Pathology & Oncology Research
https://doi.org/10.1007/s12253-020-00811-5

REVIEW

Diagnostic Accuracy of Immunohistochemistry for Mismatch Repair Proteins as Surrogate of Microsatellite Instability Molecular Testing in Endometrial Cancer

Antonio Raffone[1] · Antonio Travaglino[2] · Marco Cerbone[1] · Annarita Gencarelli[2] · Antonio Mollo[3] · Luigi Insabato[2] · Fulvio Zullo[1]

Received: 12 December 2019 / Accepted: 30 March 2020

Abstract

Microsatellite instability (MSI) defines one of the four molecular groups of endometrial carcinoma identified by The Cancer Genome Atlas (TCGA). Immunohistochemistry for mismatch repair (MMR) proteins (MLH1, MSH2, MSH6, PMS2) has been proposed as a widely applicable technique to identify this group in the common practice. However, the diagnostic accuracy of such approach has never been calculated. We aimed to assess: 1) the diagnostic accuracy of MMR proteins immunohistochemistry as surrogate of MSI molecular testing in endometrial carcinoma; 2) whether a combination of only two MMR proteins may be used as a still cheaper test. A systematic review and meta-analysis of was performed by searching electronic databases from their inception to September 2019. All studies assessing endometrial carcinoma with both MMR proteins immunohistochemistry and MSI molecular testing were included. Diagnostic accuracy was assessed as sensitivity, specificity, positive and negative likelihood ratios (LR+, LR-), diagnostic odds ratio (DOR) and area under the curve (AUC) on SROC curves. A subgroup analysis was performed for a combination of only two MMR proteins (MLH1-MSH2 vs MSH6-PMS2). Ten studies with 3097 patients were included. Out of these, 1110 were suitable for the meta-analysis. Immunohistochemistry for all the four MMR proteins showed sensitivity = 0.96, specificity = 0.95, LR + =17.7, LR- = 0.05, DOR = 429.77, and high diagnostic accuracy (AUC = 0.988). The combination of MLH1 and MSH2 showed sensitivity = 0.88, specificity = 0.96, LR + =22.36, LR- = 0.15, DOR = 200.69, and high diagnostic accuracy (AUC = 0.9838). The combination of MSH6 and PMS2 showed the same results as the complete panel of four MMR proteins. In conclusion, MMR proteins immunohistochemistry is a highly accurate surrogate of MSI molecular testing in endometrial carcinoma. A combination of MSH6 and PMS2 may allow reducing the cost without decrease in the diagnostic accuracy.

Keywords Mismatch repair · Microsatellite instability · Endometrial cancer · Risk assessment · ProMisE · TCGA

Introduction

Endometrial carcinoma is the most common cancer in women in the Western countries, and the fourth most prevalent gynecologic tumor worldwide [1–3]. The current risk assessment of patients with endometrial carcinoma is inaccurate and still based on poorly reproducible histological examination [2, 4–6]. It has been considered at the basis of the increase of incidence and mortality of endometrial carcinoma in the last decades [2, 7–9]. In fact, the poor reproducibility may lead to overtreatment and undertreatment of patients, and mistakes in patients' selection in clinical trials [2, 7–9].

In 2013, The Cancer Genome Atlas (TCGA) Research Network proposed a novel classification of endometrial carcinoma into four molecular prognostic groups:

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12253-020-00811-5) contains supplementary material, which is available to authorized users.

✉ Antonio Travaglino
antonio.travaglino@unina.it

[1] Gynecology and Obstetrics Unit, Department of Neuroscience, Reproductive Sciences and Dentistry, School of Medicine, University of Naples Federico II, Naples, Italy

[2] Anatomic Pathology Unit, Department of Advanced Biomedical Sciences, School of Medicine, School of Medicine, University of Naples Federico II, Via Sergio Pansini, 5, 80131 Naples, Italy

[3] Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Salerno, Italy

POLE/ultramutated, microsatellite instability (MSI)/hypermutated, copy-number high, and copy-number low. [10, 11]. The prognostic value of such classification has repeatedly been confirmed, offering the possibility of drastically improving the patient management [2, 7–9, 12, 13]. However, this classification appeared little applicable in the common practice, mainly due to high costs and technical difficulties of sequencing analysis [2, 7–9]. For this reason, great interest has been given to the search for more applicable immunohistochemical surrogates of molecular markers [2, 3]. In fact, immunohistochemistry has long since been used to predict genetic alterations in endometrial neoplastic lesions [14–19].

A novel classifier, the Proactive Molecular Risk Classifier for Endometrial Cancer (ProMisE), has proposed immunohistochemistry for p53 and mismatch repair (MMR) proteins as surrogates of molecular testing for copy-number status and MSI, respectively [7–9]. The MMR panel that is usually tested includes four proteins: MLH1, MSH2, MSH6 and PMS2 [20–27]. However, a combination of only MLH1 and MSH2 has been previously adopted [28], while a combination of only MSH6 and PMS2 has more recently been proposed [29].

Nevertheless, the accuracy of MMR proteins immunohistochemistry as surrogate of MSI molecular testing has never been calculated, as well as the accuracy of a combination of only two MMR proteins rather than the complete MMR panel.

This study aimed to define 1) the diagnostic accuracy of MMR proteins immunohistochemistry as surrogate of MSI molecular assay in endometrial carcinoma and 2) whether a combination of only two MMR proteins may be used instead of all the four MMR proteins to reduce the costs, through a systematic-review and meta-analysis.

Materials and Methods

Study Protocol

Protocol for all stages of the study (search strategy, study selection, risk of bias within studies assessment, data extraction and analysis) was a priori established. The study followed the Synthesizing Evidence from Diagnostic Accuracy Tests (SEDATE) guidelines [30] and the Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement [31]. All review steps were independently completed by 3 reviewers (AR, AT, MC) and disagreements were discussed with other authors.

Search Strategy

Web of Sciences, Scopus, MEDLINE, Google Scholar, EMBASE, ClinicalTrial.gov and Cochrane Library were searched from their inception to September 2019, with a combination of the following text words: "MMR"; "mismatch repair"; "MSI"; "microsatellite instability", "MLH1"; "MSH2"; "MSH6"; "PMS2"; "Lynch Syndrome"; "surrogate"; "endometrium"; "tumor"; "tumour"; "carcinoma"; "endometrial cancer"; "endometrioid adenocarcinoma"; "serous"; "undifferentiated"; "clear cell"; "endometrium"; "immunohistochemistry"; "immunohistochemical"; "marker"; "prognosis"; "Atlas"; "cancer"; "genome"; "PCR"; "sequencing"; "testing"; "assay"; "TCGA"; "PORTEC"; "TransPORTEC"; "Proactive Molecular Risk Classifier"; "ProMisE". Relevant references from each selected study were also evaluated.

Study Selection

We included all peer-reviewed studies assessing the association between MMR proteins immunohistochemistry and MSI molecular assay in endometrial carcinoma. Exclusion criteria were: reviews; case reports; studies not allowing comparisons between immunohistochemistry and molecular analysis; sample size <5 patients; study assessing the above-mentioned association in other cancers. In the case of overlapping data between studies (i.e. same institution, study period, and/or results), we only included the study with the higher study population. No language or country restriction was planned.

Risk of Bias within Studies Assessment

The risk of bias within studies assessment was performed following the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) [32]. Four domains related to the risk of bias were evaluated for each included study: 1) Patient selection (i.e. if the patient selection included consecutive or randomly selected women); 2) Index test (i.e. if the MMR proteins assessed and the results of immunohistochemistry were clearly and completely reported); 3) Reference standard (i.e. if the microsatellite markers assessed and the results of MSI molecular testing were clearly and completely reported); 4) Flow and Timing (i.e. if all women were evaluated with both index test and reference standard; if all women were evaluated with the same tests, if the results were not affected by the latency time between index test and reference standard). Authors judged every domain as "low risk," "unclear risk" or "high risk" of bias based on data were "reported and adequate", "reported but inadequate" or "not reported", respectively. For the domains 1, 2 and 3, concerns about applicability (i.e. if study methods were not applicable to our review, regardless of their correctness) were also assessed.

Data Extraction

Original data were extracted without modification. Two-by-two contingency tables were built for each included study based on two qualitative variables:

- immunohistochemical expression of MMR proteins (index test), dichotomized as "MMR-proficient" vs "MMR-deficient";
- microsatellite status assessed by molecular analysis (reference standard), dichotomized as "microsatellite-stable" (MSS) vs "microsatellite-instable" (MSI).

MMR-proficient was defined as positive nuclear staining of all MMR proteins, while MMR-deficient was defined as complete loss of staining of any of the MMR proteins, in the presence of positive internal control (lymphocytes and stroma).

MSS was defined as absence of instability in any mononucleotide or dinucleotide markers in the microsatellite panel assessed, while MSI was defined as instability in at least 30% of mononucleotide or dinucleotide markers in the microsatellite panel assessed; the presence of instability in less than 30% of mononucleotide or dinucleotide markers in the microsatellite panel assessed (usually defined as "MSI-low") was lumped together with MSS, as consistently done in the literature [20–29, 33].

Data Analysis

Cases of endometrial carcinomas with MMR-deficient and MSI were considered as true positive; cases with MMR-proficient and MSS were considered as true negative; cases with MMR-proficient and MSI were considered as false negative; cases with MMR-deficient and MSS were considered as false positive.

We calculated sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR-) and diagnostic odds ratio (DOR) of MMR proteins immunohistochemistry as a surrogate of MSI molecular testing, in each included study and as pooled estimate. Results were graphically shown on forest plots with 95% confidence interval (CI).

We also calculated area under the curve (AUC) on summary receiver operating characteristic (SROC) curves. The accuracy of MMR proteins immunohistochemistry as a surrogate of MSI molecular testing was judged as absent for $AUC \leq 0.5$, low for $0.5 < AUC \leq 0.75$, moderate for $0.75 < AUC \leq 0.9$, high for $0.9 < AUC < 0.97$, very high for $AUC \geq 0.97$.

Lastly, we calculated post-test probabilities of MSI in the case of MMR-proficient of MMR-deficient. Results were graphically shown on a Fagan's nomogram with 95% CI. The pre-test probability (i.e. prevalence of MSI in endometrial carcinoma) of 28% derived from TGCA findings [8].

Statistical heterogeneity amongst the included studies was evaluated by using the Higgins I^2 index, and judged as null for $I^2 = 0\%$, minimal for $0\% < I^2 \leq 25\%$, low for $25 < I^2 \leq 50\%$, moderate for $50 < I^2 \leq 75\%$ and high for $I^2 > 75\%$.

We adopted the random effect model of DerSimonian and Laird for all analyses regardless of the heterogeneity, according to the SEDATE guidelines [30].

Additional analysis was performed by separating data about index test into two subgroups based on the use of only two MMR proteins (MLH1 and MSH2, or MSH6 and PMS2) rather than all the four MMR proteins. We calculated sensitivity, specificity, LR+, LR-, DOR, AUC on SROC curves, and post-test probabilities for each subgroup.

The data analysis was performed by using Meta-DiSc version 1.4 (Clinical Biostatistics Unit, Ramon y Cajal Hospital, Madrid, Spain) and Review Manager 5.3 (Copenhagen: The Nordic Cochrane Centre, Cochrane Collaboration, 2014).

Results

Study Selection

303 articles were identified through database search. 238 articles remained after duplicate removal. 171 articles remained after titles screening. 111 articles were evaluated for eligibility after abstracts screening. Finally, 10 observational studies were included in the systematic review [20–29], and five studies with 1110 patients were included in the meta-analysis [21, 22, 25, 27, 29]. The whole process of study selection is reported in detail in Supplementary Fig. 1.

Study and Patient Characteristics

Among the included studies, 2 [20, 22] were prospective and 8 were retrospective (Table 1). Mean age and body mass index (BMI) were 63 years and 30.5 kg/m^2. Characteristics of the included studies are shown in Table 1. Most endometrial carcinomas (92.35%) were endometrioid adenocarcinoma, while other histotypes were: serous (2.92%), mixed (1.86%), carcinosarcoma (1.24%), clear cell (0.93%), undifferentiated (0.13%), mucinous (0.09%), unknown (0.49%). Grading was G1-G2 (low grade) in 80.5%, G3 (high grade) in 17.6% and unknown in 1.9% of cases. FIGO stage was I-II (limited to the uterus) in 88%, III in 9.4%, IV in 2.1% and unknown in 0.5% of cases. Pathological features of endometrial carcinomas are shown in Table 2.

Histological specimens were obtained by hysterectomy in all the included studies. On immunohistochemistry, eight studies assessed the complete MMR proteins panel (MLH1, MSH2, MSH6, PMS2), while one study only assessed MLH1 and MSH2 [28], and the remaining study assessed MLH1, MSH2 and MSH6 [26]. DNA was extracted from paraffin-embedded tissue in 8 studies, form fresh tissue in 1 study [25] and from both types of tissue in the remaining one [27]. MSI testing was performed by polymerase chain reaction (PCR) in all studies. Details regarding immunohistochemical

Table 1 Characteristics of the included studies

Study	Country	Setting	Study design	Period of enrolment (*Years*)	Sample size	Patients selection	BMI Mean	Meaan age (range)
Chao et al. [20]	China	Peking Union Medical College Hospital	Prospective	2017–2018 (1)	102	Consecutive	23 (19–31)	56 (32–82)
Libera et al. [21]	Italy	IRCCS Institute for Pharmacological Researches Mario Negri	Retrospective	2017–2018 (1)	35	Consecutive	25,6	51.60 (31–67)
Bruegl et al. **[22]**	Texas	University of Texas MD Anderson Cancer Center	Prospective	2012–2014 (2)	192	Consecutive	33,8 (15,5—74,7)	61.3 (23–86)
Stelloo et al. [29]	Netherlands	Leiden University Medical Centre	Retrospective	1990–1997 (7) 2002–2006 (4)	696	Consecutive	not reported	69 (41–88)
McConechy et al. [25]	Canada	Medicine, University of British Columbia and BC Cancer Agency	Retrospective	not reported	89	Consecutive	not reported	62.6
Goodfellow et al. [23]	Ohio	State University Comprehensive Cancer Center	Retrospective	2003–2007 (4)	360	Consecutive	35 (16.6–82.8)	62 (25–100)
Haraldsdottir et al. **[24]**	Ohio	The Ohio State University Comprehensive Cancer Center	Retrospective	2012–2014 (3)	15	not reported	not reported	59.5 (47–65)
Peterson et al. [27]	Minnesota	Mayo Clinic, Rochester,.	Retrospective	not reported	93	not reported	not reported	66 (42–92)
Choi et al. [28]	Republic of Korea	Chonnam National University Medical School	Retrospective	1998–2002 (*5*)	39	not reported	not reported	53.4 (30–68)
Ollikainen et al. *[26]*	Finland	University of Helsinki, Departments of Medical Genetics	Retrospective	1986–1997 (*12*)	33	Consecutive	35	62

and molecular procedures are reported in detail in Table 3 and Supplementary Table 1.

Overall, there were 32 endometrial carcinomas who resulted MSI-low at molecular testing; out of these, 19 cases showed intact MMR, 9 cases showed a loss of MLH1 and PMS2, 2 cases showed a loss of PMS2 alone and 2 cases showed a loss of MSH6 alone [21, 22, 25, 29].

Risk of Bias within Studies Assessment

In the "patient selection" domain, three studies were judged at unclear risk of bias because they did not report if the patients were consecutive or randomly selected [24, 27, 28], while the remaining studies were considered at low risk. High concerns about applicability were raised for two studies (only patients with presumed Lynch syndrome were included) [24, 26].

In the "index test" domain, two studies were considered at high risk of bias because results of MMR proteins immunohistochemistry were incompletely reported [20, 23], while the remaining studies were considered at low risk. High concerns about applicability were raised for two studies (not all MMR proteins were assessed) [26, 28].

In the "reference standard" domain, all studies were considered at low risk of bias, since results of MSI testing and the microsatellite markers assessed were clearly reported. No concerns about applicability were raised.

In the "flow and timing" domain, 3 studies were considered at unclear risk of bias, because it was unclear if all eligible patients were assessed with both index and reference standard [22, 23, 29]; all the remaining studies were considered at low risk.

Results of risk of bias within studies assessment are shown in Supplementary Fig. 2.

Diagnostic Accuracy Assessment

By using all the four MMR proteins, immunohistochemistry showed a pooled sensitivity of 0.96 (95% CI, 0.93–0.98) in detecting MSI, with moderate heterogeneity among studies (I^2 = 74.7%). Pooled specificity was 0.95 (95% CI, 0.93–0.96) with minimal heterogeneity (I^2 = 22.7%). Pooled positive and negative likelihood ratios were 17.7 (95% CI, 11.9–26.33) and 0.05 (95% CI, 0.01–0.2) respectively, with minimal heterogeneity (I^2 = 17.9%) and high heterogeneity (I^2 =

Table 2 Characteristics of endometrial carcinomas

	Tumor stage (%)					Tumor grade (%)				Histotype (%)							
Study	**I**	**II**	**III**	**IV**	**n.r.**	**G1**	**G2**	**G3**	**n.r.**	**END**	**CC**	**SER**	**MUC**	**CAS**	**MIX**	**UND**	**n.r.**
Chao et al. [20]	87	0	19	4	1	0	0	0	0	87	3	9	0	4	7	1	
	(78,4)	(0)	(17,1)	(3,6)	(0)	(0)	(0)	(0)	(0)	(78,4)	(2,7)	(8,1)	(0)	(3,6)	(6,3)	(0,9)	(0)
Libera et al. [21]	26	1	1		7	12	14	9	0	32	1	1	1	0	0		
	(74,3)	*(2,9)*	*(2,9)*	*(0)*	*(20)*	*(34,3)*	*(40)*	*(25,7)*	*(0)*	*(91,4)*	*(2,9)*	*(2,9)*	*(2,9)*	*(0)*	*(0)*	*(0)*	*(0)*
Bruegl et al. [22]	151	13	29	20		19	139	55	0	158	4	15	0	3	31	2	
	(70,9)	*(6,1)*	*(13,6)*	*(9,4)*	*(0)*	*(8,9)*	*(65,3)*	*(25,8)*	*(0)*	*(74,2)*	*(1,9)*	*(7)*	*(0)*	*(1,4)*	*(14,6)*	*(0,9)*	*(0)*
Stelloo et al. [29]	696		0	0	0	587		109	0	679	0	17	0	0	0		
	(100)		*(0)*	*(0)*	*(0)*	*(84,3)*	*(0)*	*(15,7)*	*(0)*	*(97,6)*	*(0)*	*(2,4)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*
McConechy et al. [25]	61	6	17	3	2	30	27	30	2	71	0	12	0	0	0		6
	(68,5)	*(6,7)*	*(19,1)*	*(3,4)*	*(2,2)*	*(33,7)*	*(30,3)*	*(33,7)*	*(2,2)*	*(79,8)*	*(0)*	*(13,5)*	*(0)*	*(0)*	*(0)*	*(0)*	*(7,7)*
Goodfellow et al. [23]	702	88	129	19		383	408	147	0	938	0	0	0	0	0		
	(74,8)	*(9,4)*	*(13,8)*	*(2)*	*(0)*	*(40,8)*	*(43,5)*	*(15,7)*	*(0)*	*(100)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*
Haraldsdottir et al. [24]	13		1	0	0	6	3	5	0	11	1	0	0	1	1		
	(92,9)		*(7,1)*	*(0)*	*(0)*	*(42,9)*	*(21,4)*	*(35,7)*	*(0)*	*(78,6)*	*(7,1)*	*(0)*	*(0)*	*(7,1)*	*(7,1)*	*(0)*	*(0)*
Peterson et al. [27]	0	0	0	0	0	33	16	12	35	61	2	10	0	20	3		
	(0)	*(0)*	*(0)*	*(0)*	*(0)*	*(34,4)*	*(16,7)*	*(12,5)*	*(36,5)*	*(63,5)*	*(2,1)*	*(10,4)*	*(0)*	*(20,8)*	*(3,1)*	*(0)*	*(0)*
Choi et al. [28]	33		6	0	0	20	11	8	0	30	0	0	0	0	0		9
	(84,6)		*(15,4)*	*(0)*	*(0)*	*(51,3)*	*(28,2)*	*(20,5)*	*(0)*	*(76,9)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	*(23,1)*
Ollikainen et al. [26]	23	1	1	0	0	10	9	3	3	22	0	2	1	0	0		
	(92)	*(4)*	*(4)*	*(0)*	*(0)*	*(40)*	*(36)*	*(12)*	*(12)*	*(88)*	*(0)*	*(8)*	*(4)*	*(0)*	*(0)*	*(0)*	*(0)*
TOTAL (cases)	1902		203	46	10	1837		378	40	2089	21	66	2	28	42	3	11
TOTAL %	*(88,0)*		*(9,4)*	*(2,1)*	*(0,5)*	*(80,5)*		*(17,6)*	*(1,9)*	*(92,35)*	*(0,93)*	*(2,92)*	*(0,09)*	*(1,24)*	*(1,86)*	*(0,13)*	*(0,49)*

END: endometrioid; **CC**: clear cell; **SER**: serous; **MUC**: mucinous; **CAS**: carcinosarcoma; **MIX**: mixed; **n.r.:** not reported

80.7%) respectively. Pooled DOR was 429.77 (95% CI, 145.82–1266.68), with low heterogeneity ($I^2 = 36.4\%$). The overall accuracy was very high, with an AUC of 0.988 (Fig. 1).

In the case of a positive test (negative immunohistochemical nuclear staining of any of the four MMR proteins), the post-test probability of MSI was 88% (95% CI, 83–90%), while in the case of a negative test (normal immunohistochemical nuclear staining of all the four MMR), the post-test probability was 2% (95% CI, 2–3%) (Fig. 3a).

Subgroup Analysis

By using only MLH1 and MSH2, immunohistochemistry showed a pooled sensitivity of 0.88 (95% CI, 0.84–0.91) in detecting MSI, with moderate heterogeneity among studies ($I^2 = 64.1\%$). Pooled specificity was 0.96 (95% CI, 0.95–0.97) with no heterogeneity ($I^2 = 0\%$). Pooled positive and negative likelihood ratios were 22.36 (95% CI, 15.84–31.56) and 0.15 (95% CI, 0.09–0.27) respectively, with no heterogeneity ($I^2 = 0\%$) and moderate heterogeneity ($I^2 = 67.9\%$), respectively. Pooled DOR was 200.69 (95% CI, 117.82–341.83), with no heterogeneity ($I^2 = 0\%$). The overall diagnostic accuracy was very high, with an AUC of 0.9838 (Fig. 2). In the case of a positive test (negative immunohistochemical nuclear staining of MLH1 and/or MSH2), the post-test probability of MSI was 90% (95% CI, 86–93%), while in the case of a negative test (normal immunohistochemical nuclear staining of both MLH1 and PMS2), the post-test probability was 6% (95% CI, 4–7%) (Fig. 3b).

By using only MSH6 and PMS2, there were no differences compared to the assessment of all the four MMR proteins; therefore, the results of diagnostic accuracy were the same as the main analysis (Fig. 1, Fig. 3a).

Discussion

Main Findings and Interpretation

Our study showed that immunohistochemistry for MMR proteins was a highly accurate immunohistochemical surrogate of MSI molecular testing in endometrial carcinoma, with an AUC of 0.988. The combination of MLH1 and MSH2 led to

Table 3 Details about mismatch repair (MMR) proteins immunohistochemistry and microsatellite instability (MSI) molecular testing

STUDY	MMR PROTEINS IMMUNOHISTOCHEMISTRY		MSI MOLECULAR TESTING				
	MMR PROTEINS TESTED	NUMBER	DNA EXTRACTED FROM	MOLECULAR TECHNIQUE	MICROSATELLITE MARKERS TESTED	NUMBER OF MARKERS	POSITIVE MSI TRESHOLD
Chao et al. [20]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample	Multiplex fluorescent PCR	BAT-25, BAT-26, NR-21, NR-24, NR-27, MONO-27	6	≥2 loci
Libera et al. [21]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample	Multiplex fluorescent PCR	BAT-25, BAT-26, NR-21, NR-22, NR-24	5	≥2 loci
Bruegl et al. [22]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample	Multiplex fluorescent PCR	BAT-25, BAT-26, D5S346, D2S123, D17S250, TGFBR2, NR-21,	7	≥3 loci
Stelloo et al. [29]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample	Multiplex fluorescent PCR	BAT-25, BAT-26, NR-21, NR-24, MONO-27	5	≥2 loci
McConechy et al. [25]	MLH1, MSH2, MSH6, PMS2	4	Fresh-frozen tissue	Polymerase chain reaction (PCR)	BAT-25, BAT-26, D17S250, D5S346, D2S123	5	≥2 loci
Goodfellow et al. [23]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample	Polymerase chain reaction (PCR)	BAT-25, BAT-26, DSS346, D2S123, D17S250	5	≥2 loci
Haraldsdottir et al. [24]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample	Multiplex fluorescent PCR	BAT-25, BAT-26, NR-21, NR-24, MONO-27	5	≥2 loci
Peterson et al. [27]	MLH1, MSH2, MSH6, PMS2	4	Paraffin-embedded tumor sample and Fresh-frozen tissue	Polymerase chain reaction (PCR)	BAT-25, BAT-26, D17S250, D5S346, ACTC, D18S55, BAT-40, D10S197, BAT34c4, MYCL	10	≥3 loci
Choi et al. [28]	MLH1, MSH2	2	Paraffin-embedded tumor sample	Polymerase chain reaction (PCR)	BAT-25, BAT-26, BAT-40, D2S123, D17S250, D8S554	6	≥2 loci
Ollikainen et al. [26]	MLH1, MSH2, MSH6	3	Paraffin-embedded tumor sample	Multiplex Fluorescent PCR	BAT-25, BAT-26, D5S346, D2S123, D17S250	5	≥2 loci

a slight decrease in the accuracy (AUC = 0.9838), while the combination of MSH6 and PMS2 showed the same accuracy as all the four MMR proteins.

A microsatellite is a tract of DNA in which a short sequence of one or more base pairs is repeated (usually 5–50 times). The human genome contains hundreds of thousands of microsatellite loci [34, 35]. MSI indicates a condition in which the length of microsatellites is altered due to deletion or insertion of repeating units, and represents a common condition in several human neoplasms, in particular colorectal, endometrial and gastric carcinoma [33]. MSI is typically associated with high mutational load and leads to the exposition of numerous neo-antigens, generating a strong immune response; this offers the possibility of using immunotherapy for treating MSI carcinomas [36]. On this account, the Food and Drug Administration has approved the use of immune checkpoint inhibitors in any solid tumor with MSI [37].

In endometrial carcinoma, MSI is the hallmark of one of the four prognostic subgroups identified by TCGA [10]. The MSI subgroup is mainly constituted by endometrioid carcinomas of variable grade, and typically have an intermediate prognosis [2, 7–9, 11, 12]. In fact, in early stage endometrioid carcinomas (which usually have a good prognosis), MSI appears as an unfavorable prognostic factor, while in high-risk carcinomas (which usually have a poor prognosis), MSI appears as a favorable prognostic factor [38, 39].

Given the outstanding prognostic value of the TCGA classification, it has become mandatory to introduce such

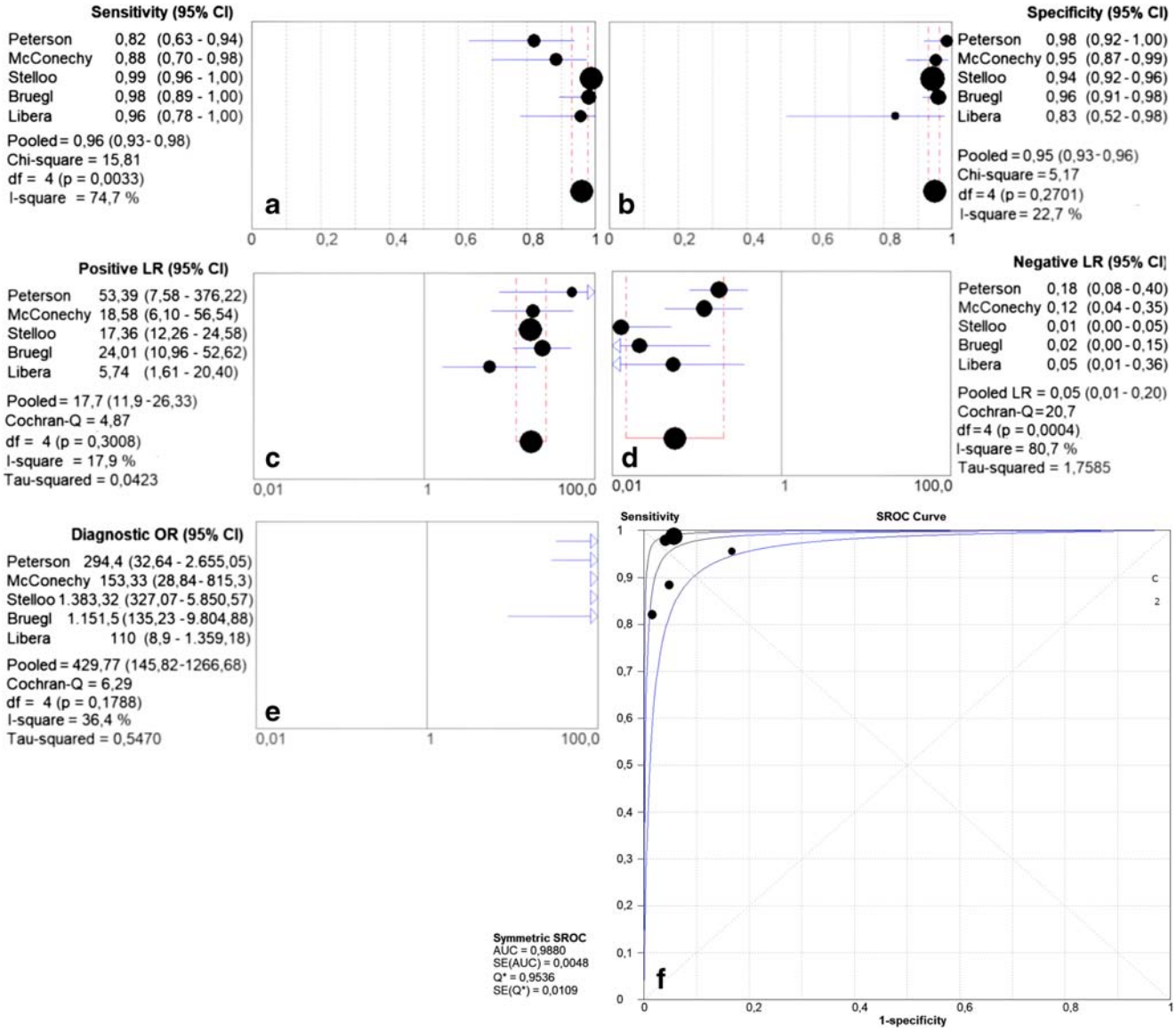

Fig. 1 Forest plots reporting sensitivity (**a**), specificity (**b**), positive and negative likelihood ratios (**c** and **d**), diagnostic odds ratio (**e**) with SROC curves (**f**) of immunohistochemistry for mismatch repair proteins (complete panel) as surrogate of microsatellite instability molecular testing

classification in the common practice, overcoming the issues related to the complexity and costs of molecular analysis; immunohistochemistry has played a crucial role in this field [2, 3, 7–9].

MMR proteins immunohistochemistry has appeared as the obvious candidate surrogate of MSI molecular testing [7–9]. Indeed, MSI is usually caused by an inefficient activity of any of the main four MMR proteins: MLH1, MSH2, MSH6 and PMS2 [33]. In this field, the most used MMR proteins have been MLH1 and MSH2, whose defect accounts for most cases of MSI. [28, 40, 41]. More recently, a combination of PMS2 and MSH6 has been proposed as a possible alternative to the complete panel of four MMR proteins [29].

However, the diagnostic accuracy of MMR proteins expression for identifying MSI in endometrial carcinoma has never been calculated. Such information may be crucial for a definitive validation of MMR immunohistochemistry as an easy, cheap and widely available testing for the MSI prognostic group. Furthermore, if substantiated by a diagnostic accuracy analysis, the use of only two MMR may further reduce the costs for the introduction of the TCGA classification in the clinical practice.

Our results showed that the immunohistochemical assessment of all the four MMR proteins was a very highly accurate surrogate of MMR testing in endometrial carcinoma, with high sensitivity (0.96) and specificity (0.95), and an AUC of about 0.99. This finding strongly confirms the usefulness of MMR proteins immunohistochemistry, supporting its practical use for identifying the MSI TCGA group.

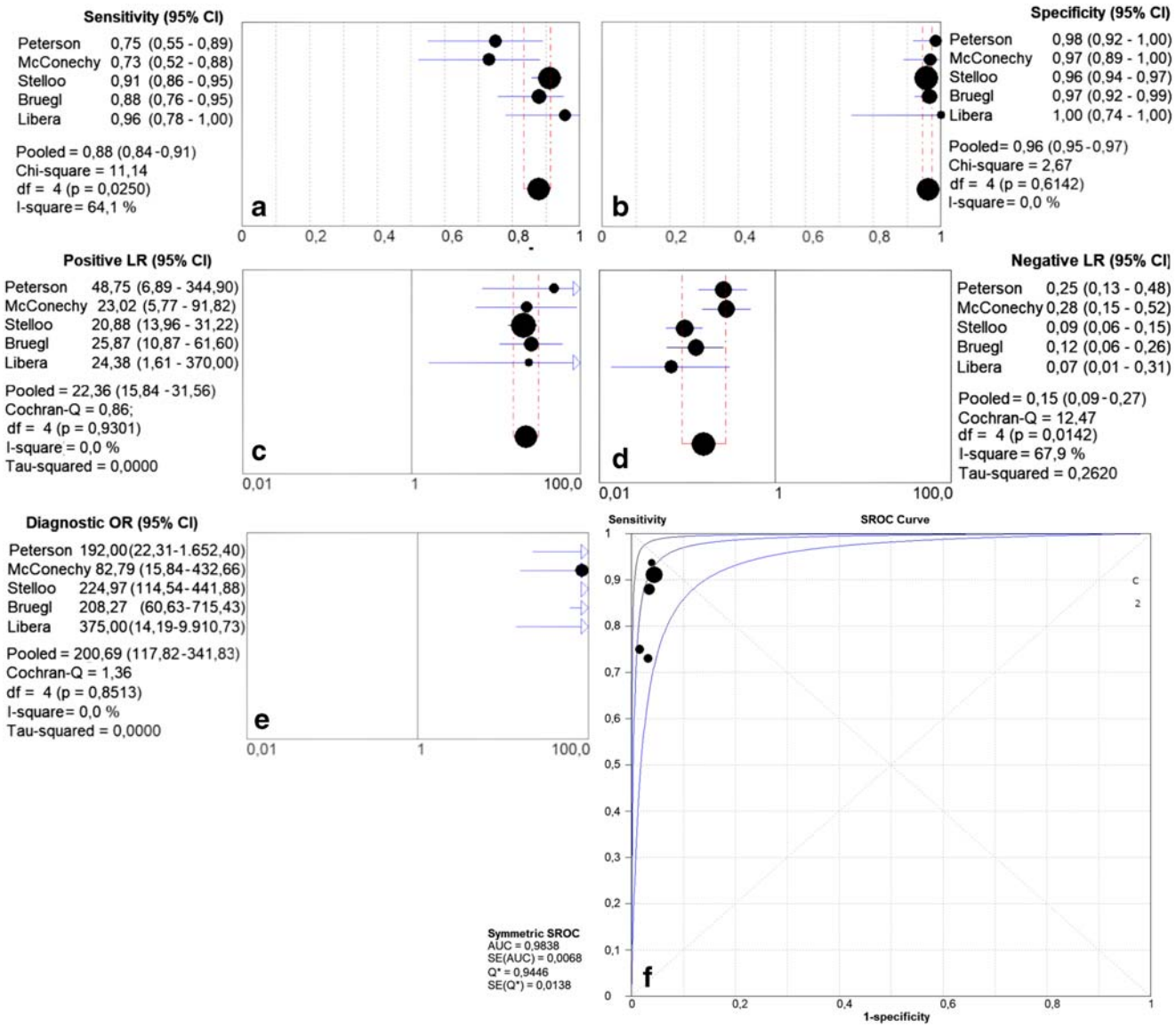

Fig. 2 Forest plots reporting sensitivity (**a**), specificity (**b**), positive and negative likelihood ratios (**c** and **d**), diagnostic odds ratio (**e**) with SROC curves (**f**) of immunohistochemistry for MLH1 and MSH2 as surrogate of microsatellite instability molecular testing

The subgroup analysis showed that a combination of only 2 MMR proteins was accurate enough to be used as a furtherly cheaper surrogate of MSI testing. Indeed, the accuracy remained very high for both MLH1/MSH2 and MSH6/PMS2 combinations. However, while the MLH1/MSH2 combination showed a decrease in sensitivity (0.88) with a slight decrease in the AUC (0.98), the MSH6/PMS2 combination showed the same results as the combination of all the four MMR proteins. This supports that the use of MSH6 and PMS2 alone may allow reducing the costs for immunohistochemistry while maintaining the same accuracy as the complete panel of MMR proteins.

The explanation for these findings appears to lie in the fact that MLH1 and MSH2 form heterodimers with PMS2 and MSH6, respectively: in this regard, it seems that a loss of MLH1 consistently leads to a concomitant loss of PMS2, as well as a loss of MSH2 consistently leads to a concomitant loss of MSH6; on the other hand, the loss of PMS2 or MSH6 does not imply the concomitant loss of MLH1 or MSH2 [42]. In the light of this evidence, reducing the immunohistochemical markers from four to two should not lead to an increased number of false negatives (i.e. patients with MSI but with normal MMR proteins expression).

Taking into account its accuracy and costs, immunohistochemistry for MSH6 and PMS2 may be introduced in the common practice as the routine test for the identifying the MSI prognostic group in endometrial carcinoma.

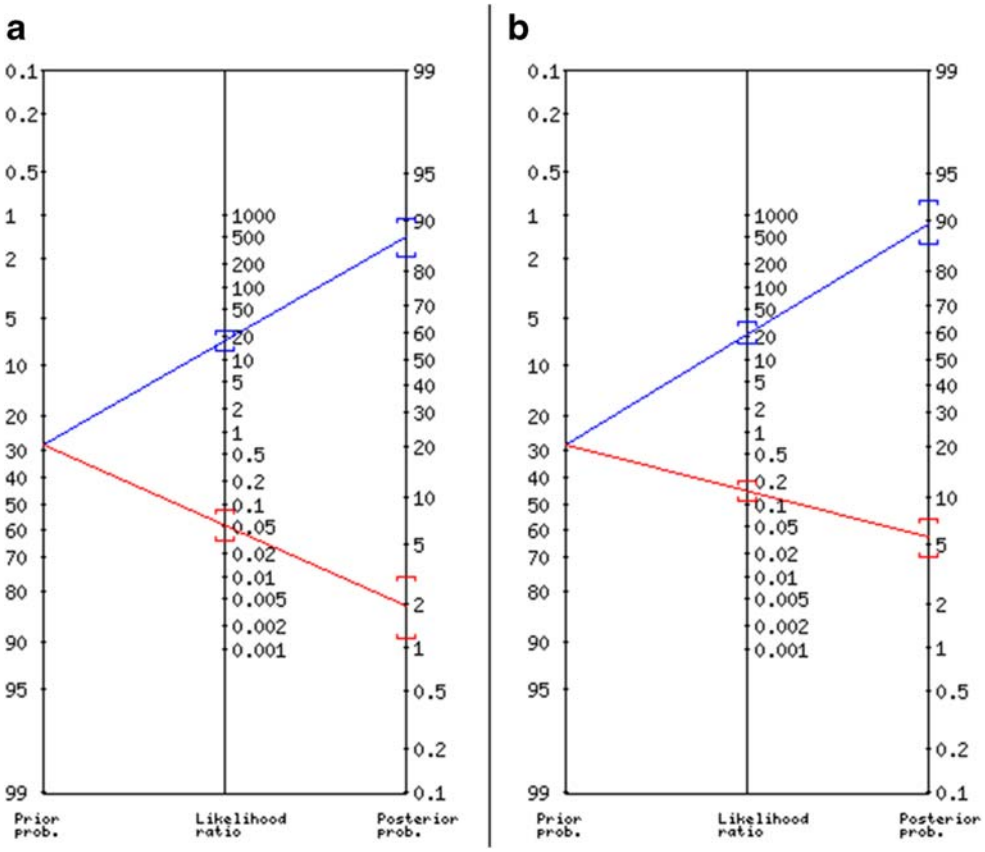

Fig. 3 Fagan's nomogram showing pre-test and post-test probability of microsatellite instability, in case of positive test (red line) or negative test (blue line) at immunohistochemistry for mismatch repair proteins (**a:** complete mismatch repair proteins panel; **b:** MLH1 and MSH2)

Strengths and Limitations

To the best of our knowledge, this may be the first systematic review and meta-analysis focused on this topic. We calculated the diagnostic accuracy of immunohistochemistry of MMR proteins as a surrogate of MSI molecular testing in endometrial carcinoma, confirming the reliability of such approach. Furthermore, we found that the use of only MSH6 and PMS2 may ensure the same accuracy with a further reduction in the costs, strengthening the evidence about the applicability of the TCGA classification.

Limitations of our results may lie in the subjectivity of the pathological assessment of MMR proteins expression and in the possibility of doubtful immunohistochemical patterns [43, 44]. Furthermore, Stelloo et al. showed that the MMR pattern may be heterogeneous across the tumor. In fact, the MSI status and the complete loss of MMR expression may be limited to some areas of the tumor ("subclonal loss"). Stelloo et al. suggested to label the tumor as "MSI" if the subclonal loss involves at least 10% of the tumor area [29]. Unfortunately, the other included studies did not specify the area required for a diagnosis of MSI.

Another limitation might be the lack of a univocal microsatellite panel for the assessment of MSI. In fact, several combinations of microsatellite marker have been proposed over time [20–29]. However, high concordance has been shown between different microsatellite panels [33].

Conclusion

Immunohistochemistry for MMR proteins (MLH1, MSH2, MSH6, PMS2) is a very highly accurate surrogate of MSI molecular testing in endometrial carcinoma. A combination of MSH6 and PMS2 may allow reducing the costs without a decrease in diagnostic accuracy. These findings support the feasibility of the introduction of the TCGA classification into the common practice.

Author Contributions AT, AR and MC independently assessed electronic search, eligibility of the studies, inclusion criteria, risk of bias, data extraction and data analysis. Disagreements were resolved by discussion with AM, LI, AG and FZ. MC, AM and LI contributed to the elaboration of methods for risk of bias assessment, data extraction and analysis. AT, AR and FZ conceived the study; AM, MC, AG and FZ worked on the design of the study; AT, AR, MC, AM, LI, AG and FZ worked on the manuscript preparation; LI and FZ supervised the whole study.

Compliance with Ethical Standards

Conflict of Interest The authors report no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A (2015) Cancer statistics, 2015. CA Cancer J Clin 65(1):5–29
2. Raffone A, Travaglino A, Mascolo M, Carbone L, Guida M, Insabato L, Zullo F (2019a) TCGA molecular groups of endometrial cancer: pooled data about prognosis. Gynecol Oncol 155(2): 374–383
3. Travaglino A, Raffone A, Saccone G, de Luca C, Mollo A, Mascolo M, de Placido G, Insabato L, Zullo F (2019a) Immunohistochemical nuclear expression of β-catenin as a surrogate of CTNNB1 exon 3 mutation in endometrial Cancer. Am J Clin Pathol 151(5):529–538
4. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, Marth C, Nout R, Querleu D, Mirza MR, Sessa C, ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group (2016) ESMO-ESGO-ESTRO consensus conference on endometrial Cancer: diagnosis, treatment and follow-up. Ann Oncol 27(1):16–41
5. Gilks CB, Oliva E, Soslow RA (2013) Poor interobserver reproducibility in the diagnosis of high-grade endometrial carcinoma. Am J Surg Pathol 37:874–881
6. Hoang LN, McConechy MK, Kobel M et al (2013) Histotype-genotype correlation in 36 high-grade endometrial carcinomas. Am J Surg Pathol 37:1421–1432
7. Talhouk A, McConechy MK, Leung S, Li-Chang HH, Kwon JS, Melnyk N, Yang W, Senz J, Boyd N, Karnezis AN, Huntsman DG, Gilks CB, McAlpine J (2015) A clinically applicable molecular-based classification for endometrial cancers. Br J Cancer 113(2): 299–310
8. Talhouk A, McConechy MK, Leung S, Yang W, Lum A, Senz J, Boyd N, Pike J, Anglesio M, Kwon JS, Karnezis AN, Huntsman DG, Gilks CB, McAlpine J (2017) Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. Cancer 123(5):802–813
9. Kommoss S, McConechy MK, Kommoss F, Leung S, Bunz A, Magrill J, Britton H, Kommoss F, Grevenkamp F, Karnezis A, Yang W, Lum A, Krämer B, Taran F, Staebler A, Lax S, Brucker SY, Huntsman DG, Gilks CB, McAlpine J, Talhouk A (2018) Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. Ann Oncol 29(5): 1180–1188
10. Network CGAR et al (2013) Integrated genomic characterization of endometrial carcinoma. Nature 497(7447):67–73
11. Travaglino A, Raffone A, Mascolo M et al (2019b) Clear cell endometrial carcinoma and the TCGA classification. Histopathology. https://doi.org/10.1111/his.13976
12. Stelloo E, Nout RA, Osse EM et al (2016) Improved risk assessment by integrating molecular and Clinicopathological factors in early-stage endometrial Cancer. Clin Cancer Res 22(16):4215–4224
13. Britton H, Huang L, Lum A et al (2019) Molecular classification defines outcomes and opportunities in young women with endometrial carcinoma. Gynecol Oncol 153(3):487–495
14. Travaglino A, Raffone A, Saccone G, Mascolo M, Pignatiello S, Mollo A, de Placido G, Insabato L, Zullo F (2019c) PTEN immunohistochemistry in endometrial hyperplasia: which are the optimal criteria for the diagnosis of precancer? APMIS 127(4):161–169
15. Raffone A, Travaglino A, Saccone G, Viggiani M, Giampaolino P, Insabato L, Mollo A, de Placido G, Zullo F (2019b) PTEN expression in endometrial hyperplasia and risk of cancer: a systematic review and meta-analysis. Arch Gynecol Obstet 299(6):1511–1524
16. Raffone A, Travaglino A, Saccone G, Mascolo M, Insabato L, Mollo A, de Placido G, Zullo F (2019c) PAX2 in endometrial carcinogenesis and in differential diagnosis of endometrial hyperplasia. A systematic review and meta-analysis of diagnostic accuracy. Acta Obstet Gynecol Scand 98(3):287–299
17. Travaglino A, Raffone A, Saccone G, Insabato L, Mollo A, de Placido G, Zullo F (2019d) Immunohistochemical predictive markers of response to conservative treatment of endometrial hyperplasia and early endometrial cancer: a systematic review. Acta Obstet Gynecol Scand 98(9):1086–1099
18. Raffone A, Travaglino A, Saccone G, Cieri M, Mascolo M, Mollo A, Insabato L, Zullo F (2019d) Diagnostic and prognostic value of ARID1A in endometrial hyperplasia: a novel marker of occult cancer. APMIS 127(9):597–606
19. Travaglino A, Raffone A, Saccone G et al (2019e) Nuclear expression of β-catenin in endometrial hyperplasia as marker of premalignancy. APMIS. https://doi.org/10.1111/apm.12988
20. Chao X, Li L, Wu M et al (2019) Comparison of screening strategies for Lynch syndrome in patients with newly diagnosed endometrial cancer: a prospective cohort study in China. Cancer Commun (Lond) 39:42
21. Libera L, Craparotta I, Sahnane N, Chiaravalli AM, Mannarino L, Cerutti R, Riva C, Marchini S, Furlan D (2018) Targeted gene sequencing of lynch syndrome-related and sporadic endometrial carcinomas. Hum Pathol 81:235–244
22. Bruegl AS, Ring KL, Daniels M et al (2017) Clinical Challenges Associated with Universal Screening for Lynch Syndrome-Associated Endometrial Cancer. Cancer Prev Res (Phila) 10:108–115
23. Goodfellow PJ, Billingsley CC, Lankes HA, Ali S, Cohn DE, Broaddus RJ, Ramirez N, Pritchard CC, Hampel H, Chassen AS, Simmons LV, Schmidt AP, Gao F, Brinton LA, Backes F, Landrum LM, Geller MA, DiSilvestro P, Pearl ML, Lele SB, Powell MA, Zaino RJ, Mutch D (2015) Combined microsatellite instability, MLH1 methylation analysis, and immunohistochemistry for lynch syndrome screening in endometrial cancers from GOG210: an NRG oncology and gynecologic oncology group study. J Clin Oncol 33:4301–4308
24. Haraldsdottir S, Hampel H, Tomsic J et al (2014) Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. Gastroenterology 147: 1308–1316.e1
25. McConechy MK, Talhouk A, Li-Chang HH, Leung S, Huntsman DG, Gilks CB, McAlpine J (2015) Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. Gynecol Oncol 137:306–310
26. Ollikainen M, Abdel-Rahman WM, Moisio AL, Lindroos A, Kariola R, Järvelä I, Pöyhönen M, Butzow R, Peltomäki P (2005) Molecular analysis of familial endometrial carcinoma: a manifestation of hereditary nonpolyposis colorectal cancer or a separate syndrome. J Clin Oncol 23:4609–4616
27. Peterson LM, Kipp BR, Halling KC, Kerr SE, Smith DI, Distad TJ, Clayton AC, Medeiros F (2012) Molecular characterization of endometrial cancer: a correlative study assessing microsatellite instability, MLH1 hypermethylation, DNA mismatch repair protein expression, and PTEN, PIK3CA, KRAS, and BRAF mutation analysis. Int J Gynecol Pathol 31:195–205
28. Choi YD, Choi J, Kim JH et al (2008) Microsatellite instability at a tetranucleotide repeat in type I endometrial carcinoma. J Exp Clin Cancer Res 27:88
29. Stelloo E, Jansen AML, Osse EM, Nout RA, Creutzberg CL, Ruano D, Church DN, Morreau H, Smit VTHBM, van Wezel T, Bosse T (2017) Practical guidance for mismatch repair-deficiency testing in endometrial cancer. Ann Oncol 28:96–102
30. Sotiriadis A, Papatheodorou SI, Martins WP (2016) Synthesizing evidence from diagnostic accuracy tests: the SEDATE guideline. Ultrasound Obstet Gynecol 47(3):386–395

31. Moher D, Shamseer L, Clarke M et al (2015) Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic Rev 4:1
32. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM, QUADAS-2 Group (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155(8):529–536
33. Murphy KM, Zhang S, Geiger T, Hafez MJ, Bacher J, Berg KD, Eshleman JR (2006) Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. J Mol Diagn 8(3): 305–311
34. Richard GF, Kerrest A, Dujon B (2008) Comparative genomics and molecular dynamics of DNA repeats in eukaryotes. Microbiol Mol Biol Rev 72(4):686–727
35. Gulcher J (2012) Microsatellite markers for linkage and association studies. Cold Spring Harb Protoc 4:425–432
36. Colle R, Cohen R, Cochereau D, Duval A, Lascols O, Lopez-Trabada D, Afchain P, Trouilloud I, Parc Y, Lefevre JH, Fléjou JF, Svrcek M, André T (2017) Immunotherapy and patients treated for cancer with microsatellite instability. Bull Cancer 104(1):42–51
37. Chang L, Chang M, Chang HM, Chang F (2018) Microsatellite instability: a predictive biomarker for Cancer immunotherapy. Appl Immunohistochem Mol Morphol 26(2):e15–e21
38. Moroney MR, Davies KD, Wilberger AC, Sheeder J, Post MD, Berning AA, Fisher C, Lefkowits C, Guntupalli SR, Behbakht K, Corr BR (2019) Molecular markers in recurrent stage I, grade 1 endometrioid endometrial cancers. Gynecol Oncol 153:517–520
39. Stelloo E, Bosse T, Nout RA, MacKay HJ, Church DN, Nijman HW, Leary A, Edmondson RJ, Powell ME, Crosbie EJ, Kitchener HC, Mileshkin L, Pollock PM, Smit VT, Creutzberg CL (2015) Refining prognosis and identifying targetable pathways for high-risk endometrial cancer; a TransPORTEC initiative. Mod Pathol 28: 836–844
40. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75(5):1027–1038
41. Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD (1994) Mutation of a mutL homolog in hereditary colon cancer. Science 263(5153):1625–1629
42. Vilar E, Gruber SB (2010) Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol 7(3):153–162. https://doi.org/10.1038/nrclinonc.2009.237
43. Sarode VR, Robinson L (2019) Screening for lynch syndrome by immunohistochemistry of mismatch repair proteins: significance of indeterminate result and correlation with mutational studies. Arch Pathol Lab Med 143(10):1225–1233
44. Watkins JC, Nucci MR, Ritterhouse LL, Howitt BE, Sholl LM (2016) Unusual mismatch repair Immunohistochemical patterns in endometrial carcinoma. Am J Surg Pathol 40(7):909–916