

## Research Article

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# Endometrial cancer and microsatellite instability status

**Abstract:** Microsatellite instability (MSI) is an important factor in the development of various cancers as an identifier of a defective DNA mismatch repair system. The objective of our study was to define the association between microsatellite instability status and traditional clinicopathologic characteristics of endometrioid type adenocarcinoma. Material and methods: MSI status of endometrial cancer was examined by employing the Promega MSI Analysis System. This system uses 5 mononucleotide markers to identify MSI in tumour and normal tissue DNA (BAT-25, BAT-26, NR-21, NR-24, and MONO-27), and 2 pentanucleotide markers (Penta C and Penta D) for specimen identification. In this study, we investigated MSI status in 109 endometrial carcinomas. Results and conclusions: One hundred (92%) of 109 endometrial cancers showed endometrioid type histology and only 9 (8%) non-endometrioid type. MSI-high was found in 17% (17/100) of endometrioid type adenocarcinomas, in 0% (0/9) of non-endometrioid carcinomas. Selected clinicopathologic parameters for endometrioid type adenocarcinomas were compared to the MSI status which was separated into two groups – MSI-high and MSI stable. The results showed that MSI-high status was related to clinicopathologic parameters such as deep myometrial invasion and higher histologic grade in endometrioid type adenocarcinomas.

**Keywords:** Endometrial cancer, Clinicopathologic parameters, Microsatellite instability

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## 1 Introduction

Endometrial cancer is one of the mostly widespread cancers (6th place) among women worldwide, contributing to 290,000 new cases in 2008, and a standardised incidence rate of 8 per 100,000 women [1]. In Europe endometrial cancer is the fourth most frequent cancers in women and the tenth most frequent reason for cancer mortality [2]. This type of cancer almost exclusively develops for women in the post-menopausal period. Its most common histological subtype is adenocarcinoma of endometrioid type or Type I (70-80% of all endometrial cancers) and another, less common, subtype is adenocarcinoma of non-endometrioid type or Type II (20-30% of all endometrial cancers) [1, 2]. Type I endometrial cancer is usually hormone sensitive, and occurs in women exposed to estrogens. It is usually well differentiated and has a low potential for myometrial invasion and metastasis [1, 3]. Type II endometrial cancer is not associated with estrogen or progestogen stimulation and arises from atrophic endometrial tissues. It is poorly differentiated, has a high probability of myometrial invasion and metastasis, and a worse prognosis [4, 5].

Specific molecular genetic alterations which are thought to be significantly affecting the development of cancer have been identified in several studies of these two different types of endometrial carcinoma [6-8]. One of the molecular alterations associated with endometrioid type (Type I endometrial cancer) is microsatellite instability (MSI), which indicates defects in the DNA mismatch repair system (MMR). Microsatellites are short repetitive nucleotide sequences in the human genome [9, 10]. Because of

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their repeat structure, microsatellites are prone to slippage or replication errors. Usually, such errors are repaired by the DNA mismatch repair system, however this system can fail resulting in microsatellite instability. Microsatellites exhibit the same number of nucleotide repeats in tumour and healthy tissue of the same individual, however loss of MMR can result in widespread changes in the number of repeat sequences. In 1998, the National Cancer Institute (USA) recommended a panel of 5 mono/or di-nucleotide markers for determination of MSI. The tumour is classified as MSI-high (MSI-H) if it shows instability in at least 2 of 5 markers; MSI-low (MSI-L) if 1 of 5 and microsatellite stable (MSS) if none of 5 [11].

This molecular phenotype was originally found in hereditary non-polyposis colon cancer (HNPCC), also known as Lynch syndrome [12-15]. The most common cancer type related to Lynch syndrome is hereditary colorectal carcinoma, which accounts for 1-3% of all colorectal cancers and the second most common type is hereditary endometrial cancer, which accounts for 2% of all endometrial carcinomas [16, 17]. Currently, MSI testing is used when detecting tumours associated with Lynch syndrome as over 90% of such tumors have high level of microsatellite instability. MSI is a well established and effective genetic marker for detection of Lynch syndrome [18].

MSI status in colorectal carcinomas has been thoroughly studied and it has been found to be important not only for detecting HNPCC, but also as a prognostic and predictive factor in sporadic colorectal carcinomas. MSI has been reported in 15% of sporadic colorectal carcinomas and was identified as an independent prognostic marker associated with older age, female sex and such clinicopathologic parameters as prevalence in proximal colon, mucinous histology, lower rate of lymph node metastasis, and systemic metastasis. It was also established that MSI tumours have a more favorable prognosis than microsatellite stable tumours [19, 20]. MSI value as a predictive marker for response to a treatment with 5-FU (5-fluorouracil), irinotecan and other chemotherapeutic agents is controversial. Studies suggest that stage II colorectal cancers with MSI- high phenotype are irresponsive to 5-FU chemotherapy and MSI-high tumours are more sensitive to treatment with irinotecan than MSS tumours [21, 22].

In endometrial carcinoma, however, the relation between clinicopathologic parameters (which are known to be related with the prognosis of the disease) and MSI status is not yet clear. MSI incidence in sporadic endometrial carcinoma in different studies has been detected in the range from 9% to 45% [23-25]. A number of studies have

examined the relation between clinicopathologic parameters and MSI status with conflicting results (reasons being small sample size, retrospective analysis, different MSI markers and failure to account for significance of histological type). The majority of published analyses indicate that the MSI-high is more common in endometrioid type histology of endometrial carcinoma than non-endometrioid type [25-27]. It was also stated that MSI-high endometrial tumours were associated with higher tumour grade, deep myometrial invasion and higher clinical stage [26, 28, 29], whereas the other studies found no relationship between tumour grade, clinical stage and MSI status [23, 24, 30]. None of the studies showed relationship between lower tumour grade and MSI-high and only a few studies observed lower clinical stage in MSI-high endometrial tumours [4]. MSI value as a prognostic factor in sporadic endometrial carcinomas is also not clear. At least one study has found a link between MSI-high status and a higher survival rate [24], in other, though, MSI-high was related with a poor prognosis but only in FIGO I stage of endometrial carcinomas [31]. However, the majority of studies have shown no prognostic advantage [4, 23, 32].

The aim of our study is to investigate whether women age at the time of endometrial cancer diagnosis and selected clinicopathologic parameters such as tumour grade, depth of myometrial invasion and stage of disease found in endometrial cancers are related to MSI-high phenotype. To avoid limitations of other studies and receive conclusive results we have selected only endometrioid type adenocarcinomas and used only mononucleotide MSI detection markers.

## 2 Material and methods

### 2.1 Patients and selection of tissue

One hundred nine patients (109 women) who were treated for endometrial carcinoma during the period between April 2010 and June 2011 at the Institute of Oncology, Vilnius University (now National Cancer Institute) were included in the study and written informed consent was obtained from all participants. The Regional Ethical Committee approval was obtained before initiation of this study (protocol No. 158200-05-180-43). All patients were without known familial history of HNPCC (known also as Lynch syndrome) and the history of other cancers. Samples for this study were collected during the surgical treatment and later examined by an expert pathologist. In

order to histologically classify the endometrial cancers, World Health Organization standards were used and to identify the stage, surgical and pathological data were examined (using the standards of the International Federation of Obstetrics and Gynecology (FIGO) which were established in the year 2010).

## 2.2 DNA extraction and MSI analysis

Specimens of the tumour tissue were extracted from formaline-fixed, paraffin-embedded sample tissue. Tumour tissue DNA was purified by organic extraction, and DNA clean-up was performed. Normal tissue (peripheral blood) DNA was obtained using DNA extraction kit (QIAamp Blood Midi, QIAGEN) according to standard procedures.

The microsatellite phenotype of each endometrial tumour was analysed by employing the Promega MSI Analysis System (version 1.2, Promega Madison, WI, USA). This system uses 5 mononucleotide markers to identify MSI in a tumour and normal tissue DNA (BAT-25, BAT-26, NR-21, NR-24, and MONO-27), and 2 pentanucleotide markers (Penta C and Penta D) to identify whether the tumour and normal DNA specimens are from the same patient. Some studies have shown, that the Promega method is better than the traditional one, which was suggested by the National Cancer Institute in 1998. The reason behind this is that in order to detect MSI-high phenotype, using only mononucleotide markers produce results with higher specificity and better sensitivity than using a combination of both mononucleotide and dinucleotide markers, as suggested by the traditional method (2 mononucleotide: BAT-25 and BAT-26, 3 dinucleotide: D2S123, D5S346 and D17S250) [33, 34]. Manufacturer's instructions (Promega) were used to perform the MSI analysis. ABI Prism 3100 Genetic Analyzer (Applied Biosystems) was used to separate the products obtained from fluorescently labeled

polymerase chain reaction, and the data were analysed using GeneMapper 3.0 Software (Applied Biosystems).

Tumours were classified by the following method: if more than 2 out of 5 markers demonstrated size alterations or shifts in the tumour DNA with respect to the normal tissue DNA, this cancer was identified as MSI-high. Tumours with only one marker showing instability were classified as MSI-low while tumours with none of the markers showing instability were classified as microsatellite stable. When doing statistical analysis, MSI-low and microsatellite stable tumours were both regarded as MSI stable.

## 2.3 Statistical analysis

In order to compare MSI-high and MSI stable groups by women age and selected clinicopathologic characteristics (tumour grade, depth of myometrial invasion and stage of disease), the t test and Fisher's exact test were used. Differences were regarded as statistically significant for  $p < 0.05$ .

# 3 Results

## 3.1 MSI analysis in endometrial carcinomas

The mean age of women at the time of endometrial cancer diagnosis was 64.0 years (range 40-83). 100 of 109 (92%) endometrial carcinomas showed endometrioid type histology and only 9 (8%) non-endometrioid types. Microsatellite instability analysis was successful for all the tumours of the patients involved in the study with 16% (17/109) of the cases being classified as MSI-high (Table 1). MSI-high was only found in endometrioid type adenocar-

**Table 1:** Microsatellite instability (MSI) status according to the histological type of cancer

Histological type	Number of patients	MSI-high n (%)	MSI stable n (%)
Total	109	17 (15.6%)	92 (84.4%)
Endometrioid adenocarcinoma	100	17 (17.0%)	83 (83.0%)
Serous adenocarcinoma	3	0	3 (100%)
Clear cell carcinoma	1	0	1 (100%)
Adenosarcoma	5	0	5 (100%)

**Table 2:** Clinicopathologic characteristics according to the microsatellite instability (MSI) status in endometrioid type adenocarcinomas

Characteristics	Number of patients	MSI status		p value
		MSI-high n (%)	MSI stable n (%)	
Total	100	17 (17%)	83 (83%)	
Tumour grade				
G 1	40	2 (5%)	38 (95%)	p=0.007
G 2-3	60	15 (25%)	45 (75%)	
Myometrial invasion				
<50%	62	7 (11%)	55 (89%)	p=0.049
≥50%	38	10 (26%)	28 (74%)	
Pathological tumour (T), stage*				
T1 confined to the corpus uteri	88	14 (16%)	74 (84%)	p=0.33
T2-T4 expansion beyond the uterus	12	3 (25%)	9 (75%)	

\*T1 – corresponds to FIGO I stage, T2-T4 – FIGO II-IV stage

cinomas: there were 17 out of 100 (17%) cases for endometrioid type and 0 out of 9 (0%) for non-endometrioid type. Note, as the number of non-endometrioid type cancers was so low, reliable statistical conclusions for this type could not be drawn.

### 3.2 The clinicopathologic characteristics of MSI-high status

The relationship of clinicopathologic characteristics with MSI status was analysed only for the endometrioid type adenocarcinomas. The results are shown in the Table 2. Characteristics of each MSI-high sample are shown in Table 3.

The mean age of women with MSI-high tumor phenotype was 65.7 years (range: 53-80) and was not statistically different from women with MSI stable phenotype (63.6 years; range: 40-83) ( $p=0.214$ ). None of the women in <50 years old group (a total of 6 cases) had a MSI-high tumour.

There was a highly statistically significant ( $p=0.007$ ) association between MSI-high phenotype and a higher tumour grade in endometrioid type adenocarcinomas. The frequency of MSI-high status in tumour grade 1 was

5% (2/40) as distinct from 25% (15/60) in grade 2 and 3 combined.

A statistically significant association between deep myometrial invasion and MSI-high status was identified ( $p=0.049$ ). In tumours with deep myometrial invasion ( $\geq 50\%$ ) the MSI-high phenotype was detected in 10 of the 38 cases (26%), whereas in tumours which do not have a myometrial invasion or have a superficial myometrial invasion (<50%) only 7 of the 62 cases (11%) showed MSI-high status.

The association between the higher clinical cancer stage and MSI-high status was not statistically significant ( $p=0.33$ ). However, MSI-high phenotype was more frequent in cases where tumour was expanded beyond the uterus (3/12 – 25%) than in cases where tumour was only confined to corpus uteri (14/88 – 16%).

## 4 Discussion

In the present study we investigated selected clinical pathological parameters of their association with MSI status in sporadic endometrial carcinomas using mononucleotide MSI detection markers. In our study the statistical relationship between the mean age of women at the

**Table 3:** Characteristics of each MSI-high sample in endometrioid type adenocarcinomas

Patient No.	Age at diagnosis (y)	Characteristics			No. of positive MSI markers
		Tumour grade (G)	FIGO stage	Myometrial invasion (%)*	
1	71	G2	IA	No	2/5
2	68	G2	IA	No	4/5
3	54	G1	IA	No	3/5
4	74	G2	IA	< 50	4/5
5	72	G1	IA	< 50	2/5
6	61	G2	IA	< 50	5/5
7	63	G2	IA	< 50	2/5
8	72	G2	IB	≥ 50	3/5
9	67	G2	IB	≥ 50	2/5
10	58	G2	IB	≥ 50	3/5
11	80	G2	IB	≥ 50	2/5
12	66	G2	IB	≥ 50	3/5
13	73	G3	IB	≥ 50	2/5
14	61	G2	IB	≥ 50	4/5
15	63	G2	IIIC	≥ 50	4/5
16	53	G3	IIIC	≥ 50	4/5
17	61	G2	IVB	≥ 50	3/5

\*No – invasion only in endometrium

time of endometrial cancer diagnosis and MSI status was not significant. No previous study has reported any relationship between age and MSI status in sporadic endometrial carcinomas [14, 26, 30].

MSI-high was found in 17% of the endometrioid type adenocarcinomas (17/100), in non-endometrioid carcinomas MSI-high was not detected (0/9). Similar results were reported by the other researchers – MSI-high was detected more frequently in endometrioid histologic type than non-endometrioid type [26, 27]. Ours and previously done studies show that MSI is not involved in the majority of cases of non-endometrioid type adenocarcinomas and thus it can be concluded that in case of sporadic endometrioid carcinoma, MSI is almost exclusive to Type I cancers.

In this study the clinicopathologic factors known to be related with the prognosis of the disease were analysed between MSI-high and MSI stable cases only in the endometrioid type adenocarcinomas. In the present study

we found that the deep myometrial invasion and higher histologic tumour grade was significantly related to MSI-high status. This relationship with MSI-high phenotype was reported in the most previous studies [26, 29, 32]. In our study, we also observed relationship between MSI-high phenotype and higher clinical stage. However, this relationship was statistically insignificant, but MSI-high phenotype was more frequently detected (almost twice) in cases where tumour had spread outside the uterus than in cases where tumour was found only in corpus uteri. It is known that the presence of higher histologic tumour grade, deep myometrial invasion and higher clinical cancer stage are well-known prognostic markers which provide higher recurrence rate and worse survival in endometrial carcinomas. Therefore, it can be suggested that MSI-high phenotype is an unfavourable prognostic marker in sporadic endometrial carcinomas. However, there were many reports where no differences in distribution of MSI-high phenotype by tumour grade, myometrial

invasion and clinical cancer stage were found [23,24,32]. In contrast to colorectal cancers, it can not be concluded that MSI-high phenotype is an independent prognostic indicator for sporadic endometrioid type adenocarcinoma.

## 5 Conclusions

It was found that MSI-high status is related to endometrioid type adenocarcinoma while none of the non-endometrioid type adenocarcinomas showed MSI-high phenotype. The study identified a statistically significant relationship between MSI-high phenotype and clinicopathologic parameters, such as high histologic grade and deep myometrial invasion in endometrioid type adenocarcinoma. Potential conclusion could be drawn that MSI status is related with unfavorable clinicopathologic prognostic factors for endometrioid type adenocarcinoma, however it is inconclusive whether MSI status is an independent prognostic factor. More research in this field needs to be done with larger sample sizes, using standardised MSI markers to receive more conclusive results.

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