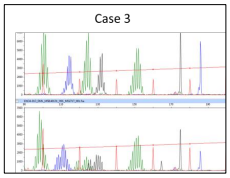
Case 1





Case 3





Case 5