Lack of association between deficient mismatch repair expression and outcome in endometrial carcinomas of the endometrioid type

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Abstract

Objective: Endometrial carcinomas of the endometrioid type (EEC) are associated with a good prognosis. However, about 20% of them recur and new prognostic markers are needed. Microsatellite instability (MSI), associated with mismatch repair (MMR) deficiency, is a frequent alteration in EECs that has been associated with prognosis. However, its prognostic impact on EECs remains unclear. The aim of the present study was to clarify the relationship between MMR deficiency and outcome in a large cohort of well classified EECs.

Methods: A total of 212 EEC samples were analyzed by immunohistochemistry for the MMR genes MLH-1, MSH-2, MSH-6 and PMS-2. Kaplan-Meier survival analysis and log-rank tests were performed to study the prognostic significance of MMR deficiency taking into account clinical and pathological parameters.

Results: We observed no association between MMR deficiency and OS or FFS in our 212 EEC patients (p-value=0.6565 and 0.4380, respectively). When we performed the analysis in different FIGO-stage groups, we did not find association between MMR and OS or FFS in stage I, I/II or III/IV. When we analyzed the specific group of patients with lymphatic invasion separately, MMR expression was not associated with OS or FFS either.

Conclusions: MMR deficiency does not seem to be a good prognostic marker in endometrioid type endometrial carcinomas.

Keywords

Mismatch repair, endometrioid carcinomas, immunohistochemistry, outcome

Introduction

Endometrial carcinoma (EC) is the most common malignant tumour of the female genital tract. From a pathogenic viewpoint, EC falls into two different types: type I and II [1]. Type I ECs are represented by low grade endometrioid carcinomas (EECs), which account for approximately 80% of all ECs. These tumors are estrogen related, occur in premenopausal and perimenopausal women, arise in a background of endometrial hyperplasia and are associated with good prognosis [2]. However, about 20% of EECs recur. Therefore, several studies have aimed to find new prognostic markers in order to detect from the beginning patients at high risk of recurrence [3].

EECs frequently have genetic alterations such as microsatellite instability (MSI) [4]. MSI is thought to result from the accumulation of mutations during DNA replication and to be associated with inactivating mutations in mismatch repair (MMR) genes [5]. Presumably, loss of MMR gene expression leads to increased genomic instability. There are four MMR genes of clinical interest: MLH1, MSH2, MSH6 and PMS2 [6].

Clinicopathologic impact of MMR deficiency or MSI on EECs remains unclear. As far as we know, four studies have been performed with EEC patients. Two studies have associated MSI+ with poor prognosis in EECs. Nout et al found association with decreased survival in 65 FIGO stage I EEC patients [7]. An et al found association with poor prognostic indicators in 86 EECs [8]. By contrast, Zighelboim et al did not find any association with outcome in 446 EECs [9]. Mackay et al did not find association with survival in 131 EECs but when only the 78 FIGO stage I/II patients were considered, there was association with a decreased survival [10]. In addition, other studies including EECs in combination with other EC tumours have reported

contradictory associations with better [6, 11] or worse outcome [12] or no association [13].

The aim of the present study was to clarify the relationship between MMR deficiency and outcome in a large cohort of well classified EECs.

Methods

For the current study, 212 EEC samples of paraffin-embedded specimens collected during the period 2001–2007 were retrieved from the archives at the Department of Pathology at University Hospital Donostia. Ethical committee approval was obtained. Clinical and pathological data were obtained from medical records, including age, histological grade, FIGO-stage, myometrial infiltration, lymphatic invasion and outcome.

There are two equivalent screening methods to assess MMR deficiency: PCR based microsatellite instability and immunohistochemistry (loss of MLH1, MSH2, MSH6 or PMS2). The agreement between both methods is excellent (>90%). Our election was Immunohistochemistry because it is described as more accessible, inexpensive and undergoes stringent inter-laboratory quality assurance [14].

Eight tissue microarrays (TMA) were constructed from paraffin-embedded blocks of 212 EEC cases. Representative tumor areas were selected from hematoxilin-eosin sections and marked on individual paraffin blocks. Two tissue cores (1mm diameter) were obtained for each tumor. They were arrayed in a new paraffin block using Manual Tissue Arrayer MTA-1 (Beecher Instruments, Wisconsin, USA).

Four micrometer sections of TMAs were analyzed by immunohistochemistry using antibodies for MLH-1 (ref. 790-4535, Ventana, Tucson, AZ), MSH-2 (ref. 760-4531, Ventana), MSH-6 (ref. 790-4455, Roche) and PMS-2 (ref. 760-4531, Ventana). The staining was performed using a Bench Mark ULTRA system (Ventana).

MLH1, MSH2, MSH6 and PMS2 protein expression was considered lost when less than 5% of tumour cells stained positive in presence of an internal positive control of normal lymphocytes and/or stromal cells [6]. Furthermore, when tumours showed loss of MLH1, MSH2, MSH6 or PMS2 in the TMA samples, full sections of tumour were stained to avoid false-negative interpretations (due to heterogeneous positivity of the tumours for this markers) (Fig. 1). A tumour sample was considered MMR deficient (dMMR) when at least one of the MMR proteins was lost.

The association between MMR protein deficiency and clinical parameters was analyzed with chi-square test. Kaplan-Meier survival analysis and log-rank tests were performed in order to study the prognostic significance of dMMR. Overall survival (OS) was calculated from the date of diagnosis until last follow-up or death, while failure-free survival (FFS) was calculated from date of diagnosis to progression, end of the follow-up period or death. The significance level was set at 5%. Stata/SE 8.0 software was applied for all calculations.

Results

Clinical data were available for all the 212 patients and are summarized in table 1.

Among the 212 patients analyzed, 64 (30.2%) were considered MMR deficient (dMMR). The other 148 patients were MMR normal (nMMR). Association between MMR deficiency and clinical parameters is summarized in table 2.

There was not statistically significant association between MMR deficiency and age, grade, FIGO-stage or myometrial infiltration.

Using long-rank test, we observed no association between MMR deficiency and OS or FFS in our 212 EEC patients (p = 0.6565 and 0.4380 respectively) (Fig 2).

We also analyzed the prognostic value of MMR expression in different FIGO-stage groups. In the 135 patients with FIGO-stage I, we did not find association between MMR and OS or FFS (p = 0.6104 and 0.1711 respectively) (fig. 3). When we analyzed patients in stage I/II (163 patients) and in stage III/IV (35 patients), MMR expression did not show association with OS (p = 0.9032 in stage I/II patients and p = 0.3772 in stage III/IV patients) or FFS (p = 0.2409 in stage I/II patients and p = 0.8361 in stage III/IV patients).

Finally, we analyzed the specific group of patients with lymphatic invasion separately. Only 13 out of our 212 EEC patients had lymphatic invasion described. MMR expression was not associated with OS or FFS in these 13 patients (p = 0.4524 and 0.9097 respectively). However, cases with deficient MMR expression tended to have lower OS (fig. 4).

Discussion

Although low grade endometrioid carcinomas (EECs) are associated with a general good prognosis, about 20% of them recur [3]. Therefore, new prognostic markers are needed, in order to detect patients at risk of recurrence from the beginning. Considering that microsatellite instability (MSI) is a frequent alteration in EECs (30%), its prognostic value has been studied. However, the prognostic significance of MSI or the associated dMMR in EECs remains controversial [1]. Contradictory results among studies could be due to small or non-homogeneous populations, differences in treatment protocols among studies, or the use of different prognostic criteria. In the present study, we evaluated the clinicopathologic and prognostic impact of MMR deficiency in a large and well-characterized population of 212 EECs.

In the present study, we have not found any association between MMR deficiency and OS or DFS in EEC patients. This result is in agreement with two previous studies carried out in 131 and 446 EECs respectively, in which they did not find association between MSI and survival [9, 10]. On the other hand, An et al, in a smaller population of 93 EECs, found association between MSI and poor prognosis indicators [8]. They did not directly analyze survival, which could be one of the reasons for differences in results. In their study, they included higher histological grade, lymphovascular invasion, deep myometrial invasion, higher clinical stages and higher cyclin A and skp2 immunoreactivity. When we analyzed the relationship between MMR deficiency and some of those clinicopathologic parameters in a larger population of 212 EEC patients, we did not find correlation with histological grade, myometrial invasion or clinical stage.

Considering that the association with survival could be stage-specific, we stratified our population according to FIGO stage and performed the analysis for each group. We did not find association between dMMR and survival, OS or FFS, in the 135 FIGO stage I patients or in the 163 FIGO stage I/II patients. By contrast, two previous studies had shown associations in such low stage patients. Nout et al, found associations between MSI and lower survival in 65 FIGO stage I EECs [7] and Mackay et al found the same result in 83 EECs in stage I/II but not in 53 stage III/IV or in the combined population [10]. Nevertheless, these studies were carried out with smaller sample sizes and their statistical power might be limited.

Finally, other studies including ECCs in combination with other EC tumours have reported contradictory associations with better [6, 11] or worse outcome [12] or no association at all [13]. However, this could be due to the inclusion of patients with other more aggressive histology. In particular, Terada et al, described an association with better survival in presence of dMMR in 66 EC patients with lymphatic invasion [6]. Therefore, we studied the subgroup of patients with described lymphatic invasion in our group of well-characterized EECs and we did not find any association with OS or FFS. In this case, our population included 13 patients. Despite the low number of cases included, we found a tendency to decreased OS, which is the opposite. Also, differences in results could be due to the inclusion of cases with non-endometrioid histology in the study by Terada et al, as mentioned above.

Taking our results and previous reports into account, we consider that MMR deficiency does not seem to be a good prognostic marker in endometrial carcinomas of the endometrioid type.

Disclosure Statement

Authors reported no potential conflicts of interest.

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References

1- Matias-Guiu X, Prat J. Molecular pathology of endometrial carcinoma. Histopathology 2013;62:111-23.

2- Alvarez T, Miller E, Duska and Oliva E. Molecular profile of grade 3 endometrioid endometrial carcinoma: is it a type I or type II endometrial carcinoma?. Am J Surg Pathol 2012;36:753-61.

3- Salvesen HB, Haldorsen I, Trovic J. Markers for individualised therapy in endometrial carcinoma. Lancet oncol 2013;13:353-61.

4- Yeremian A, Moreno-Bueno G, Dolcet X, Catasus L, Abal M et al. Endometrial carcinoma: molecular alterations envolved in tumor development and progression. Oncogene 2012;1-11.

5- Banno K, Oiri K, Yanokura M, Tsuni K, Masuda K et al. Biomarkers in endometrial cancer: Possible clinical applications. Oncol Lett 2012;3:1175-80.

6- Terada KY, Black M, Terada LH, Davis J, Shimizu DM. Survival of endometrial cancer patients with lymphatic invasion and deficient mismatch repair expression. Gynecol Oncol 2013;

7- Nout R, Bosse T, Creutzberg CL, Jürgenliemk-Schulz IM, Jobsen JJ et al. Improved risk assessment of endometrial cancer by combined analysis of MSI, PI3K-AKT, Wnt/β-catenin and P53 pathway activation. Gynecol oncol 2012;126:466-73.

8- An HJ, Kim KI, Kim JY, Shim JY, Kang H et al. Microsatellite Instability in endometrioid type endometrial adenocarcinoma is associated with poor prognostic indicators. Am J Surg Pathol 2007; 31: 846-53.

9- Zighelboim I, Goodfellow PJ, Gao F, Gibb RK, Powell MA et al. Microsatellite Instability and Epigenetic Inactivation of MLH1 and outcome of patients with endometrial carcinomas of the endometrioid type. J Clin Oncol 2007;15: 2042-8.

227 10- Mackay HJ, Gallinger S, Tsao MS, McLachlin CM, Tu D et al. Prognostic value of
228 microsatellite instability (MSI) and PTEN expression in women with endometrial
229 cancer: Results from studies of the NCIC Clinical Trials Group (NCIC CTG). Eur J
230 Cancer 2010; 46: 1365-73.
231 11- Black D, Soslow RA, Levine DA, Tornos C, Chen SC et al. Clinicopathologic
232 significance of defective DNA mismatch repsir in endometrial carcinoma. J Clin Oncol
233 2006; 24(11): 1745-53.
234 12- Cohn DE, Frankel WL, Resnick KE, Zanagnolo VL, Copeland LJ et al. Improved
235 survival with an intact DNA mismatch repair system in endometrial cancer. Obstetr
236 Gynecol 2006;105(5):1208-15.
237 13- Nelson GS, Pink A, Lee S, Han G, Morrin D et al. MMR deficiency is common in
238 hight-grade endometrioid carcinomas and is associated with an unfavourable outcome.
239 Gynecol Oncol 2013; 131: 309-314.
240 14- Clarke BA, Cooper K. Identifying Lynch Syndrome patients with endometrial
241 carcinoma: shortcomings of morphologic and clinical schemas. Adv Anat Pathol 2012;
242 19: 231-8.
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Table legends

Table 1: Clinical features of patients

OS, overall survival; FFS, failure free survival

Table 2: Association between MMR deficiency and clinical parameters

Figure legends

Fig. 1. Cases positive for MLH-1, MSH-2, MSH-6 and PMS-2, respectively, in the immunohistochemical analysis.

Fig. 2. Kaplan-Meier analysis of overall survival and failure-free survival based on MMR protein expression in 212 EEC patients.

Fig. 3. Kaplan-Meier analysis of overall survival and failure-free survival based on MMR protein expression in 135 patients with FIGO-stage I.

Fig. 4. Kaplan-Meier analysis of overall survival and failure-free survival based on MMR protein expression in 13 EEC patients with lymphatic invasion.

Tables

Table 1: Clinical features of patients

Parameter		N (%)
Age	≤ 65 years	116 (54.7)
	> 65 years	96 (45.3)
Grade	I	93 (44.1)
	II	96 (45.5)
	III	22 (10.4)
FIGO stage	I	135 (68.2)
	II	28 (14.1)
	III	17 (8.6)
	IV	18 (9.1)
Myometrial infiltration	<50%	155 (73.1)
	≥50%	57 (26.9)
Lymphatic invasion	Yes	13 (6.1)
	No / not described	199 (93.9)
OS 5 years	Yes	181 (95.8)
	No	8 (4.2)
FFS 5 years	Yes	169 (88)
	No	23 (12)

OS, overall survival; FFS, failure free survival

262

Table 2: Association between MMR deficiency and clinical parameters

Parameter	MMR deficient (N, %)	MMR normal (N, %)	P value
Age			
<65 years	36 (56.3)	80 (54)	0.768
≥65 years	28 (43.7)	68 (46)	
Grade			
I	29 (46)	64 (43.2)	0.734
II	29 (46)	67 (45.3)	
III	5 (8)	17 (11.5)	
FIGO stage			
I	44 (73.3)	91 (65.9)	
II	6 (10)	22 (15.9)	0.609
III	4 (6.7)	13 (9.5)	
IV	6 (10)	12 (8.7)	
Myometrial infiltration			
<50%	50 (78.1)	105 (70.9)	0.279
≥50%	14 (21.9)	43 (29.1)	

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Figures

Figure 1

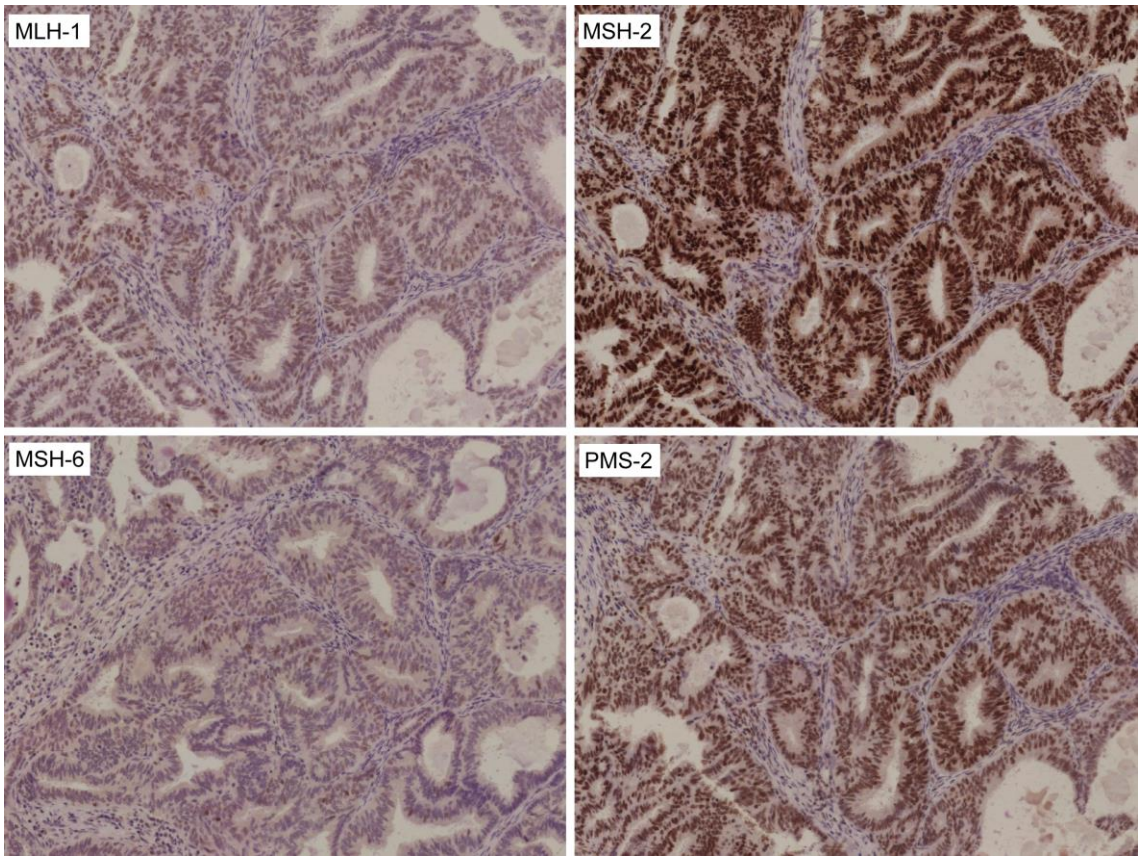
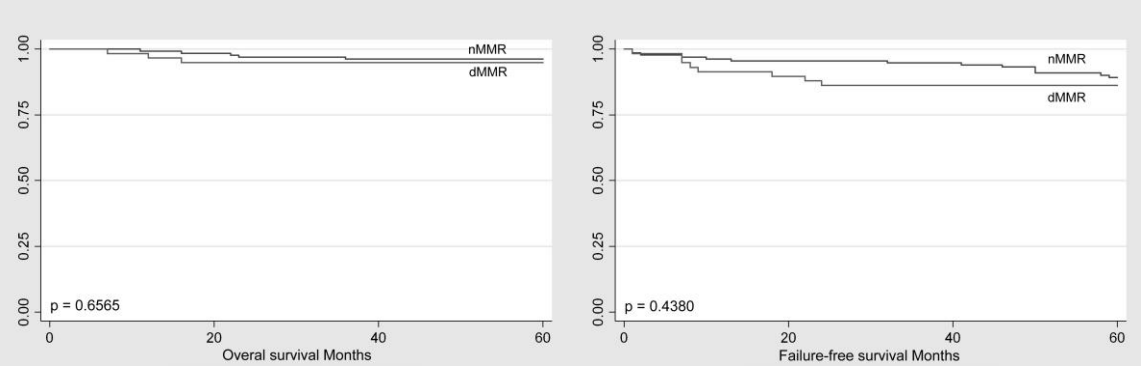


Figure 2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0.75
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nMMR
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Overal survival Months
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nMMR
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p = 0.4380
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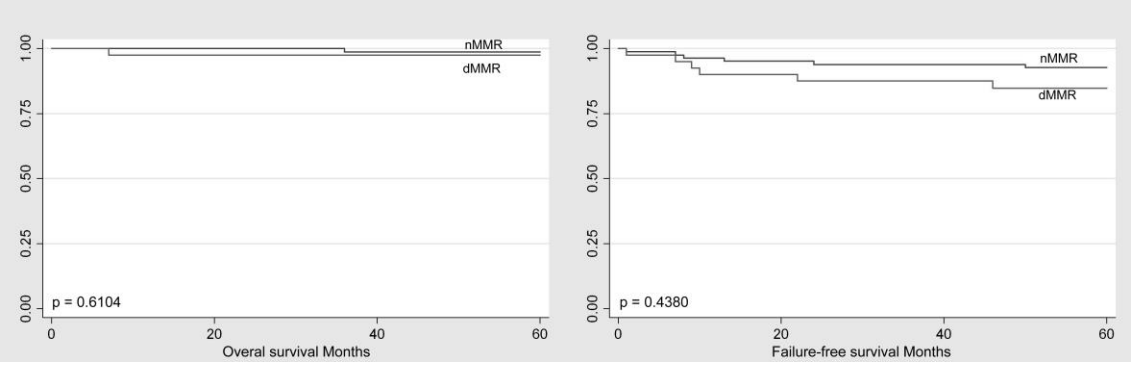
60
Failure-free survival Months

Figure 3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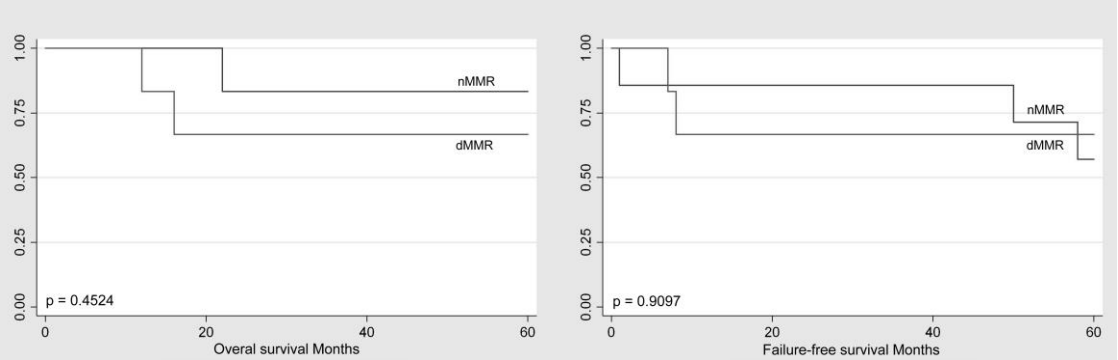
Failure-free survival Months

Figure 4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Failure-free survival Months

Highlights

- Deficient mismatch repair is not associated with outcome in endometrioid carcinomas
- Mismatch repair deficiency is not associated with outcome in FIGO stage subgroups