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Original Research Article

Impact of microsatellite instability on survival of endometrial cancer patients

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ABSTRACT

Background and objective: Endometrial cancer (EC) is the most commonly diagnosed gynecologic malignancy among women worldwide and may be classified on the basis of different molecular, pathologic and genetic alterations, including microsatellite instability (MSI). Although MSI is associated with a more favorable outcome in colorectal cancer, its relationship with prognosis in EC cancer is not yet clear. The aim of our study is to identify whether MSI correlates with survival of patients in EC.

Materials and methods: We examined MSI status and survival of 109 women. MSI was detected by employing the Promega MSI Analysis System, which used 5 mononucleotides markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) to identify MSI in a tumor and normal tissue DNA and 2 pentanucleotide markers (Penta C and Penta D) for specimen identification. Median follow-up of patients was 40.4 months (range 5.2–47.9). Survival was estimated by the Kaplan–Meier method and Cox regression analysis was used to assess the effects of different variables on patient survival.

Results: MSI-high was detected in 15.6% EC cases, all of which were associated with endometrioid type histology. Kaplan–Meier survival analysis showed no statistically significant differences between patients with MSI-high and MSI stable tumors ($P = 0.4$) and multivariate analysis concluded that MSI status remained insignificant after stage, histology and tumor grade adjustment ($P = 0.5$).

Conclusions: Our study showed no statistically significant relationship between MSI-high and survival of endometrial cancer patients.

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1. Introduction

Endometrial cancer is one of mostly widespread cancers (6th place) among women worldwide, contributing to 290 000 new cases in 2008, and a standardized incidence rate of 8 per 100 000 women [1]. In Europe, endometrial cancer is the fourth most frequent cancer in women and the tenth most frequent cancer among cancer deaths. About 81 500 women are affected every year in the European Union and the incidence is increasing [2]. In Lithuania endometrial cancer is the third most common cancer and causes 4% of cancer deaths among women [3]. According to the recent EUROCARE report 5-year survival rate for endometrial cancer in Europe was more than 75% [4].

Based on the tumor histology, biology and clinical features, endometrial cancers are classified into two clinicopathologic types: type I or endometrioid cancer (70%–80% of all endometrial cancers) and another, less common, is type II or non-endometrioid cancer (20%–30% of all endometrial cancers) which includes serous and clear cell histology [5]. Type I endometrial cancer is usually hormone sensitive, and more commonly occurs in women exposed to estrogens. It is usually associated with a high level of tumor differentiation, low potential for myometrial invasion and favorable prognosis. 5-year overall survival for women with type-I endometrial cancer is about 75% and for women with stage I disease is approximately 90% [2]. On the contrary, type II endometrial cancer is not hormone dependent. It is characterized by poor level of differentiation, high probability of myometrial invasion, aggressive clinical course and worse prognosis [6]. Patients with type II endometrial cancer have a very poor 5-year overall survival of only 18%–27%. Survival is reported 35%–50% for patients with clinical or pathological stage I–II and 0%–15% in stage III–IV [7,8].

In addition to being classified into two types, endometrial cancers can also be divided on the basis of specific genetic alterations, which are thought to be significantly affecting the development of cancer. One of the molecular alterations often associated with type I (endometrioid) cancer is microsatellite instability (MSI). Microsatellites are short repetitive nucleotide sequences of DNA, consisting of 1–5 nucleotides [9]. Because of their repeat structure, microsatellites are prone to slippage or replication errors. Usually, such errors are repaired by the DNA mismatch repair system (MMR); however, this system can fail resulting in microsatellite instability. Microsatellites exhibit the same number of nucleotide repeats in tumor and healthy tissue of the same individual, however, loss of MMR can result in widespread changes in the number of repeat sequences [10]. In 1998, the National Cancer Institute (USA) recommended a panel of 5 mono/or dinucleotide markers for the determination of MSI. The tumor 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].

The majority of MSI in colorectal and endometrial cancers is caused by defects in DNA mismatch repair genes, mainly *MLH1*, *MSH2*, *MSH6* and *PMS2* [12].

Although MSI is an independent prognostic marker of a favorable outcome in colorectal cancer [13–15], it is feasible that MSI may also influence endometrial cancer survival. Evidence of MSI association with prognosis in endometrial cancer is controversial. Some studies have shown that MSI is associated with a more favorable outcome [16], whereas others have reported the opposite – a worse prognosis [17,18]. However, some studies have reported that MSI has no prognostic value at all [19].

The objective of our study is to identify, whether MSI correlates with survival of endometrial cancer patients.

2. Materials and methods

2.1. *Data collection*

One hundred nine primary patients (109 women) who were treated for endometrial cancer during the period between April 2010 and June 2011 at the Institute of Oncology, Vilnius University 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). Samples for this study were collected during the surgical treatment (hysterectomy) and later examined by an expert pathologist. The histological type was classified using World Health Organization criteria and surgical staging were determined using the standards of the International Federation of Obstetrics and Gynecology (FIGO 2009). The vital status of the study group was assessed as of March 30, 2014.

2.2. *DNA extraction and MSI analysis*

Specimens of the tumor tissue were extracted from formalin fixed, paraffin-embedded sample tissue. Tumor 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 tumor was analyzed 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 tumor 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 tumor 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 sensitivity and specificity 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) [20,21]. MSI analysis was performed according to the manufacturer's instructions (Promega). ABI Prism 3100 Genetic Analyzer (Applied Biosystems) was used to separate the products obtained from fluorescently labeled polymerase chain reaction, and the data were analyzed using GeneMapper 3.0 Software (Applied Biosystems).

Tumors were classified by the following method: if more than 2 out of 5 markers demonstrated size alterations or shifts

Table 1 – Demographic and clinical characteristics of a study group by MSI status (n = 109 patients).

Variable	No. of patients	% of total	No. of patients, MSI stable	% of total	No. of patients, MSI-high	% of total
Total	109	100.00	92	100.00	17	100.00
Age, years						
<64	53	48.62	45	48.91	8	47.06
≥64	56	51.38	47	51.09	9	52.94
Menopause						
No	7	6.42	7	7.61	0	–
Yes	102	93.58	85	92.39	17	100.00
FIGO stage						
I	88	80.73	75	81.52	13	76.47
II	8	7.34	9	9.78		–
III	9	8.26	5	5.43	3	17.65
IV	4	3.67	3	3.26	1	5.88
Tumor grade						
G1	41	37.61	39	42.39	2	11.76
G2	50	45.87	37	40.22	13	76.47
G3	14	12.84	12	13.04	2	11.76
Not applicable	4	3.67	4	4.35	0	–
Tumor histology						
Endometrioid adenocarcinoma	100	91.74	83	90.22	17	100.00
Other (including serous adenocarcinoma, clear cell carcinoma, adenosarcoma)	9	8.26	9	9.78	0	–

in the tumor DNA with respect to the normal tissue DNA this cancer was identified as MSI-high. Tumors with only one marker showing instability were classified as MSI-low while tumors with none of the markers showing instability were classified as microsatellite stable. In statistical analysis, MSI-low and microsatellite stable tumors were both regarded as MSI stable.

2.3. *Statistical analysis*

Demographic and clinical data are summarized descriptively as proportions. Survival was estimated by the Kaplan–Meier method and Cox regression analysis was used to assess the effects of different variables on patient survival. Survival curves were compared using the log-rank test. $P < 0.05$ indicated a significant statistical difference. All statistical analyses were performed using Stata Statistical Software version 11.0 (StataCorp. 2009, Stata Statistical Software: Release 11.0, College Station, TX, USA).

3. Results

Demographic and clinical characteristics of the study group are presented in Table 1. The average age of patients was 64.3 ± 9.9 years (median 64; range 40–83 years). Median follow-up was 40.4 months (range 5.2–7.9). 100 of 109 (91.7%) cases of endometrial cancers showed endometrioid type histology and only 9 (8.3%) non-endometrioid types. Microsatellite instability analysis was successful for all the tumors of the patients involved in the study with 15.6% (17/109) of the cases being classified as MSI-high. MSI-high was only found in

Table 2 – Observed survival and results of univariate survival analysis.

Variable	3-year survival, %	95% confidence intervals	Log-rank, P
Age			
<64	92.45	81.13–97.10	0.89
≥64	90.96	79.63–96.14	
Menopause			
No	85.71	33.41–97.86	0.82
Yes	92.09	84.80–95.97	
FIGO stage			
I	95.40	88.34–98.27	0.003
II	66.67	28.17–87.83	
III	87.50	38.70–98.14	
IV	75.00	12.79–96.05	
Tumor grade			
G1	100.00	–	<0.001
G2	95.87	84.46–98.95	
G3	64.29	34.33–83.31	
Not applicable	50.00	5.78–84.49	
Tumor histology			
Endometrioid adenocarcinoma	95.93	89.52–98.46	<0.001
Other (incl. serous adenocarcinoma, clear cell carcinoma, adenosarcoma)	44.44	13.59–71.93	
MSI			
MSI stable	90.15	81.92–94.75	0.40
MSI-high	100.00	–	

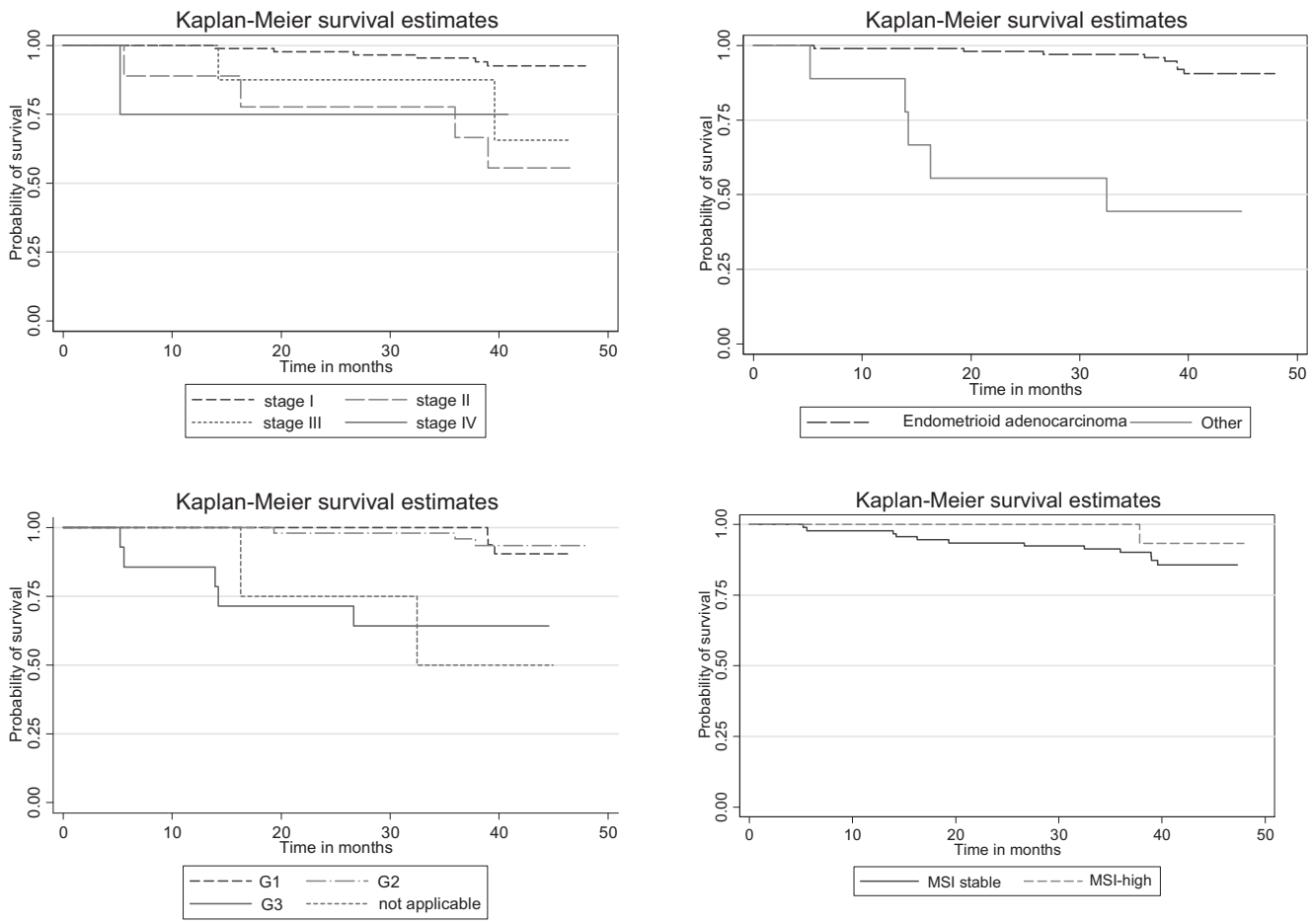

Figure – Kaplan–Meier survival analysis of endometrial carcinoma by stage, histology, grade of differentiation and MSI status.

endometrioid type cancers: there were 17 out of 100 (17.0%) cases for endometrioid type and 0 out of 9 (0%) for non-endometrioid type.

The results of Kaplan–Meier univariate survival analysis as well as 3-years survival are presented in Table 2. Better survival was related to the earlier cancer stage, higher level of tumor differentiation and endometrioid histology. Statistically significant results are presented in Figure as well as survival curves by MSI status. Kaplan–Meier survival analysis showed no statistically significant differences between patients with MSI-high and MSI stable tumors ($P = 0.4$), however, survival of patients with MSI stable tumors was lower.

Cox multivariate survival analysis was conducted to determine the prognostic value of the significant clinical and pathological features (Table 3). In the multivariate analysis MSI status remained insignificant after stage, histology and tumor grade adjustment ($P = 0.5$).

4. Discussion

Modern research in the mechanisms of carcinogenesis stimulates the search for molecular biomarkers which would help to provide better prognosis for disease development. One such biomarker detected in colorectal cancer is microsatellite instability (MSI), which was found to be as an independent prognostic indicator for this cancer. MSI correlation with clinicopathological features and disease prognosis in cancers of different types was reviewed in detail in the article in the press [22]. In recent years MSI was intensively studied as a

Table 3 – Results of multivariate survival analysis.

Variable	Hazard ratio	95% confidence intervals	P
FIGO stage			
I	1.00	Ref.	
II	3.52	0.89–13.88	0.07
III	3.60	0.62–20.67	0.2
IV	3.68	0.39–34.48	0.3
Tumor grade			
G1	1.00	Ref.	
G2	1.02	0.18–5.54	0.9
G3	4.78	1.02–22.45	<0.05
Not applicable	1.39	0.16–11.92	0.8
Tumor histology			
Endometrioid adenocarcinoma	1.00	Ref.	
Other	4.53	0.97–21.07	0.05
MSI			
MSI stable	1.00	Ref.	
MSI-high	0.46	0.05–4.77	0.5

prognostic marker in endometrial cancer, but the results were contradictory.

In endometrial cancer the majority of data is collected describing the relationship between MSI and clinicopathological tumor characteristics [16,23,24]. Most of the research conclude that MSI-high phenotype is related with type I or endometrioid cancer, higher tumor grade, deep myometrial invasion and more advanced stage of the disease [23,25]. It must be noticed, that only a few studies observed the relationship between MSI and earlier stages of the disease [26]. However, according to the other studies, no correlation between MSI and tumor clinicopathological characteristics was found [27,28].

In colorectal cancer MSI is associated with older age of patients, female sex, and such tumor clinicopathological parameters as a prevalence in proximal colon, mucinous differentiation, lymphocytic infiltration and low pathologic stage [14,15,29]. A number of studies showed that MSI-high tumors have a more favorable prognosis and are less prone to lymph node metastasis, and systemic metastasis than MSI stable tumors [13,29]. MSI-high is an independent favorable prognostic marker for overall survival in colon cancer. Several studies support this further by confirming that MSI-high tumors were strongly correlated with longer disease free survival and overall survival than MSI stable tumors in colon cancers [29,30].

There are a limited number of studies done on the MSI and survival in endometrial cancer and the data is controversial. Karamurzin and Rutgers [31] stated that MSI is one of the molecular characteristics associated with endometrioid type of cancer, however, MSI has not been demonstrated to have an independent prognostic significance. It was confirmed by a previously done study [19] which includes 446 patients only with type I or endometrioid cancer. In contrast, some studies [16] found MSI to be associated with a more favorable outcome. However, other authors [17] detected the association of MSI with poor prognosis for the disease.

In the present study, we investigated MSI status in endometrial cancer using mononucleotide MSI detection markers and the frequency of MSI-high was detected in 15.6% of cases (17 out of 109). Data from different studies showed that frequency of MSI in endometrial cancer ranged from 9% to 43% [16,19]. Such differences might be due to several reasons: quantitatively small study groups, retrospective analysis, different panels of markers for MSI detection and failure to account the influence of histological type.

In our study MSI-high phenotype was only found in endometrioid type of cancer (17/100), while in non-endometrioid type MSI-high was not detected (0/9). Similar results were reported by the other researchers – MSI-high was found to be more frequent in endometrioid cancers [16,23].

Although MSI is correlated with a favorable prognosis in colorectal cancer patients, the MSI overall impact to endometrial cancer survival is still controversial. According to ours and other studies, MSI-high phenotype is related to higher tumor grade, deep myometrial invasion and higher stage of the disease, thus it might be also related to worse survival in endometrial cancer. However, in our study after adjusting for other known prognostic variables MSI was not identified as a significant prognostic factor for survival of endometrial cancer patients. This, though, could be explained by the study limitation – a small sample size. The conflicting results showed that it is necessary to continue the investigation including a greater number of patients with MSI-high tumors.

5. Conclusions

In our study 109 endometrial cancer cases were analyzed and 15.6% were detected with MSI-high tumors. There was no statistical significance for MSI-high relationship with survival of endometrial cancer patients.

Conflict of interest statement

The authors state no conflict of interest.

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