Analytic Validity

Data Extraction for Analytic Validity

Study: (Author, year, UI)

Inclusion criteria (all must be yes)

	Yes	No
Did study evaluate biological material from patients with CRC considered to be at risk for HNPCC?		
Did the study report ANY of the following? (check which one below)		
1) Proportion MSI-H with NIH markers versus other markers		
2) Sensitivity or specificity of MSI-H using NIH markers compared with a reference standard that the study claims is better		
3) Sensitivity or specificity of IHC compared with an immunohistochemical standard that study claims is better		
4) Sensitivity or specificity of a genetic technique compared with a reference standard (or combination of standards)		
5) Reliability of MSI/IHC/genetic method across laboratories or within a laboratory		
Are data (proportions or 2 X 2 tables) extractable or reliability data extractable?		

*NIH markers are BAT-25, BAT-26 D2S123, DS346 and D17S250

Exclusion criteria (exclude if yes)

	Yes	No
Did the study include the index test in the reference standard?		

Describe source of biological materials (and whether patients were known to have an HNPCC phenotype)	Summarize how materials were processed and analyzed

MSI Proportion (add additional 2 X 2 tables where relevant)

Proportion MSI-H using NIH markers (≥2 markers)	Proportion MSI-H using other markers

MSI with a reference standard

		MSI-H using another reference standard	Describe
		Positive	**Negative**
MSI-H using NIH markers	**MSI-H (≥2 markers)**		
	MSI-S or MSI-L		

IHC with a reference standard

		IHC using another immunohistochemical reference standard	Describe
		Positive	**Negative**
IHC	**Positive**		
Describe	**Negative**		

Genetic technique with a reference standard

		Reference standard genetic technique	Describe
		Positive	**Negative**
Index genetic technique	**Positive**		
Descirbe	**Negative**		

Intra or inter-hospital reliability data	Describe
	Of 18 participating centers 2 were excluded: one because slides were damaged in transit and the other because of insufficient staining. Sensitivity for detecting loss of hMSH2 2 expression ranged from 84 to 100%; 10 centers identified all six. 5/6 false positive results were in the same case suggesting that staining or interpretation were not random. 14/16 laboratories showed 100% specificity (one laboratory had 93% specificity due to staining failure on one slide and one lab demonstrated 45% specificity due to weak or absent staining in most cases. Re-review of returned hMSH2 slides shoed lack of

Intra or inter-hospital reliability data	Describe
	internal positive control staining in at least 2 of the 6 hMSH2-negative cases from 8 of 16 centers. The other 8 centers had 100% sensitivity and 93-100% specificity on re-review. The slides that lacked internal positive control staining wre largely accounted for by two cases suggesting the possibility of fixation or processing variation. Variation of staining quality and interpretation was much greater for hMLH1 than for hMSH2. individual centers reported 0 to 100% sensitivity and 40 to 100% specificity. Re-review of the returned slides resulted in sensitivities of 0-90%. 12 centers experienced difficulty with lack of internal positive control or high background. Overall, four laboratories performed relatively well with both hMLH1 and hMSH2 staining protocols. The key element common to these and distinguishing them from the rest was a heated antigen retrieval step. Steam treatment in the presence of EDTA provided the best results although steam and citrate buffer also provided acceptable results.

	Study Quality	Yes	No	Unc
1	Was the description of how MSI or IHC and other genetic techniques described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
2	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
3	Was MSI, IHC, other genetic testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
4	Was there a clear description of which mismatch repair mutations were being tested for?			
5	Were quality control methods described for the molecular and genetic tests?			
6	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
7	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			
8	Was microdissection performed?			
9	Did the study specify whether the biological tissues were from patients known to have HNPCC clinically?			
10	Did the study include a control group in which biological material was obtained from patients known not to have HNPCC clinically			
11	**Overall rating (A B C)**			

Clinical Validity

Study: (Author, year, UI)

Inclusion/exclusion criteria for clinical validity (all must be checked "yes" for study to be included)

	Yes	No
Did study enroll patients with CRC?		
Was genetic testing compared with an index test (must have *at least* one of the following: suggestive family history, MSI, or IHC)?		
Was a minimum of hMLH1 and hMSH2 sequencing performed?		

Characteristics of Design

Population inclusion/exclusion criteria; country, single or multicenter,	**Design and analytic strategy** *(brief description of strategy used for testing patients with CRC)*	**Which MMR mutations were sought?**	**Characteristics of laboratory testing (see definitions below)**				**Predictors analyzed (check all that apply)**		**How were deleterious, missense/variants mutations defined (ND if not described)**
				Y	N	?	Am 1		
			≥5 MSI markers used?				Am R		
			MSI-H defined by ≥ 2 markers?				Beth 1		
			Microdissection?				Beth R		
			Gene screening?				MSI-H		
			Deletion analysis?				MSI-L		
			Conversion analysis?				IHC		
							Age <50		
							Suggestive family history		
							Specify		
							Other		
							Specify		

Am 1= original Amsterdam criteria; Am 2 = revised Amsterdam criteria; Beth 1= original Bethesda Guidelines; Beth 2 = Revised Bethesda Guidelines
MSI-H = Microsattelite instability high; MSI-L = Microsatellite instability low; IHC= immunohistochemistry (staining for mismatch repair proteins); ?=unclear

Results

N enrolled, Mean age, %male dropouts, reasons for dropouts	**Proportion with a mismatch repair mutation (describe which MMR gene).** *(e.g. 23 patients with MMR/1000 patients with CRC 18 MLH1 5 MSH2)*	**Proportion of overall population positive for each predictor** *(e.g. of 1000 patients with CRC, 5% positive AM, 10% positive Beth, 12% suggestive family history)*	**Correlation of predictors to one another (e.g. IHC versus MSI)** *Report what study found such as correlation coefficients or other measures of correlation*	**Study conclusion** *(What did the authors conclude about the testing strategy or other major findings).*	**Implications to family /other findings or comments from authors relevant to any key question**	**Quality grade (see checklist below) and add specific comments about study quality/concerns here**

	How was Lynch Syndrome defined (check all that apply)?		**Specify numerator and denominator and any comments (ND if not described)**
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had an MMR gene mutation?	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had MSI (high or low, please specify)	Amsterdam 1		
	Amsterdam R		
	Other (specify)		
Among patients with the Lynch syndrome defined clinically (i.e. fulfillment of the Amsterdam criteria) what proportion had abnormal IHC	Amsterdam 1		
	Amsterdam R		
	Other (specify)		

	Yes	No
Did the study perform testing only on patients who fulfilled clinical criteria (i.e. when clinical suspicion for HNPCC was increased?		

If yes, which clinical criteria (check all that apply)?	
Am 1 +	
Am R +	
Beth 1 +	
Beth R +	
Age <50	
Suggestive family history (specify)	
Other (specify)	

COPY A NEW 2 X 2 TABLE FOR EACH INDEX TEST

What was the population (i.e. from table above)? "ND" if not a restricted population *(e.g. Patients with CRC who had a suggestive family history)*	Index tests	Check ***ONE***	Index test	Number with MMR+	Number with MMR-
	Am 1 +		+	(A)	(B)
	Am R +		-	(C)	(D)
	Beth 1 +				
	Beth R +				
	MSI-H*				
	MSI-L*				
	Age <50				
	IHC (no staining)				
	Other (specify)**				

***Note** comparator to MSI-H or MSI-L is MSI-stable

**Add combinations of tests under "other" category (eg. MSI-H plus positive IHC for MLH1; MSI-H plus negative results on methylation analysis).

Grade	Explanation for Quality Scoring
A	Most or all of the criteria are fulfilled and the conclusions of the study would be very unlikely to be affected by those that are not.
B	Some of the criteria are fulfilled and the conclusions of the study would be unlikely to be affected by those that are not
C	Few or no criteria were fulfilled and the conclusions of the study would be thought likely or very likely to be altered by multiple omissions in the required criteria for an acceptable study

Item	Criteria	Yes	No	Unclr
	General Quality Criteria			
1	Were unselected patients with CRC included? (i.e. were representative of patients seen in clinical practice {not selected based upon a suggestive family history or other criteria that may cause selection bias)			
2	Inclusion criteria clear?			
3	Did the whole sample or a random selection of the sample (i.e. total population of patients with CRC) receive verification using gene sequencing?			
4	Were the results of IHC or MSI or other predictors interpreted without knowledge of the results of sequencing? (i.e. was there blinding).			
5	Were the results of sequencing interpreted without knowledge of the results of the index test results (i.e. was there blinding)			
6	Did authors describe how uninterpretable or intermediate results were analyzed (e.g. badly stained tissues etc)?			
7	Were withdrawals from the study explained?			
8	Did the authors report ***AND*** analyze results for deleterious MMR mutants			
	Analytic Validity			
9	Was the description of how MSI or IHC or other predictors described in sufficient detail that others could replicate it (e.g. either a full description or relevant references)?			
10	Did authors describe what specimens were tested (e.g., blood, tumor tissue etc.)?			
11	Was MSI, IHC, sequencing or other testing performed at a similar time interval between specimen collection and processing for all subjects evaluated?			
12	Was there a clear description of which mismatch repair mutations were being tested for?			
13	Was there a clear description of percentage of eligible subjects for whom valid genotypic data were obtained across study groups (e.g., the proportion of patients who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI who underwent sequencing….i.e. avoid verification bias)?			
14	Were quality control methods described for the molecular and genetic tests?			
15	Did the authors attempt to address the reproducibility of results (reliability of tests)?			
16	Did the authors specify that samples from each group of subjects (e.g., those who fulfilled and did not fulfill clinical criteria or those who did or did not have MSI) included in each batch analyzed? (i.e. this helps minimize the effect of random errors).			

Genetic and molecular testing methods

	Examples of tests
Genetic screening methods	Single-stranded conformation polymorphism (SSCP) Conformation sensitive gel electrophoresis (CSGE) Denaturing gradient gel electrophoresis (DGGE) Denaturing high-pressure liquid chromatography (DHPLC)
Deletion analysis	Southern blotting Multiplex Ligation-dependent Probe Amplification (MLPA)
Conversion analysis	Process of converting diploid cells to haploid cells. This is potentially important in HNPCC since the presence of a normal allele can sometimes make it difficult to identify a mutation in the mutant allele
MSI methods	Should have testing for five or more MSI markers. The five markers are "BAT25, BAT26, D2S123, D5S346 AND D17S250"
IHC	All methods ok
Microdissection	Whether or not the tumor tissue used for MSI is microdissected to reduce the amount of DNA contributed by non-tumor cells definitely affects the sensitivity of detecting MSI because the non-tumor cells are microsatellite stable and reduce the unstable peak signals as a percentage of the total signal, not allowing detection of MSI at many loci.

Benefits and Harms

Data Extraction Form For Benefits/Harms

Au, Year, UI, Country Single or Multicenter?

Study description (N enrolled)	How was HNPCC defined?	Inclusion/exclusion criteria	Intervention(s)
	Did all patients have a personal history of an HNPCC-related cancer? Check one Yes / No / Uncl		

Outcomes	Dropouts, explanations, other comments	Study Conclusions	Quality score* (comments)

Place an X in boxes that the study is relevant

Perspective	Benefits/Harms screening (e.g. family history, Amsterdam, Bethesda other)	Benefits/Harms genetic testing	Benefits/Harms counseling/informing	Efficacy of counseling (e.g., ability to accurately convey risks and options, minimize anxiety, inform decisions to take tests)	Accuracy/penetrance testing for HNPCC for HNPCC-related cancers/factors such as race, age sex, comorbidities that may be related to accuracy/penetrance	Benefits/Harms management options individuals with a mutation/Outcomes (e.g. early detection, mortality/morbidity, decision-making by patients, family members and providers, or public health/policy?)
Proband with CRC	**2,3**	**5**			**8c**	**1,6a,6b,7,10**
Family member/other high risk individual	**2,3**	**5**	**9**	**8a**	**8b,8c**	**1,6b,9,10,11**
Public health or policy	**2,3**	**5**	**9**	**8a**	**8b,8c**	**1,6,a,6b,7,10**

***Score Overall Quality of Study as Follows**

(A) Most overall quality rating scores are an "A" and the results of the study are considered to provide strong evidence
(B) Most overall quality rating scores are a "B" and the results of the study are considered to provide moderate evidence
(C) Most overall quality rating scores are a "C" and the results of the study are considered to provide weak evidence

Domain/question	***Place an "X" in one***					***Overall rating***		
Selection Bias						A (strong)	B (moderate)	C (weak)
Are individuals selected to participate likely to be representative of target population?	Very likely	Somewhat likely	Not likely					
What % of selected individuals agreed to participate?	80-100	60-79	<60	ND	NA			
Allocation Bias						A (strong)	B (moderate)	C (weak)

(RCTs only, for quasi-experimental, case-control/before/after, no control group or other skip to "Confounders")								
Is the method of random allocation stated?	Yes	No						
If the method of random allocation is stated, is it appropriate?	Yes	No						
Was the method of random allocation reported as concealed?	Yes	No						
Confounders						A (strong)	B (moderate)	C (weak)
Prior to the intervention, were there between group differences for important confounders reported in the paper?	Yes	No	Can't tell					
If there were differences between groups for important confounders, were they adequately managed in the analysis?	Yes	No	NA					
Were there important confounders NOT reported in the paper (describe above under quality score)?	Yes	No						
Blinding						A (strong)	B (moderate)	C (weak)
Was (were) the outcome assessor(s) blinded to the intervention or exposure status of the participants?	Yes	No	ND	NA				
Data Collection methods						A (strong)	B (moderate)	C (weak)
Were data collection tools shown or are they known to be valid?	Yes	No						
Were data collection tools shown or are they known to be reliable?	Yes	No						
Withdrawals and Dropouts						A (strong)	B (moderate)	C (weak)
Indicate the % of participants completing the study. (If the % differs by groups, record the lowest).	80-100	60-79	<60	ND	NA			
Analysis						A (strong)	B (moderate)	C (weak)
Is there a sample size calculation or power calculation?	Yes	Partially	No					
Is there a statistically significant difference between groups?	Yes	No	ND					
Are the statistical methods appropriate?	Yes	No	ND					
Indicate the unit of allocation	Community	Organization/group	Provider	Client	Institution			
Indicate the unit of analysis	Community	Organization/group	Provider	Client	Institution			
If the unit of allocation and analysis differed, was the cluster analysis done?	Yes	No	NA					
Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?	Yes	No	Can't tell					
Intervention Integrity						A (strong)	B (moderate)	C (weak)
What % of participants received the allocated intervention or exposure of interest?	80-100	60-79	<60	ND	NA			

Was the consistency of the intervention measured (i.e. intervention was provided to all participants in the same way)?	Yes	No	ND	NA				
Is it likely that subjects received an unintended intervention (contamination or cointervention) that may influence the results?	Yes	No	Can't tell					