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Clinical Cancer Research

Imaging, Diagnosis, Prognosis

Stathmin Overexpression Identifies High-Risk Patients and Lymph Node Metastasis in Endometrial Cancer

Jone Trovik[1,2], Elisabeth Wik[2], Ingunn M. Stefansson[3,4], Janusz Marcickiewicz[5], Solveig Tingulstad[6], Anne C. Staff[7,8], Tormund S. Njolstad[2], MoMaTec Study Group[9–13], Ingrid Vandenput[14], Frederic Amant[14], Lars A. Akslen[3,4], and Helga B. Salvesen[1,2]

Abstract

Purpose: Overexpression of the oncogen *Stathmin* has been linked to aggressive endometrial carcinoma and a potential for PI3Kinase inhibitors in this disease. We wanted to validate the prognostic value of Stathmin expression in a large prospective multicenter setting. As lymph node sampling is part of current surgical staging, we also aimed to test if Stathmin expression in endometrial curettage specimens could predict lymph node metastasis.

Experimental Design: A total of 1,076 endometrial cancer patients have been recruited from 10 centers to investigate the biological tumor marker Stathmin in relation to clinicopathologic variables, including lymph node status and survival. Stathmin immunohistochemical staining was carried out in 477 hysterectomy and 818 curettage specimens.

Results: Seventy-one percent of the patients ($n = 763$) were subjected to lymph node sampling, of which 12% had metastatic nodes ($n = 94$). Overexpression of Stathmin was detected in 37% (302 of 818) of the curettage and in 18% (84 of 477) of the hysterectomy specimens investigated. Stathmin overexpression in curettage and hysterectomy specimens were highly correlated and significantly associated with none-ndometrioid histology, high grade, and aneuploidy. Stathmin analysis in preoperative curettage samples significantly correlated with, and was an independent predictor of, lymph node metastases. High Stathmin expression was associated with poor disease-specific survival ($P \leq 0.002$) both in curettage and hysterectomy specimens.

Conclusions: Stathmin immunohistochemical staining identifies endometrial carcinomas with lymph node metastases and poor survival. The value, as a predictive marker for response to PI3Kinase inhibition and as a tool to stratify patients for lymph node sampling in endometrial carcinomas, remains to be determined. *Clin Cancer Res; 17(10); 3368–77. ©2011 AACR.*

Introduction

Endometrial cancer is the most common gynecological malignancy in the western world and the incidence is rising in several countries (1). 15% to 20% of patients with presumed localized disease at primary treatment recur (2, 3). Of all patients dying from this disease, one third was initially classified as early-stage disease (4). Although metastatic lymph nodes detected as part of the surgical staging procedure identifies patients with poor prognosis, the procedure has been associated with higher complication rates (5). Routine pelvic lymph node sampling does not contribute to a survival benefit for the patients subjected to such procedure (5, 6). We are still in need of better prognosticators to tailor treatment for high-risk groups. Identification of new reliable molecular markers in preoperative curettage specimens would contribute to such individualized surgical and adjuvant treatment.

The oncoprotein Stathmin is known to be a microtubule destabilizer, promoting cell proliferation, mobility, metastasis, and resistance to antimicrotubule therapy (7, 8). Immunohistochemical staining has shown Stathmin protein expression to be correlated with aggressive clinicopathologic variables and poor prognosis in endometrial cancer (9) and several other malignancies such as breast (10, 11), ovarian (12), and cervical cancer (13).

Authors' Affiliations: [1]Department of Obstetrics and Gynecology, Haukeland University Hospital; [2]Department of Clinical Medicine, and [3]The Gade Institute, University of Bergen; [4]Department of Pathology, Haukeland University Hospital, Bergen, Norway; [5]Center for Gynecological Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden; [6]Department of Gynecology, St.Olav's Hospital, Trondheim; [7]Department of Obstetrics and Gynecology, Oslo University Hospital Ulleval; [8]Faculty of Medicine, University of Oslo; [9]Department of Obstetrics and Gynecology, Akershus University Hospital, Oslo; [10]Department of Obstetrics and Gynecology, Haugesund Hospital, Haugesund; [11]Department of Obstetrics and Gynecology, Hospital of Vestfold, Toensberg; [12]Department of Obstetrics and Gynecology, Foerde Central Hospital, Foerde; [13]Department of Obstetrics and Gynecology, AAlesund Hospital, AAlesund, Norway; and [14]Department of Gynecological Oncology, UZGasthuisberg, KULeuven, Leuven, Belgium

The members of MoMaTEC study group are M.E. Engh[9], K. Oddenes[10], J.A. Rokne[11], J.T. Jugum[12], M.S. Lode[13].

Corresponding Author: Jone Trovik, Department of Obstetrics and Gynecology, Haukeland University Hospital, 5021 Bergen, Norway. Phone: 00-475-597-4200; Fax 00-475-529-7412. E-mail: jone.trovik@helse-bergen.no

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Translational Relevance

Endometrial cancers are generally diagnosed at early stage with good outcome. Still, one third of patients dying from cancer were initially classified as early stage. Recurring tumors have limited response to conventional systemic therapy. Thus, patients with localized endometrial cancer have 2 major needs: (1) adjuvant therapies that will reduce recurrence rate, and (2) improved tools targeting these therapies to patients most likely to recur and respond. In this large international prospective multicenter trial, including more than 1,000 patients, we find that overexpression of the oncogene *Stathmin* identifies endometrial carcinomas with lymph node metastases and poor survival. Stathmin overexpression in preoperative curettage specimens independently predicts risk of lymph node metastasis, and may in the future aid the selection of patients for lymph node sampling. Stathmin overexpression is also linked to PI3Kinase signaling activation and studies of Stathmin as a predictor for response to PI3Kinase inhibitors needs to be determined.

The PI3Kinase signaling pathway is one of the major oncogenic pathways found to be upregulated in cancer in general, and in aggressive types in particular (14, 15). On this background, PI3Kinase inhibitors are promising drugs presently entering clinical trials (16). In a standard clinical setting, immunohistochemical markers would be more applicable than estimation of PI3Kinase activation in fresh tissues. Stathmin overexpression has been shown to be associated with PI3Kinase activation in breast (10) and endometrial cancer (9), indicating that Stathmin staining may have a potential as a marker to select patients more likely to respond to treatment with PI3Kinase inhibitors.

The objective of the present study is to validate the prognostic value of Stathmin expression in a large prospective multicenter setting. To our knowledge, this is the first study to investigate, in a prospective multicenter setting, the role of Stathmin as a prognostic marker in relation to traditional clinicopathologic factors including lymph node status. We also wanted to investigate to what extent Stathmin tested in preoperative curettage specimen could identify patients with high risk of lymph node metastasis. Accordingly, the occurrence of lymph node metastases and death due to endometrial cancer are applied as the major study endpoints.

Materials and Methods

Patients

In total, 1,076 endometrial cancer patients have been recruited, and tissue samples and clinical information collected (MoMaTEC; ref. 17). After written consent, 553 prospectively collected consecutive endometrial cancer patients, treated at the Department of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway, were included from May 2001 through 2009. From January 2007 through 2009, 9 other centers included additionally 523 patients primarily treated for endometrial carcinomas at their institutions. The formalin-fixed and paraffin-embedded tumor tissue from curettage was collected from all centers, and corresponding samples from the hysterectomy specimens were collected at Haukeland University Hospital. Clinicopathologic data including age at diagnosis, International Federation of Gynecology and Obstetrics (FIGO) stage according to the 1988 criteria (18), histologic type and grade and treatment modalities were recorded. Because the study objective was to evaluate the impact of Stathmin expression in a patient group treated routinely in a prospective multicenter setting, we applied the routine histologic reports for grading and subtyping. The distribution of histopathologic subtype for a patient series from the same region previously subjected to histopathologic revision (19) showed a similar proportion of 10.1% serous papillary/clear cell carcinomas compared with 13.7% in current patient series not subjected to histopathologic revision, and with a similar 5-year survival difference of 42% and 43% for these subgroups versus their endometrioid counterparts respectively. This is in line with the strong focus on correct histopathologic subtyping in the standard treatment planning of these patients over the last decade. To relate the biomarker's value as a preoperative predictor of lymph node metastases in relation to other factors available before primary surgery, the histology report from the original preoperative curettage was collected and was reported for 920 patients. In 48 patients, the preoperative diagnose was either benign ($n = 6$) or hyperplasia ($n = 42$), still 20 of these patients had lymph node sampling carried out. In these cases, macroscopic tumor visible in the excised uterus was considered sufficiently highly cancer suspect to prompt lymph node sampling if the patient was found fit for extended procedure. In some cases, cancer was verified by peroperativ frozen section pathology examination.

Pelvic lymphadenectomy as part of surgical staging was conducted in the majority of cases (763: 71%). In this period, the routine lymph node sampling has been pelvic, not para-aortic. The sampling was extended outside the pelvis only if suspicious para-aortic nodes were encountered during the operation. Range of nodes removed was 1 to 72 (median = 14). The aim was initially to remove 10 nodes or more, this was achieved in 555 patients, being 73% of those sampled. The final decision of extent of sampling was made by the responsible surgeon, based on an overall assessment of the patient's risk of recurrence balanced by the general comorbidity.

Follow-up data regarding recurrence and survival have been collected from patient records and correspondence with primary physicians or gynecologists

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responsible for outpatient controls. Date of last follow-up was November 10, 2010. The mean follow-up for survivors was 30 months (range 0–90). The treatment for these patients mainly consisted of hysterectomy and bilateral salpingo-oophorectomy unless surgery was contraindicated owing to severe medical conditions. For the Norwegian patients, adjuvant treatment was not routinely given with FIGO stage I or IIa, but local radiation was administered for IIb during the first half of the study period. Swedish and Belgian patients received radiation or chemoradiation in higher frequencies also in stage II. In total 32% (198/889) of FIGO I/II category patients received adjuvant treatment versus 84% (158/187) in FIGO III /IV. A total of 105 patients died of endometrial cancer. For further patient characteristics see Table 1.

Tissue microarray

Hematoxylin and eosin stained slides from individual hysterectomy and curettage specimen were evaluated to identify the area with representative tissue of highest tumor grade. Three 0.6 mm tissue cylinders from the selected area of each specimen were punched out and mounted in a recipient paraffin block by using a custom-made precision instrument (Beecher Instruments). This method for producing tissue microarrays (TMA) has been described and validated before (20, 21). TMAs with representative tumor were available in 477 hysterectomy (Haukeland University Hospital only) and 818 curettage specimens (multicenter setting). Even in 38 cases with original curettage diagnosis as benign or hyperplasia, we were able to detect adequate areas with suspected cancer for TMA preparation. TMA sections of 5 μm were subsequently dewaxed with xylene/ethanol for immunohistochemical staining.

Immunohistochemistry

Antigen retrieval was done by microwaves for 10 minutes at 750 W and for 15 minutes at 350 W in citrate buffer at pH 6. Slides were blocked for peroxidase (Dako S-2032) for 8 minutes and incubated for 60 minutes at room temperature with a polyclonal Stathmin antibody (3352 Cell Signaling) diluted 1:50. Staining procedures were carried out by using the DAKO Autostainer (no. 3400–9567) automated slide processing equipment. The EnVision + rabbit HRP-labeled polymer method was used for adding secondary antibody as recommended by DakoCytomation. Finally, slides were briefly counterstained with Dako Real Hematoxylin. Samples of malignant melanoma and seminoma known to yield positive staining for Stathmin (22) were used as positive controls. Substituting the primary antibody with diluent only was used as a negative control.

Evaluation of staining

Blinded for patient characteristics and outcome, the slides were evaluated by 1 of the authors (J. Trovik) by using a standard light microscope. A semiquantitative grading system incorporating staining intensity (score 0–3) and area of tumor with positive staining (0, no staining; 1, <10%; 2, 10%–50%; and 3, >50% of tumor cells) was applied. Staining index was calculated as the product of

Table 1. Characteristics for 1,076 endometrial cancer MoMaTEC trial patients at time of primary treatment

Characteristics	*n*	%
Mean age, y	66	
Range	28–94	
Menopausal status		
Pre/perimenopausal	111	10.3
Postmenopausal	965	89.7
FIGO stage		
I	784	72.9
II	105	9.8
III	136	12.6
IV	51	4.7
Histologic type[a]		
Endometrioid	881	81.9
Nonendometrioid	195	18.1
Histologic differentiation[b]		
Grade 1 and 2	736	68.8
Grade 3	333	31.2
Primary surgery		
Hysterectomy and oophorectomy	1,037	96.4
Diagnostic curettage only	32	3.0
Palliative surgery	7	0.7
Lymph node sampling		
Not carried out	313	29.1
Carried out	763	70.9
Adjuvant primary treatment[c]		
None	721	67.0
Radiation	144	13.4
Chemotherapy	134	12.5
Chemo radiation	58	5.4
Hormonal treatment	18	1.7
Status at last follow-up[d]		
Alive without disease	782	77.5
Alive with recurrent disease	75	7.4
Dead without disease	38	3.8
Dead with but not from disease	9	0.9
Dead because of disease	105	10.4

NOTE: MoMaTEC, Molecular Markers for Treatment of Endometrial Cancer, NCT00598845.

[a]Endometrioid group including 12 adenosquamous cancers. Nonendometrioid: clear cell 42, serous papillary 89, carcinosarcoma 47, and undifferentiated carcinoma 17.

[b]Missing information about grade in 7 cases.

[c]One patient missing information concerning additional treatment.

[d]A total of 67 patients have not yet been evaluated for follow-up.

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staining intensity and staining area, range 0–9, as described in several publications (23, 24). Values defined by the upper quartile for the data set were considered positive, in line with other immunohistochemical studies done from our center (23, 24). The overall staining was stronger in hysterectomy than curettage specimens, corresponding to staining index 6 and 9 as cutoff for upper quartile/strong expression in the curettage set versus Index 9 in the hysterectomy set. To evaluate interobserver reproducibility, a subset of random TMA slides have been evaluated independently by 2 of the authors (H.B.S. and J.T.) blinded for the result of the other evaluator. 168 curettage and 88 hysterectomy specimens were thus rescored. Complete categorical agreement was found in 87% for the curettage ($\kappa = 0.72$) and 95% for the hysterectomy specimens ($\kappa = 0.86$).

To further explore a possible mechanism for staining differences between curettage and hysterectomy, pathology reports from 126 randomly selected corresponding curettage and hysterectomy specimens have been evaluated regarding fixation time (time from when tissue have been put in formalin until macroscopic pathologic examination and paraffin processing are conducted). Curettings had a significant shorter fixation time, median 1 day, as compared with hysterectomy specimens with a median of 6 days. Staining index did not correlate with fixation time, however, neither in curettage nor in hysterectomy specimen, with χ^2 test, $P = 0.43$ and $P = 0.40$, respectively.

Images for illustration were captured by using a Leica DC300 digital camera with a 40× magnification lens.

Statistical analysis

Statistics were conducted by the statistical program SPSS15.0. Associations between categorical variables were evaluated by Pearson's chi-square test. *P* values represent 2-sided tests and are of statistical significance when $P < 0.05$. Univariate analyses of time to recurrence (recurrence-free survival) and death due to endometrial carcinoma (disease-specific survival) were carried out by using the Kaplan–Meier method and differences were estimated by the Mantel–Cox (log rank) test. Binary logistic regression was used to evaluate the ORs for lymph node metastasis. Cox' proportional hazards method was used for multivariate survival analysis.

Power calculation

Estimation of sample size was done by chi-square test by using software East4, 2005, Cytel Software Corp. To achieve 90% power to detect a 20% higher occurrence of positive markers in patients with metastatic lymph nodes (25% vs. 5%) at a 5% significance level, 101 patients were needed for inclusion. With an estimated prevalence for lymph node metastases of 20%, 500 cases should be included to achieve sufficient power. To reach 90% power to detect a 30% difference in 5-year survival (90% for patients with negative marker vs. 60% with positive marker) at a 5% level of significance, 65 patients were needed, assuming the positive to negative ratio for the marker was 1:3.

Approvals

The study has been approved by the Norwegian Data Inspectorate (961478-2), the Norwegian Social Science Data Services (15501), and the local Institutional Review Board (REKIII nr. 052.01).

Results

Stathmin expression and clinicopathologic features

Stathmin staining was mainly cytoplasmatic. Strong expression was seen in 84 (18%) of hysterectomy and in 302 (37%) of curettage specimens. Overexpression of Stathmin in curettage was significantly correlated with aggressive clinicopathologic characteristics including high FIGO stage, nonendometrioid histologic type, high histologic grade, aneuploidy, and presence of lymph node metastasis (Table 2). For Stathmin expression in hysterectomy specimens, the correlations were similar in relation to aggressive histologic type, grade, and ploidy. No correlation was seen with age or menopausal status. Stathmin expressions in hysterectomy and curettage specimens were highly significantly correlated although not completely overlapping; 251 (67%) were cordant and 126 (33%) discordant ($P = 0.002$).

Stathmin curettage expression predicts lymph node metastasis

We also examined if Stathmin could predict lymph node metastasis independently of pathologic information available before primary surgical therapy. Preoperative curettage/endometrial biopsy report diagnoses were categorized as high risk if either histologic type was reported as serous papillary, clear cell or carcinosarcoma, or if the histologic grade was reported as grade 3 or undifferentiated. Other histologic diagnoses were categorized as low risk including benign endometrium, hyperplasia, and well/moderately differentiated endometrioid carcinoma.

Histologic type and grade as well as Stathmin expression in curettage specimens all correlated significantly with the occurrence of lymph node metastasis, but Stathmin was the strongest and the only independent predictor of lymph node metastasis (Table 3) with a sensitivity = 0.47, specificity = 0.66, positive predictive value (PPV) = 0.17, and negative predictive value (NPV) = 0.89. Analyzing the low-risk curettage group separately, the multivariate OR is 1.82 (95% CI: 0.93–3.57), sensitivity = 0.44, specificity = 0.70, PPV = 0.13, and NPV = 0.92, for high-risk curettage the respective values are 1.42 (95% CI: 0.57–3.53), sensitivity = 0.67, specificity = 0.41, PPV = 0.27, and NPV = 0.79.

Stathmin expression in hysterectomy specimen had a weaker correlation with lymph node metastasis with OR 1.84 (95% CI: 0.87–3.86).

Stathmin predicts survival

Disease-specific survival was significantly related to age, menopausal status, FIGO stage, histologic subtype and grade, ploidy and lymph node status in univariate

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Table 2. Stathmin protein expression in curettage ($n = 818$) and hysterectomy ($n = 477$) specimens from patients with endometrial cancer related to clinicopathologic variables

Variable	Stathmin expression curettage			Stathmin expression hysterectomy		
	Weak *n* (%)	Strong[a] *n* (%)	*P*	Weak *n* (%)	Strong[b] *n* (%)	*P*
FIGO stage			0.024			0.257
I, II	437 (65)	237 (35)		329 (83)	66 (17)	
III, IV	79 (55)	65 (45)		64 (78)	18 (22)	
Histologic type			0.001			0.012
Endometrioid	452 (66)	238 (35)		331 (84)	61 (16)	
Nonendometrioid	64 (50)	64 (50)		62 (73)	23 (27)	
Histologic grade[c]			<0.001			0.001
Grade 1, 2	409 (71)	168 (29)		281 (87)	43 (13)	
Grade 3	104 (44)	133 (56)		110 (74)	39 (26)	
Ploidy[d]			0.013			0.047
Diploid	145 (64)	83 (36)		212 (82)	48 (19)	
Aneuploid	27 (46)	32 (54)		45 (70)	19 (30)	
Lymph node metastasis[e]			0.020			0.104
Negative	343 (66)	176 (34)		292 (85)	53 (15)	
Positive	41 (53)	37 (47)		33 (75)	11 (25)	

[a]Upper quartile, indices 6 and 9.
[b]Upper quartile, index = 9.
[c]Missing cases 4 in curettage and hysterectomy group.
[d]Ploidy data available for 287 cases in curettage group 324 in hysterectomy group.
[e]Lymph node sampling done in 597 cases in curettage group and 389 in hysterectomy group.

analyses (Table 4). Strong Stathmin expression significantly influenced survival both when measured in curettage and hysterectomy specimens, $P < 0.001$ and $P = 0.002$, respectively, Figure 1. Curettage Stathmin analysis as a predictor for death due to endometrial cancer has a specificity of 0.54, sensitivity = 0.64, PPV = 0.15, and NPV = 0.92. When analyzing the endometrioid group separately, Stathmin in curettage specimens yielded a significant correlation with disease-specific survival, $P = 0.002$, whereas the staining in hysterectomy specimen showed the same tendency ($P = 0.06$). In cases with low risk preoperatively curettage, Stathmin expression has a sensitivity of 0.47, specificity = 0.70, PPV = 0.09, and NPV = 0.95 whereas in high risk patients the respective values are 0.60, 0.39, 0.26, and 0.73.

In multivariate Cox survival analysis, strong expression of Stathmin in curettage showed independent prognostic value with an adjusted HR of 1.68 (95% CI: 1.05–2.67),

Table 3. Prediction of lymph node metastases in 521 patients with endometrial cancer, uni- and multivariate logistic regression

Variable	*n*	Univariate OR	95% CI	*P*	Multivariate OR	95% CI	*P*
Histologic type curettage[a]				<0.001			0.123
Low risk	448	1					
High risk	73	3.62	2.00–6.54		2.23	0.80–6.21	
Histologic grade curettage				<0.001			0.390
1, 2	412	1					
3	109	3.15	1.83–5.44		1.53	0.59–4.01	
Stathmin curettage				0.005			0.046
Weak expression	327	1					
Strong expression	194	2.10	1.25–3.54		1.75	1.01–3.02	

NOTE: Cases with data available for all variables included in uni- and multivariate analyses.
[a]Low risk: benign, hyperplasia, endometrioid; high risk: serous papillary, clear cell, carcinosarcoma, and undifferentiated carcinomas.

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Table 4. Cause specific survival for 1,009 endometrial cancer patients related to clinicopathologic variables and Stathmin expression

Variable	*n*	Number of deaths	% 5-year survival	*P*
Age, y				<0.001
<66	499	28	92	
≥66	510	77	75	
Menopause status				0.003
Pre/perimenopausal	105	3	96	
Postmenopausal	904	102	83	
FIGO				<0.001
I, II	831	37	93	
III, IV	178	68	42	
Histologic type				<0.001
Endometrioid	825	44	91	
Nonendometrioid	184	61	51	
Histologic grade[a]				<0.001
Grade 1, 2	686	28	93	
Grade 3	317	77	65	
Ploidy[b]				<0.001
Diploid	294	24	89	
Aneuploid	76	19	69	
Lymph node metastasis[c]				<0.001
Negative	619	28	92	
Positive	89	28	49	
Stathmin curettage[d]				<0.001
Weak expression	487	37	88	
Strong expression	291	43	77	
Stathmin hysterectomy[e]				0.002
Weak expression	393	44	88	
Strong expression	84	20	69	

NOTE: Of 1,076 patients, 67 not yet attended follow-up.
[a]Missing cases 6.
[b]Ploidy data available for 370 patients.
[c]Lymph node sampling conducted in 708 cases.
[d]Stathmin curettage available for 778 patients.
[e]Stathmin hysterectomy available in 477 cases.

adjusted for age, FIGO stage, histologic type, and grade (Table 5). Strong expression of Stathmin in hysterectomy specimens yielded a similar result with an HR of 1.68 (95% CI: 1.13–3.35; $P = 0.02$).

Strong Stathmin expression was significantly more prevalent in the group of patients receiving primary adjuvant radiation or chemotherapy ($P \leq 0.003$). Stathmin curettage retained a significant HR of 1.63, CI 1.02–2.60 even when adjusted for supplementary treatment in a multivariate Cox model. Adjuvant treatment was not a statistical independent factor with HR 1.38, CI 0.80–2.39.

Discussion

The protein expression of Stathmin has been explored and found to correlate to clinicopathologic factors and poor prognosis in several cancers in different tissues such as brain (25), oral mucosa (26), breast (10, 11, 27), urothelial (28) as well as ovarian (12), and uterine cervix (13). Recently, Stathmin has been identified as a marker for PI3Kinase signaling pathway activation and poor prognosis also in endometrial carcinomas (9). In the present study, we validate the applicability of immunohistochemical staining for Stathmin as a marker applied in a prospective, multicenter setting to detect endometrial cancer patients with an aggressive phenotype.

In endometrial carcinoma, the tumor is easily accessible for biopsy by curettage prior to primary surgical treatment. Parameters routinely investigated in curettage specimens, such as histologic type and grade, yield prognostic information relevant for the planned surgical procedure. Still, relatively poor correlation between curettage and

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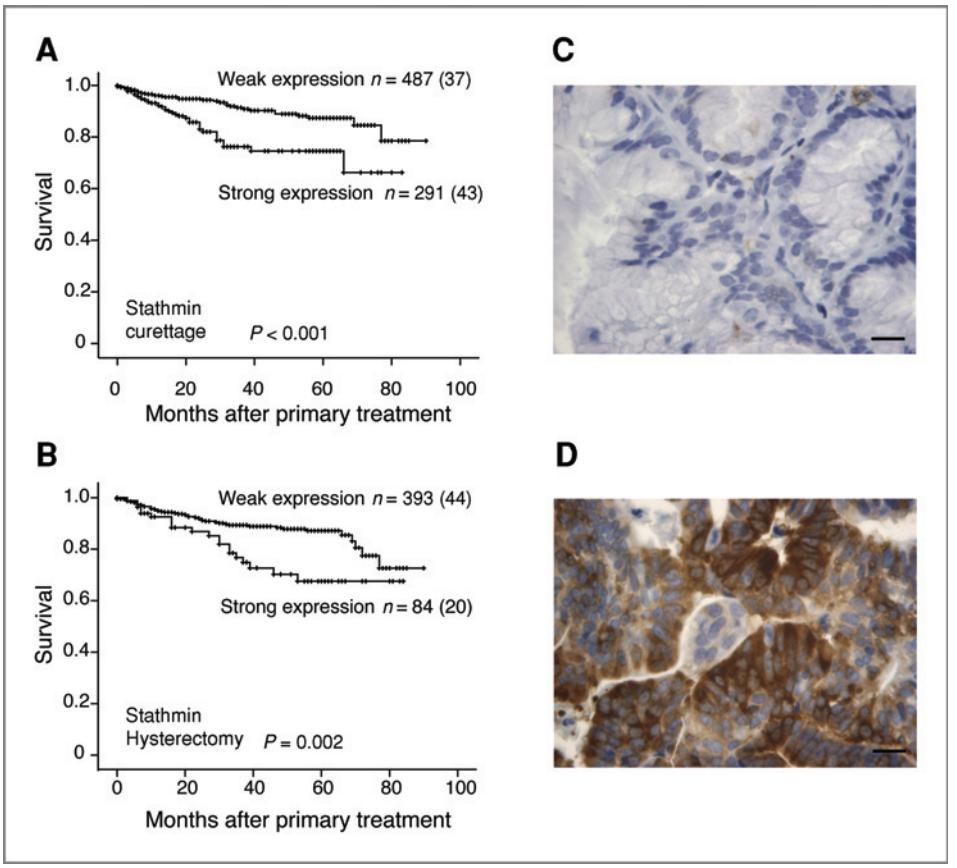

Figure 1. Disease-specific survival for patients with endometrial carcinoma (Kaplan–Meier estimation), related to expression of Stathmin immunostaining in A, curettage and B, primary tumor specimens. Immunohistochemical staining showing C, weak Stathmin expression, and D, strong Stathmin expression. Bar represents 20 μm.

hysterectomy findings has motivated the search for improved measures to define high and low-risk patients (29). Markers for hormone receptor and p53 status in tumor based on investigation of curettage specimens have consistently shown to add prognostic information (24, 30–32). We have in an earlier, smaller, retrospective single-center study (9) investigated Stathmin in hysterectomy specimens. Their upper quartile as cutoff corresponded

Table 5. Multivariate survival analysis of 775 endometrial cancer patients according to Cox' proportional hazards regression model

Variable	*n*	Unadjusted HR	95% CI	*P*	Adjusted HR	95% CI	*P*
Age	775	1.08	1.05–1.10	<0.001	1.05	1.03–1.08	<0.001
FIGO stage				<0.001			
I, II	638	1			8.62	5.26–14.14	<0.001
III, IV	137	13.15	8.23–21.00				
Histologic type				<0.001			
Endometrioid	652	1			2.21	1.19–4.13	0.012
Nonendometrioid	123	7.28	4.69–11.31				
Histologic grade				<0.001			
1, 2	546	1			1.63	0.82–3.25	0.162
3	229	6.44	4.01–10.36				
Stathmin curettage				<0.001			
Weak expression	485	1			1.68	1.05–2.67	0.030
Strong expression	290	2.43	1.56–3.78				

NOTE: Cases with data available for all variables included in uni- and multivariate analyses.

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to staining index of 6 and 9. In this validating multicenter study, we found a significant stronger staining in hysterectomy versus curettage specimens, in spite of the fact that both sets have been stained in parallel (same run) and the same procedure for TMA preparation and processing of sections were carried out. Thus different staining index groups defined the upper quartile cutoff in the 2 sets. The stronger Stathmin staining of hysterectomy specimens were not explained by different fixation time. While preparing TMAs, we opted for the most aggressive looking (lowest differentiated) part of the specimen. Curettings represent superficial parts of the tumor and are in general of smaller volume than the tumor available from hysterectomy specimens. Because there may be significant tumor heterogeneity, it is possible that the hysterectomy TMA more often contains undifferentiated tumor, this could be reflected in higher staining indexes. Although in this study, we validate Stathmin as an independent predictor of poor survival, the optimal staining procedure and cutoff is yet to be defined and more work is needed to optimize and develop a standardized test potentially applicable in a routine setting. One clinical example illustrating such cumbersome process, is the time frame from discovery of the neu oncogene in 1984 to the publication of guidelines for standardized HER-2 neu testing in breast cancer in 2007 by the American Society of Clinical Oncology and the College of American Pathologists (33).

Pelvic lymph node sampling has long been an integral part of the staging of endometrial cancer (18). It is well documented that the presence of lymph node metastasis is a predictor of poor survival (2, 34), although randomized controlled trials have not proven a survival benefit for lymph node sampling (5, 6). Depth of myometrial tumor infiltration, histologic type and grade in hysterectomy specimen can predict lymph node spread (2, 35), and different algorithms have been explored in the process for optimal selection of patients for sampling (36). Unfortunately, such assessment pre- and perioperatively have been less reliable than these assessments in hysterectomy specimen (29, 37), indicating that they are suboptimal as guidance regarding the need for lymph node sampling. Only few studies have investigated curettage markers for lymph node metastases adjusted for standard preoperative risk assessment based on histopathologic evaluation of curettage. Mariani and colleagues (38) reported p53 as an independent predictor of lymph node metastasis. The present study identifying Stathmin expression in curettage as an independent predictor for lymph node metastases is, to the best of our knowledge, the first of its kind, and is also a large prospective multicenter trial.

The high NPV of 0.92 indicates that Stathmin might have a potential of adding to the diagnostic security and permit more safely to omit lymph node sampling in low-risk patients with weak curettage Stathmin staining. The relation between Stathmin expression in hysterectomy specimen and occurrence of lymph node metastasis did not reach statistical significance. This could be due to the study being underpowered concerning this aspect (500 cases needed whereas only 389 hysterectomy specimens from patients with lymphadenectomy were available for Stathmin analysis). The possibility that such a correlation does exists therefore should not be excluded.

From clinical trials of targeted therapies in breast, lung, and colorectal cancer, we have learned that biomarkers should be applied to design future clinical trials (39–41). Endometrial cancers are heterogeneous in clinical presentation, histopathologic features, and molecular characteristics (9). Ignoring this diversity by sticking to the "one-size-fits-all" approach in future design of clinical trials may prevent us from realizing the potential of targeted therapies in management of endometrial cancers.

Taxane is one of the main chemotherapeutics applied in metastatic endometrial carcinoma (42). Taxane induces polymerization of tubules and stabilization of microtubules and thereby hinder mitosis (43). It is shown that *Stathmin* overexpression in breast cancer cell lines promotes Taxane resistance (8). When *Stathmin* expression was blocked, the Taxane resistance was opposed (44). For ovarian cancer patients, strong *Stathmin* expression has been linked to worse survival for patients who received Taxane in combination with platinum, in contrast to what was seen for patients treated with platinum alone (12). The potential for Stathmin expression as a predictive marker for Taxane response in endometrial cancer needs to be explored.

The PIK3Kinase/mTOR pathway is often upregulated in endometrial carcinoma (9, 45), and PIK3Kinase and mTOR inhibitors are presently entering clinical trials (16). The fact that activation of this signature has been found to associate with aggressive endometrial cancer and Stathmin overexpression (9), suggests Stathmin as a potential predictive marker for response. This needs to be evaluated in further clinical testing of drugs targeting the PI3Kinase pathway.

Conclusion

Stathmin immunohistochemical staining identifies endometrial carcinomas with lymph node metastases and poor survival in a large prospective multicenter setting. The value as predictive marker for response to Taxanes and PI3Kinase inhibition, and as a tool to stratify for lymph node sampling remains to be determined.

Disclosure of Potential Conflicts of Interests

H.B. Salvesen and L.A. Akslen: an option agreement has been entered into in February 2010 between Bergen Teknologioverføring AS (BTO; on behalf of the inventor's employer the University of Bergen) and Ovagene Oncology Lt regarding Stathmin expression and phenotype in endometrial cancer. This agreement grants Ovagene Oncology 6 months' exclusive right to negotiate a license for commercial application of the invention. This option period has now expired and no commercial agreement has been signed at present.

Provisional patent application in the United States (NO 61/310,109) about Stathmin expression and phenotype was submitted by BTO in March 2010.

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Jone Trovik, Elisabeth Wik, Ingunn M. Stefansson, et al.

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