Positive N-Cadherin immunostaining in uterine endometrioid carcinoma is associated with better survival

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Original Article

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ABSTRACT

Objectives: The objective of the current study is to define the immunostaining pattern of N-cadherin in uterine endometrioid carcinoma and its relation to clinicopathological features and its prognostic significance.

Design: A retrospective study of N-Cadherin immunostaining in uterine endometrioid carcinoma and non-neoplastic endometrial tissue.

Setting: Paraffin embedded tissue block were retrieved from the archive of department of Pathology, King Abdulaziz university hospital, Jeddah Saudi Arabia.

Subjects: 71 uterine endometrioid carcinomas and 30 non-neoplastic endometria were included in the study.

Interventions: Tissue microarrays were constructed. Immunostaining for N-Cadherin was done

Main Outcome Measure: statistical analysis of the immunostaining results to address the prognostic significance of N-Cadherin immunostaining**.**

Results: In non- neoplastic tissues, positive immunostaining was observed in 13.3%. In endometrioid carcinoma, positive immunostaining was seen in 40.8%. The positive immunostaining was higher in endometrioid carcinoma than in non-neoplastic tissues. In endometrioid carcinoma, positive immunostaining showed no relation with most clinicopathological feature. On the other hand, positive immunostaining was associated with better survival outcomes for overall survival (p=0.007) and disease-free survival (p=0.028).

Conclusion: In summary, we show positive N-cadherin immunostaining in uterine endometrioid carcinoma that is associated with better survival outcomes. This finding is novel and contradicting many other studies in other organs. Our finding is challenging and needs more highlight to the pattern of N-cadherin in uterine endometrioid carcinoma using more cohort of cases and molecular pathology studies to confirm its exact role.

KEYWORDS: Endometrium, Tissue Microarray, Immunohistochemistry, N-Cadherin

INTRODUCTION

Endometrial carcinoma (EC) is the most common malignancy of the female genital tract [1] with an approximate life risk of 3% [2]. EC has an increased in incidence and has doubled its death rate [3]. EC comprises 4% of all cancers in women globally [4]. In Saudi Arabia, EC constitutes 2.9% of newly diagnosed malignancies in females [5]. The 5 year overall survival in patients without metastasis ranges between 74 to 91%, while it reaches to as low as 20% in cases with metastasis [6]. Most of the deaths associated are caused by chemotherapy-resistant metastases. Therefore, investigation of the molecular mechanisms behind endometrial cancer metastasis would provide insight for the development of improved therapies [7].

The epithelial to mesenchymal transition (EMT) is a key molecular mechanism predicting cancer metastasis. EMT is characterized by loss of the epithelial marker E-cadherin, an increase in the

mesenchymal markers vimentin and N-cadherin [8]. N-cadherin is a member of cadherin superfamily that mediates cell-cell interaction in epithelial tissue [9]. For example, N-cadherin overexpression has been correlated with tumour aggressiveness and metastasis in prostate cancer, melanoma, breast cancer, and colon cancer [10-14]. N-cadherin expression could help in deciding prognosis of cancer patients; patients with high N-cadherin expression have a significantly lower overall survival and event-free survival rate than those with low N-cadherin expression [15].

There is little known about N-cadherin expression in EC with limited conclusions. The objective of the current study is to define the immunostaining pattern of N-cadherin in EC and its relation to clinicopathological features and its prognostic significance.

MATERIALS AND METHODS

Patients

The study included paraffin wax tumour blocks from 71 patients diagnosed with uterine endometrioid carcinoma in the period from 2003-2012. Also, paraffin blocks from non-neoplastic endometria of 30 patients in the period from 1995-1998 were included (20 proliferative endometrium and 10 secretory endometrium). All blocks were retrieved from the archives of the Department of Pathology at King Abdulaziz University, Jeddah, Saudi Arabia. Some clinicopathological characteristics of patients are listed in Table I. For statistical purpose, FIGO stages were classified into limited to uterine corpus (FIGO Stage I and II) and beyond the uterine corpus (FIGO III and IV). Also, grade was reclassified as low grade (grade I) and (grades II and III). Data is shown in table (1). The study was performed in accordance with the ethics committee of Faculty of Medicine, King Abdulaziz University, Saudi Arabia, and declaration of Helsinki.

Tissue Microarray

Archival paraffin-embedded tumour samples and neoplastic tissues were selected, and representative areas were marked on haematoxylin and eosin stained slides. Two tissue cylinders (cores) with a diameter of 1.5 mm was punched from morphologically representative tissue areas of each ‘donor’ tissue block and brought into new recipient paraffin blocks by using a tissue microarrayer instrument (TMA Master 1.14 SP3 (3D Histech Ltd. Budapest, Hungary). Placenta tissue was used for orientation [16].

Immunohistochemistry

TMA blocks of tumours were cut at 4 µm and mounted on positive-charged slides (Leica Microsystems Plus Slides). Sections were deparaffinised in xylene and rehydrated in an automated immunostainer (BenchMark XT, Ventana® Medical Systems Inc., Tucson, AZ, USA). Pre-treatment was done using CC1 (prediluted cell conditioning solution) for 60 min. Monoclonal mouse Anti-human N-Cadherin antibody (Clone 6G11 from Dako) was used at dilution 1:50 with incubation time 30 minutes. Ventana® I-view DAB detection kit was used according to kit manufacturer instructions. Subsequently, slides were

washed, counterstained with Mayer's haematoxylin and mounted. Negative control and positive control slides were included.

Interpretation of N-cadherin Immunostaining

N-cadherin membranous immunostaining was reported as the percentage of positive cells. The percentage of positive cells ≥ 5% indicates N-cadherin-positive expression and <5% N-cadherin-negative expression [17].

Statistical analysis

Differences between groups of patients were tested by using Mann Whitney test (in case of two groups) and Kruskal Wallis test (in case of three or more groups). Wilcoxon rank sum test was used to test difference between two related groups of paired variables Non-parametric chi-square was used to test variance along one variable. The Kaplan-Meier procedure was used to calculate the survival probabilities and the Log Rank test was used to compare the difference between survivals. Statistical procedures were performed using SPSS® Release 16.0. Statistical significance was determined at *p* value of ≤0.05 and was 2-sided.

RESULTS

Immunostaining of N-Cadherin was indicated by membranous brown colour in non-neoplastic and neoplastic endometrial (figure 1). In non-neoplastic endometrial tissues, positive immunostaining was observed in 13.3%. The occurrence of negative immunostaining was statistically more than positive immunostaining (p= <0.001). In proliferative endometrium, positive N-cadherin immunostaining was shown in 15% (3/20), while in secretory endometrium only one showed positive immunostaining (10%). In endometrioid carcinoma, positive N-cadherin immunostaining was seen in 40.8%. The occurrence of negative immunostaining was statistically more than positive immunostaining (p= <0.001). The positive N-Cadherin immunostaining was higher in endometrioid carcinoma than in non-neoplastic tissues. Data is shown in table (2).

In endometrioid carcinoma, positive N-cadherin immunostaining showed no relation with most clinicopathological features (table 3). On the other hand, positive N-cadherin immunostaining was associated with better survival outcomes for overall survival (Log Rank (Mantel-Cox) = 7.35, P=0.007) (figure 2) and better disease free survival (Log Rank (Mantel-Cox) = 4.824, p=0.028) (figure 3).

DISCUSSION

During tumour progression, cancer cells undergo major changes in the expression of the adhesion molecules resulting in detachment from original tissue and acquisition of a highly motile and invasive phenotype [18]. The adhesion of the cells is influenced by cadherin which are calcium-dependent cell molecules involved in maintaining the epithelial structure and normal tissue architecture [19].

Previous studies claimed that the abnormal level of E-cadherin is associated with tumour progression and metastasis [20], while N-cadherin is associated with a heightened invasive potential in cancer [21,22].

In the present study, N-cadherin was highly expressed in endometrioid carcinoma (40.8%), which comes in congruence with what had been reported by Xie *et al.,* [23]. This finding could raise the importance of N-cadherin expression as a possible indicator for the clinical evaluation and prognosis of endometrial cancer. The N-cadherin is abnormally expressed in some epithelial tumours, and its ability to enhance the invasion and metastasis of tumour cells is more evident than that of the E-cadherin [22], as it has an essential role in the maturation and stabilisation of normal vessels and tumour-associated angiogenic vessels [18]. Moreover, it had been suggested that E-cadherin may be, in some of the biological phases of the tumour, converted to N-cadherin [22].

Regarding N-cadherin expression in endometrial epithelium, Tsuchiya *et al.,* addressed that there is a total difference between proliferative and secretory phases; while N-cadherin is strongly presented in the epithelium of the endometrial gland in the proliferative phase, no N-cadherin is observed in the early and late secretory phases [24]. That might partially explain our findings where positive N-cadherin immunostaining was shown in 15% (3/20) in proliferative endometrium, while in secretory endometrium only one showed positive immunostaining (10%). In a study conducted by Prudkin *et al.,* they examined cadherin expression in cases with lung cancer, found that squamous cell carcinoma had reduced E-cadherin expression and increased N-cadherin cytoplasmic expression, and this phenotype was associated with relatively few clinicopathological features [25], which supports our findings, where positive N-cadherin immunostaining showed no association with most clinicopathological feature in endometrioid carcinoma. On the other side, in another study carried out by Luo *et al*., the authors concluded that high expression of cytoplasmic and nuclear N-cadherin was associated with a majority of the clinicopathological variables, including lymph node metastasis, distant metastasis and clinical stage [26]. Our study showed that positive N-cadherin immunostaining was associated with better survival outcomes expressed as overall survival and disease free survival. In this respect, the review of published literature showed that the nature of the link between N-cadherin expression and prognosis of the cancer patients varies between different researches. For example, Tothill *et al.,* reported that patients with overexpressed N-cadherin had obvious lower overall survival rate than those with moderate and low expression, and patients with low expression had a better survival rate than those with moderate and high expression, they concluded that high N-cadherin expression may lead to tumour aggressiveness and metastatic potential in colorectal cancer, and may prove to be a possible prognostic factor [27]. Quattrocchi *et al* revealed significant inverse correlation between N-cadherin expression and 36-month overall survival and significant negative association between high expression of N-cadherin and progression-free survival [28]. On the same line, Nakashima showed that N-cadherin overexpression was associated with poor outcome in patients [29], which came in agreement with previous studies [21,30,31], it has been demonstrated that there is a significant positive association between high N-cadherin expression and poor overall survival. On the other side, Abufaraj argued that N-cadherin expression is associated with higher probabilities of disease recurrence but not progression or survival outcomes [32],

in contrast to Lascombe who addressed that N-cadherin expression is an independent prognostic marker for tumour progression [33]. Despite of these discrepancies in the findings, N-cadherin is viewed as an important therapeutic target among experts [18].

The discrepancy between our findings and other N-cadherin studies especially in the endometrium may be due to the immunohistochemistry technique which depends on multiple variables, such as the fixation method, preservation technique, specimen handling, choice of antibodies, and variation in the scoring methods. The limitations of our study were the relatively low number of specimens (neoplastic and non-neoplastic).

CONCLUSION

In summary, our study confirms the overexpression of N-cadherin in uterine endometrioid carcinoma. However, it is not associated with any clinicopathological parameter. On the other hand, positive N-cadherin is associated with high survival probabilities. This finding is novel and contradicting many other studies in other organs. Our finding is challenging and needs more highlight to the pattern of N-cadherin in uterine endometrioid carcinoma using more cohort of cases and molecular pathology studies to confirm its exact role.

ACKNOWLEDGMENT

WG contributed to tissue microarray design, scoring of immunostaining, statistical analysis, and drafted the manuscript. IZ shared in scoring of immunostaining and drafting of the manuscript. BM shared in data collection and immunostaining. JM contributed to histological examination and selection of paraffin blocks included in study, contributed to the design of the study, and revised the manuscript. The manuscript has been read and approved by all authors, the requirements for authorship have been met, and each author believes that the manuscript represents honest work.

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Table 1: Clinicopathological features of endometrioid carcinoma (n=71)

Parameter		Number (%)
Age	< 60 years	49 (69%)
	≥ 60 years	22 (31%)
FIGO tumour grade	Grade 1	44 (62%)
	Grade 2	16 (22.5%)
	Grade 3	11 (15.5%)
Tumour size	≤ 2 cm	35 (49.3%)
	> 2 cm	36 (50.7%)
Myometrial invasion	< 50%	57 (80.3%)
	≥ 50%	14 (19.7%)
Lymphovascular	Absent	68 (95.8%)
	Present	3 (4.2%)
Surgical resection margin	Free	67 (94.4%)
	Involved	4 (5.6%)
Lymph node metastasis	Absent	33 (46.5%)
	Present	4 (5.6%)
	Not Sampled	34 (47.9%)
FIGO Staging	I	51 (71.8%)
	II	7 (9.85%)
	III	7 (9.85%)
	IV	6 (8.5%)
Local Recurrence	Absent	60 (84.5%)
	Present	11 (15.5%)

FIGO (International Federation of Gynaecology and Obstetrics)

Stage I: Tumour confined to corpus uteri

IA: Tumour limited to endometrium or invades less than one-half of the myometrium

IB: Tumour invades one-half or more of the myometrium

Stage II: Tumour invades stromal connective tissue of the cervix but does not extend beyond uterus

Stage III: There is regional tumour spread.

IIIA: Tumour involves serosa and/or adnexa (direct extension or metastasis)

IIIB: Vaginal involvement (direct extension or metastasis) or parametrial involvement

IIIC: The tumour involves regional lymph nodes

IIIC1: Regional lymph node metastasis to pelvic lymph nodes

IIIC2: Regional lymph node metastasis to para-aortic lymph nodes, with or without positive pelvic lymph nodes

Stage IV: The tumour invades contiguous organs or has metastasized to remote organ sites.

IVA: Tumour invades bladder mucosa and/or bowel mucosa (bullous oedema is not sufficient to classify a tumour as T4)

IVB: Distant metastasis

Table 2: Categories of immunostaining in primary tumours and non-neoplastic endometrium

Immunostaining Pattern	Primary tumour (*n*=71)	Non-neoplastic endometrium (*n*=30)	*p* value
Low immunostaining	42 (59.2%)	26 (86.7%)	0.034❝
High immunostaining	29 (40.8%)	4 (13.3%)	
p value	<0.001*	<0.001*	

*One sample non-parametric chi-square test

❝ Mann-Whitney test

Table 3: Relation between clinicopathological features and N-Cadherin immunostaining in tumours (n=71)

Parameter		P-value
Age	< 60 years	0.122
	≥ 60 years	
FIGO tumour grade	Low (FIGO Grade I)	0.989
	High (FIGO Grade II and III)	
Tumour size	≤ 2 cm	0.887
	> 2 cm	
Myometrial invasion	< 50%	0.665
	≥ 50%	
Lymphovascular	Absent	0.356
	Present	
Surgical resection margin	Free	0.703
	Involved	
Lymph node metastasis	Absent	0.400
	Present	
	Not Sampled	
FIGO staging	Early (FIGO Stage I and II)	0.245
	Late (FIGO Stage III and IV)	
Local recurrence	Absent	0.318
	Present	

FIGO (International Federation of Gynaecology and Obstetrics)

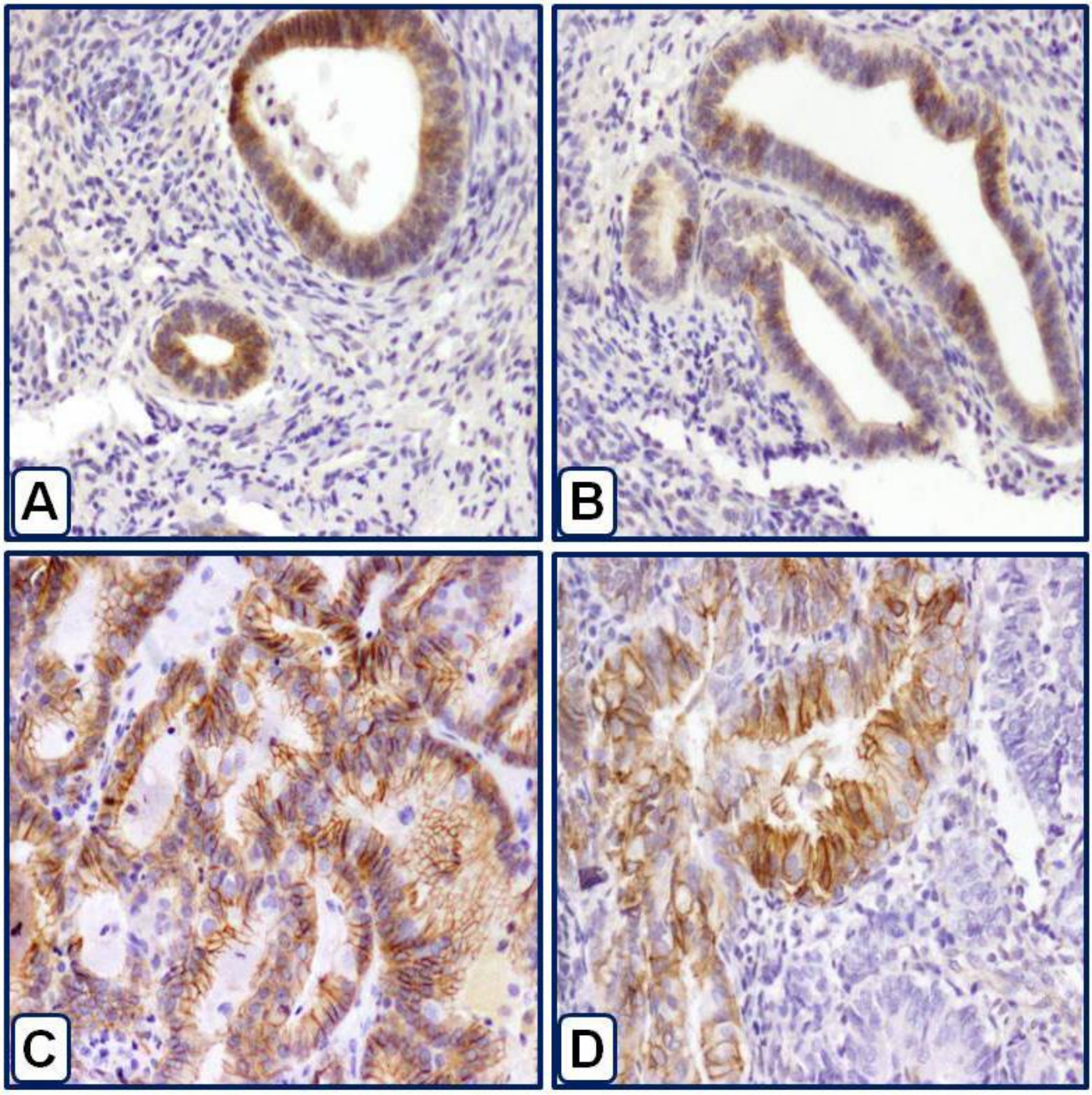

Figure 1: Immunostaining of N-cadherin in non-neoplastic endometrium and endometrioid carcinoma

Membranous immunostaining is detected proliferative endometrium (A-100X), secretory endometrium (B-100X), well-differentiated endometrioid carcinoma (C-100X), and in moderately differentiated endometrioid carcinoma (D-200X). Immunohistochemical labelling was done using the anti N-Cadherin antibody and diaminobenzidine used as the chromogen and haematoxylin as counterstain.

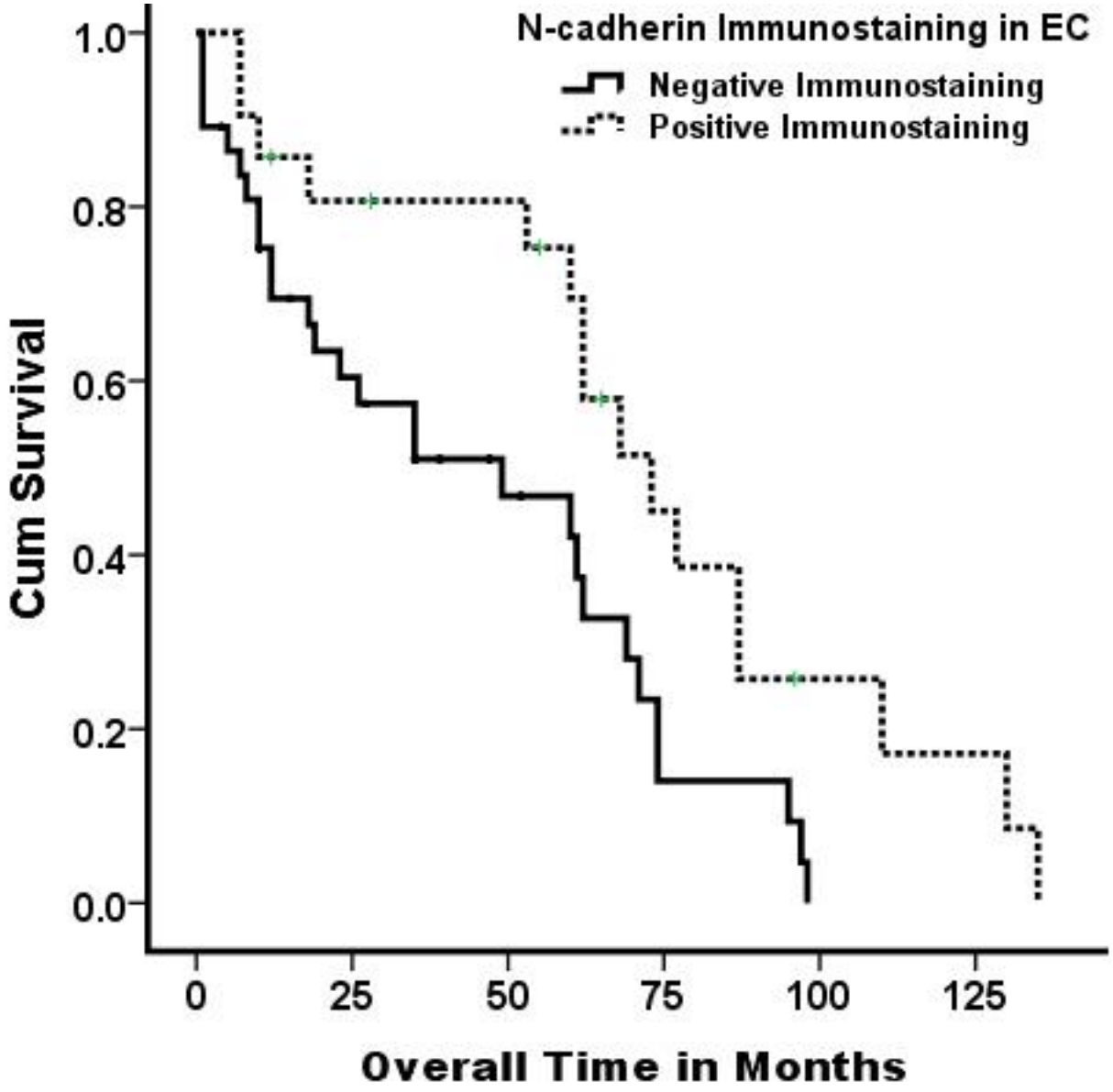

Figure 2: Overall survival curve (Kaplan Meier) according to N-cadherin immunostaining.

Positive N-cadherin immunostaining is associated with better overall survival {Log Rank (Mantel-Cox) = 7.35, p=0.007)}.

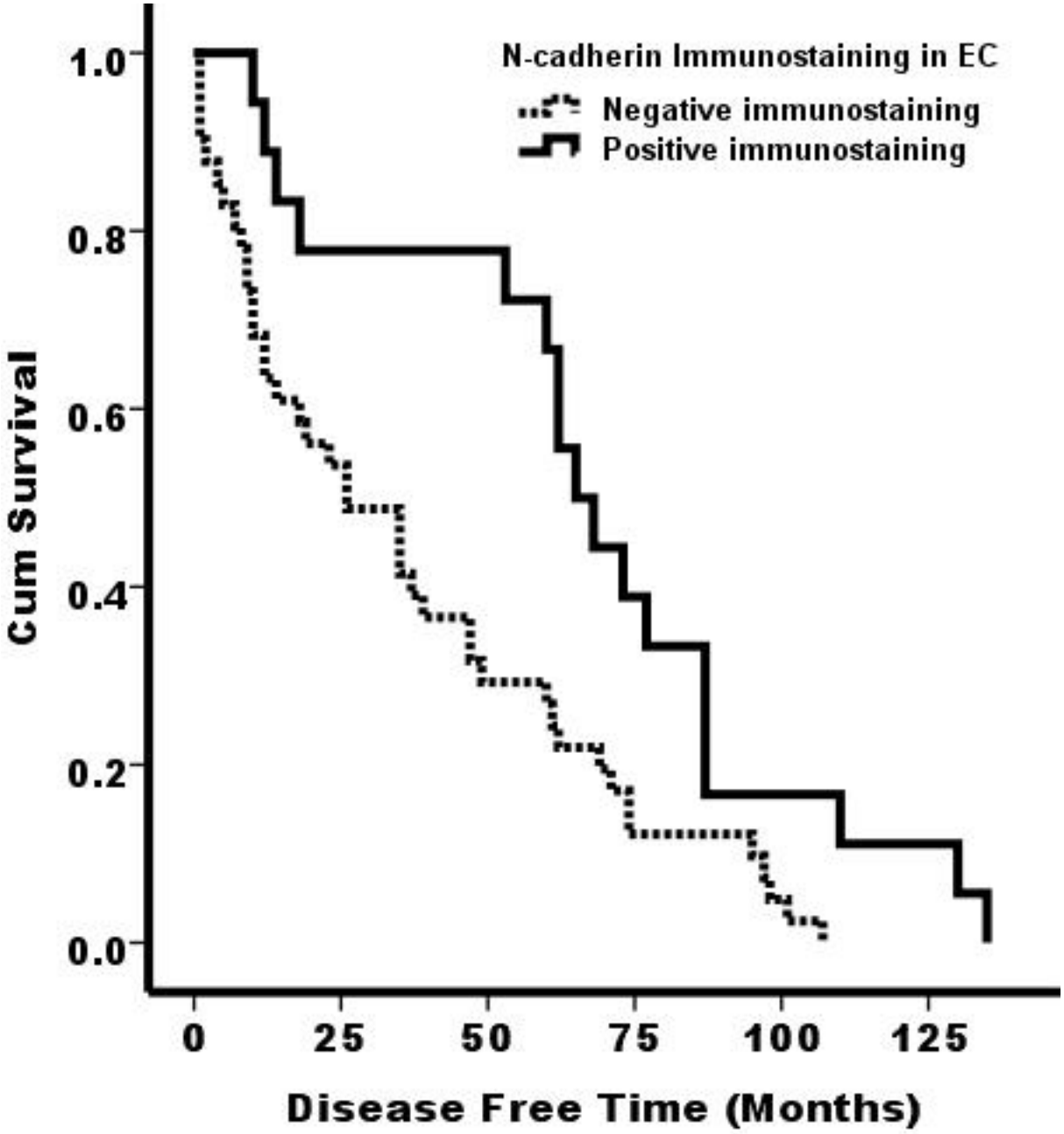

Figure 3: Disease survival curve (Kaplan Meier) according to N-cadherin immunostaining.

Positive N-cadherin immunostaining is associated with better disease-free survival {Log Rank (Mantel-Cox) = 4.824, p=0.028)}.