ANTICANCER RESEARCH *25*: 1747-1754 (2005)

S100 as an Immunohistochemically-detected Marker with Prognostic Significance in Endometrial Carcinoma

A. HONIG, N. SCHALLER, J. DIETL, J. BACKE and U. KAMMERER

Department of Obstetrics and Gynaecology, University of Wuerzburg, Germany

Abstract. *Background: Several studies have indicated that dendritic cells (DC) participate in anti-tumor immunity, possibly influencing the course of malignant disease. We tested whether tumor infiltration by $S100^+$ DC could be a prognostic marker for endometrial cancer. Materials and Methods: A retrospective study analyzing 115 tissue samples from patients with endometrial carcinoma and known histological grading as well as hormone receptor, Ki-67, Her-2/neu and p53 expression. Sections of paraffin-embedded endometrial tissue were immunohistochemically-stained with anti S100 antibody. Tumor infiltrating $S100^+$ DC were counted via microscopic examination and calculated as $S100^+$ DC per mm^2 of tissue. Results: Samples were divided into group 1: less than 10 $S100^+DC/mm^2$ ($n=44$) and group 2: 10 or more $S100^+$ DC/mm^2 ($n=71$). Correlation with clinico-pathological markers was calculated by Chi-square test. Compared to group 1, the DC-rich group 2 showed a higher level of differentiation ($p=0.045$), a lower overexpression of p53 ($p=0.021$) and less proliferation ($p=0.028$). DC infiltration was not correlated with Her-2/neu, hormone receptor status and FIGO-stage. Although no significant correlation could be seen, the DC-poor group samples seemed to correlate with a higher FIGO-stage compared to the DC-rich group. In uni- and multivariate analysis, DC infiltration proved to be a significant prognostic marker for adjusted survival but not for overall survival. Conclusion: Our results indicate that the immunohistochemical determination of $S100^+$ DC could contribute to the identification of a high-risk subgroup and, therefore, would be a favorable prognostic factor for endometrial carcinoma. Our observation that pronounced DC infiltration is associated with good prognosis points to an important role of the host's immune system response for the clinical course of endometrial cancer.*

Correspondence to: Dr U. Kammerer, Josef-Schneider Str.4, 97080 Wuerzburg, Germany. e-mail: frak057@mail.uni.wuerzburg.de

Key Words: Endometrial cancer, dendritic cells, S100 protein, prognostic factor.

The fight between the host's immune response and the tumor has been found to be an important factor for the clinical outcome of tumor patients. Several studies have indicated that dendritic cells (DC) participate in anti-tumor immunity and tumor surveillance (15). The dendritic cell family includes Langerhans' cells (CD1a-positive DC of the skin), antigen-presenting cells that are found in the lymphoreticular system and cells widely distributed throughout most parenchymal organs (15). DC are nature's best antigen-presenting cells (APC) and fulfill sentinel functions in most of these locations. As immature cells, they take up antigens in peripheral tissues, process them into proteolytic peptides, and load these peptides onto major histocompatibility complex (MHC) class I and II molecules (7, 18). During this antigen processing, DC mature and, while maturing, migrate to secondary lymphoid organs and become competent to present antigens to T lymphocytes. Thus they initiate antigen-specific immune responses (for review see 14).

Tumor-infiltrating DC are expected to capture and process antigens shed by adjacent tumor cells, thus possibly activating tumor antigen-specific T lymphocytes. Considering this important role of DC raises the question as to whether the response of DC towards a tumor could be quantitatively analyzed and would allow a prognostic statement. The presence of a large number of DC within a tumor might then imply a favorable prognosis.

In contrast, there is also evidence that DC can loose their capacity to activate T cells if exposed to malignant cells (16) and/or do not mature and migrate into lymph nodes. In such a setting, they might not fulfill their function as T cell-activating APC, inducing anti-tumor immunity, but might mediate tumor-induced tolerance *via* antagonizing T cells (17). In the latter case, a large number of DC in the tumor area might reflect their accumulation due to inhibited migration and this could then imply a bad prognosis.

To provide further evidence that the number of DC infiltrating an endometrial carcinoma is important for the clinical course of this disease, we established a study correlating the number of DC found in tissue sections of endometrial cancer by immunohistochemistry with prognosis

Table I. *Parameters of tissue samples/patients in the study.*

Clinical parameters:	n (% of n=115)
Died	80 (69.6 %)
Died of tumor	44 (38.3 %)
Relapse	19 (16.5 %)
Histological parameters:	n (% of n=101)
Adenocarcinoma (endometroid) **endometrioid*	70 (69.3 %)
Adenocarcinoma (squamous metaplasia)	17 (16.8 %)
Adenosquamous carcinoma	4 (4 %)
Serous carcinoma	3 (3 %)
Clear cell carcinoma	4 (4 %)
Undifferentiated carcinoma	3 (3 %)
Nuclear grading (n=102)	
GI	23 (22.5 %)
GII	44 (43.1 %)
GIII	35 (34.3 %)
Immunohistochemical parameters:	
Ki-67 (MIB-1) (n=95)	40 (42.1 %)
0-20% positive cells (mild)	39 (41.1 %)
21-50% positive cells (moderate)	16 (16.8 %)
51-100% positive cells (high)	
p53-overexpression (n=93)	64 (68.8 %)
IRS 0-2	29 (31.2 %)
IRS 3-4	
S100+ dendritic cells (n=115)	44 (38.3 %)
0-10 DC/mm^2	71 (61.7 %)
<10 DC/mm^2	
Her-2/neu expression (n=98)	
0-2	48 (49%)
3-12	50 (51%)

of this malignancy. None of the typical DC markers lead to reliable staining results on paraffin tissue sections and, therefore, we selected S100 as a marker since it leads to adequate staining results on these tissue sections (13, 19). Apart from DC, S100 is also expressed in peripheral neuronal tissue, but this did not interfere with our investigation because endometrium is devoid of neural structures. Investigating 115 endometrial tumor samples in this study, we demonstrated that the number of S100$^+$ DC could be a prognostic marker for endometrial carcinoma.

Materials and Methods

Patients and tissue samples. A total of 115 cases of endometrial carcinoma, diagnosed at the Department of Obstetrics and Gynecology of the University of Wuerzburg from 1980 to 1985, were reviewed and reclassified according to the FIGO criteria (1) and the WHO classification (2). Information about patients' follow-up data, including the time and cause of patients' death, were collected from the hospital records, gynecologists in practice and from the patients' families. The median age of the patients at diagnosis was 68 years (range 30-94 years). Treatment consisted of abdominal hysterectomy and bilateral salpingo-oophorectomy in 72 cases (62.6%) and primary radiation therapy (intracavitary radiation and additional percutaneous radiation in 43 cases) (36.4%). The median duration of follow-up was 4 years.

The following parameters had previously been evaluated in the tissue material: histologic type, grade of differentiation, stage, depth of myometrial invasion. Immunohistochemically-detected parameters, were of Ki-67, p53 and Her-2/neu, as well as receptors for estrogen and progesterone (3).

Immunohistochemistry. The polyclonal antibody against S100 applied in the study was obtained from Novocastra (Loxo, Deissenheim, Germany). Serial longitudinal sections were cut at 2 μm from paraffin-embedded uterine tissue specimens and placed onto APES (3-amino-propyltriethoxy-silane; Roth, Karlsruhe, Germany)-coated slides, dewaxed in xylene, rehydrated in graded ethanol and distilled water, and subjected to heat pretreatment by boiling in 0.2 M sodium citrate buffer (pH 6.0) for 15 min in a microwave oven (750 W/sec). The sections were treated with H_2O_2 prior to immunostaining to block endogenous peroxidase activity. For immunohistochemical staining, the sections were incubated with the primary antibody diluted 1:500 in phosphate-buffered saline, followed by the horseradish-peroxidase (HRP)-labelled LSAB kit system (biotin-streptavidin system, Dako, Hamburg, Germany). 3,3'-Diaminobenzidine (Sigma, Deisenhofen, Germany) was used as the chromogen. The sections were counterstained with hematoxylin.

Evaluation of results. S100$^+$ DC were counted in the entire tumor regions *via* microscopic examination at high magnification (x 400) and calculated as number of S100$^+$ cells per mm^2 of tissue by two independent observers. Potential differences in the findings of the two observers were investigated with the Student's *t*-test, but no statistically significant differences between their findings were noted. The samples were divided into group 1: less than 10 S100$^+$ cells/mm^2 tissue and group 2: 10 or more S100$^+$ cells/mm^2 tissue.

Statistical analysis. Statistical analysis of the data obtained was performed using the SPSS (Version 11) and MEDAS (Würzburg, Germany) software program. Correlation of the number of S100$^+$ cells/mm^2 tissue with nuclear grading, rate of proliferation (Ki-67) and overexpression of p53 were calculated by Chi-square test. Univariate analysis was performed on overall and tumor-specific survival in correlation to number of S100$^+$ cells and all other markers. Multivariate regression analysis utilized the proportional hazard's model of Cox.

Results

Tissue parameters (histology and immunohistochemistry) are summarized in Table I.

Histology. Retrospective tumor-typing could be performed in 101 tumors; 70 were classified as endometrioid carcinomas and 31 as non-endometrioid (17 adenocarcinoma with squamous metaplasia, 4 adenosquamous, 3 serous, 4 clear cell, 3 undifferentiated carcinomas). No mucinous adenocarcinomas were detected. The depth of myometrial infiltration was determined in 46 cases. In 25 it was less than 50%, and in 21

cases it was more than 50% of the myometrium. The histologic grade was established retrospectively in 102 tumors; 23 (22.5 %) were well-differentiated (Grade I), 44 (43.1 %) moderately- differentiated (Grade II) and 35 (34.3 %), poorly-differentiated (Grade III). It was possible to FIGO-stage 48 patients, of whom 39 had FIGO-stage I tumors, 4 had FIGO-stage II and 5 had FIGO-stage III tumors

Immunohistochemical marker detection. Immunohistochemical data was available in 95 carcinoma samples for Ki-67 (MIB-1): 40 cases showed a mild (0-20% positive cells), 39 a moderate (21-50% positive cells) and 16 a high (51-100% positive) proliferative activity as determined by Ki-67 staining. P53 expression was evaluated in 93 cases with negative p53 expression in 64 cases and 29 with p53 overexpression (Table I). Thirty out of 56 (53.6%) tumors were positive for estrogen receptor and 52 out of 95 tumors (54.7%) were progesterone receptor-positive. In 50 (51%) out of 98 endometrial carcinomas HER-2/neu-expression was positive and graded as described in Backe *et al.* (5).

S100 staining. Based on the analysis of DC, samples were divided into groups, one with less than 10 DC per mm^2 and the other with 10 or more DC per mm^2. In 71 (61.7%) out of 115 cases, more than 10 DC per mm^2 were found and 44 (38.3%) cases showed less than 10 DC per mm^2, as determined by S100 staining (Figure 1).

Parameter correlation. The occurrence of more than 10 DC in the tumor was correlated with a higher differentiation of the nuclei, negative p53 overexpression and low Ki-67 expression, all characteristic of endometrial carcinoma with better prognosis.

The following markers turned out to be statistically significant in univariate analysis if adjusted survival was investigated: $S100^+$ DC infiltrating the tumor/tumor edge, FIGO-stage, expression of estrogen receptor (although only half of the tumors could be evaluated), nuclear grading, proliferative activity as determined by Ki-67 expression and p53 overexpression (Table II).

Univariate analysis of overall survival revealed that proliferative activity, estrogen receptor expression and the FIGO-stage were significant prognostic factors (Table II).

DC infiltration turned out not to be a significant prognostic marker in univariate analysis or in multivariate analysis of adjusted survival (Table III). Other prognostic factors that were statistically significant for adjusted survival in multivariate analysis in our patient group were the FIGO-stage and nuclear grading. The FIGO-stage was the only statistically significant prognostic marker when multivariate analysis for overall survival was performed.

We also evaluated whether tumoral DC infiltration correlated with other clinicopathological parameters in endometrial cancer (Table IV). DC infiltration was positively correlated with histological grading, low rates of proliferation (Ki-67) and with overexpression of p53, but not with Her-2 status, FIGO-staging or hormone receptor status (not shown).

Correlation with survival rates. The correlation between the number of $S100^+$ cells and overall and adjusted survival rates are shown in Figure 2. The life-table analysis revealed a tendency towards a higher overall survival rate for patients with endometrial carcinoma containing more than 10 $S100^+$ DC per mm^2, although this was not statistically significant ($p=0.063$). In the Kaplan-Meier plot for adjusted survival, a significantly higher ($p<0.01$) survival rate for patients with endometrial carcinoma containing more than 10 $S100^+$ DC per mm^2 was demonstrated.

Discussion

DC play a crucial role in many human cancers (8). Elagöz *et al.* (2000) suggested that infiltration of tumors with DC might be a reliable marker correlating with prognosis of patients with endometrial cancer. To support this idea, studies were undertaken to investigate the relationship between the number of DC found within tumor tissue and the prognosis of patients. Unfortunately, none of the antibodies normally used for immunohistochemical characterization and enumeration of DC led to reliable staining results on paraffin-embedded material, thus forcing the investigators to select S100 as a marker for DC. Apart from DC, S100 is also expressed in peripheral neuronal tissue, but this did not interfere with out investigations of endometrial carcinoma because the endometrium is devoid of neural structures. A significant correlation was found between the infiltration of endometrial carcinomas with $S100^+$ DC and adjusted survival in the univariate and multivariate analysis. Multivariate analysis with regard to overall survival only yielded FIGO-stage as a significant prognostic marker. In addition to FIGO-stage, nuclear grading and degree of DC infiltration turned out to be the only significant prognostic markers in multivariate analysis for adjusted survival. Regarding the univariate analysis, the panel of clinicopathological markers gave an ambiguous picture. In this analysis, DC infiltration was not significantly associated with overall survival and FIGO-stage was once again the superior prognostic factor.

In addition to survival, we correlated the degree of DC infiltration with clinicopathological parameters. A higher degree DC infiltration was correlated with a better tumor differentiation in our investigation. Our results differ from those reported in gastric or in uterine cervix carcinomas, in which no significant associations between the degree of infiltration by DC and tumor differentiation were observed (20, 19) Similarly to our findings concerning the

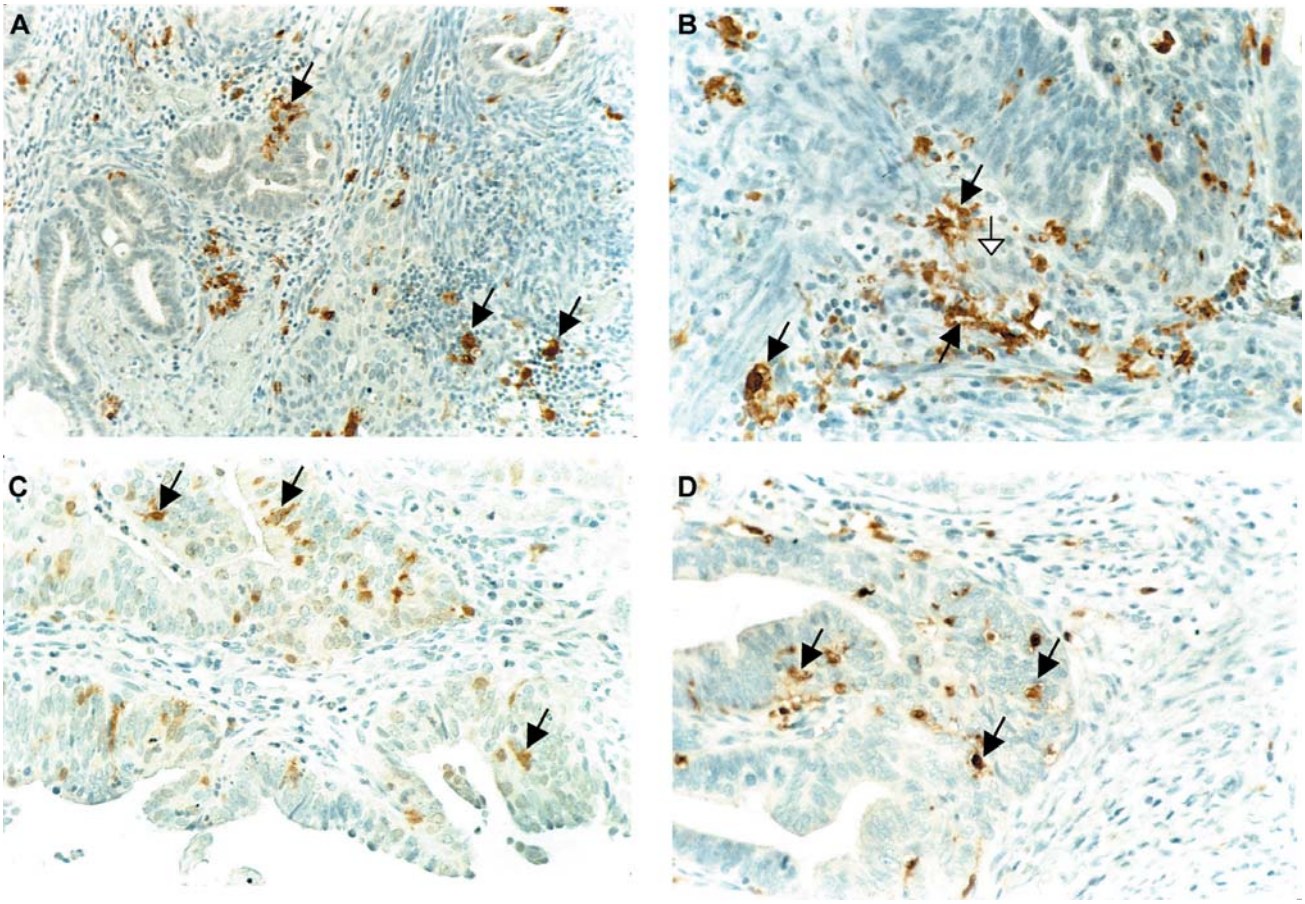

Figure 1. *Immunohistochemistry of S100$^+$ dendritic cells (DC; brown, DAB; arrow) in endometrial carcinoma tissue. Sections are counterstained with hematoxylin. A: Typical staining pattern of S100$^+$ DC shows the cells to be located directly in the tumor regions. Magnification x250. B: On higher magnification, the strong association of the DC with the tumor cells (white head arrow) is shown. Magnification x400. C+D: S100$^+$ DC invading the glandular cells in endometrial carcinoma. Magnification x400.*

interrelationship between S100$^+$ DC infiltration and proliferative activity, Maehara *et al.* (21) observed a negative correlation between tumor infiltration by S100$^+$ DC and the proliferation rate as measured with PCNA-labelling index in gastric cancer (21).

An increased number of DC, as indicated by positive S100 protein staining, has been shown to correlate with good prognosis in colorectal, lung and esophageal cancers (9, 22, 23). We were able to confirm the results of Coppola *et al.* (8) in a larger study group, showing that DC infiltration might be a favorable prognostic factor in endometrial cancer and that a higher degree of DC infiltration was associated with better prognosis. In a similar cancerous disease like adenocarcinoma of the cervix, Oka *et al.* (6) correlated S100 protein expression with prognosis in patients who were treated with radiation therapy. The investigators found that the outcome of the patients with a noticeable DC infiltration was significantly better than that of women whose tumors did not show an infiltration by S100$^+$ cells. Interestingly, in colorectal cancer only S100$^+$ cells at the peripheral area of the tumor were investigated (9). Nevertheless, the result was similar in that the presence of a large number of S100$^+$ cells was associated with low stage tumors and good prognosis. In other tumor types that have been investigated to date, infiltration by DC does not correlate with prognosis or stage. In laryngeal squamous cell carcinoma (LSCC), for example, the S100 results did not associate with grade, tumor stage or survival (10). The prognostic value of S100$^+$ cells for the outcome of renal and breast cancer patients also seems to be limited (11, 13).

Overall, studies evaluating S100$^+$ DC in different malignancies point to a very heterogeneous recruitment and stimulation processes in the setting of different tumor entities.

Table II. *Univariate analysis of survival.*

Prognostic factor	Overall survival	Adjusted survival
FIGO-stage	0.000003 ***	0.00000 ***
Nuclear grading	0.06	0.0026 *
S100$^+$ DC/mm^2	**0.063**	**0.0031 ****
Estrogen receptor	0.035*	0.041*
Progesterone receptor	0.39	0.47
Proliferation rate (Ki-67)	0.0045 **	0.0012 **
P53 overexpression	0.088	0.023*
Her-2/neu	0.74	0.55

Table III. *Multivariate analysis of survival.*

Prognostic factor	Overall survival	Adjusted survival
FIGO-stage	0.000003 ***	0.00062 ***
Nuclear grading	0.06	0.021 *
S100$^+$ DC/mm^2	**0.063**	**0.013 ***

Table IV. *Comparison of nuclear grading, proliferation rate and p53 overexpression with number of S100$^+$ cells/mm^2.*

Marker	Number of S100 cells (%)		
	all	0-10	10
Nuclear grading			
GI	22.5	10.8	29.2
GII	43.1	43.2	43.1
GIII	34.3	45.9	27.7
P53 overexpression			
neg.	68.8	54.1	78.6
pos.	31.2	45.9	21.4
Proliferation rate (Ki-67)			
mild	42.1	31.6	49.1
moderate	41.1	39.5	42.1
high	16.8	28.9	8.8

Which stimuli lead to the homing of DC in endometrial cancer or how and if those DC are activated and able to stimulate anti-tumor immunity is an intriguing question, which has not yet been clarified. Findings regarding the host's immune response might also relate to therapy for future patients, which was exemplified by Hamada *et al.* (11), who showed that degree of DC infiltration can serve as a predictive factor for immune therapies like IFN-alpha therapy for renal cell carcinoma. Attempts to use DC to vaccinate against cancer have not yielded satisfactory results. Nevertheless, there is agreement on the importance of DC for the course of malignant disease (15).

Our results reveal that S100$^+$ DC infiltrating endometrial cancer are associated with a better outcome of the affected patients. Furthermore, based on our investigation, DC infiltration could be interpreted as an indicator of anti-tumor immunoreactivity in endometrial carcinoma. Infiltrating DC warrant future research efforts to elucidate more about the host's immune response towards solid human tumors.

References

1 FIGO stages: 1988 revision. Gynecol Oncol *35*: 125-127, 1989.

2 World Health Organization: Histologic typing of female genital tract tumours. Int Histol Class Tumours *13*, 1994.

3 Backe J, Gassel AM, Hauber K, Krebs S, Bartek J, Caffier H, Kreipe H-H, Müller-Hermelink H-K and Dietl J: p53 protein expression in endometrial cancer is related to proliferation activity and prognosis but not to expression of p21 protein. Int J Gyn Pathol *16*: 361-368, 1997.

4 Backe J, Gassel AM, Muller T, Schon S and Kaesemann H: Rate of proliferation as a prognostic criterion in endometrial carcinoma – an immunohistochemical analysis with the monoclonal antibody KI-S1. Zentralbl Gynakol *119(7)*: 334-342, 1997.

5 Backe J, Gassel AM, Krebs S, Muller T and Caffier H: Immunohistochemically detected HER-2/neu-expression and prognosis in endometrial carcinoma. Arch Gynecol Obstet *259(4)*: 189-195, 1997.

6 Oka K, Nakano T and Arai T: Adenocarcinoma of the cervix treated with radiation alone: prognostic significance of S-100 protein and vimentin immunostaining. Obstet Gynecol *79(3)*: 347-350, 1992.

7 Whiteside TL and Odoux C: Dendritic cell biology and cancer therapy. Cancer Immunol Immunother, in press.

8 Coppola D, Fu L, Nicosia SV, Kounelis S and Jones M: Prognostic significance of p53, bcl-2, vimentin, and S100 protein-positive Langerhans cells in endometrial carcinoma. Hum Pathol *29(5)*: 455-462, 1998.

9 Nakayama Y, Inoue Y, Minagawa N, Katsuki T, Nagashima N, Onitsuka K, Tsurudome Y, Sako T, Hirata K, Nagata N and Itoh H: Relationships between S-100 protein-positive cells and clinicopathological factors in patients with colorectal cancer. Anticancer Res *23(6a)*: 4423-4426, 2003.

10 Karakok M, Bayazit YA, Ucak R, Ozer E, Kanlikama M, Mumbuc S and Sari I: Langerhans cell related inflammatory reaction in laryngeal squamous cell carcinoma. Auris Nasus Larynx *30(1)*: 81-84, 2003.

11 Hamada I, Kato M, Yamasaki T, Iwabuchi K, Watanabe T, Yamada T, Itoyama S, Ito H and Okada K: Clinical effects of tumor-associated macrophages and dendritic cells on renal cell carcinoma. Anticancer Res *22(6C)*: 4281-4284, 2002.

12 Hammar S, Bockus D, Remington F and Maureen B: The widespread distribution of Langerhans cells in pathologic tissues. Hum Pathology *17*: 894-905, 1986.

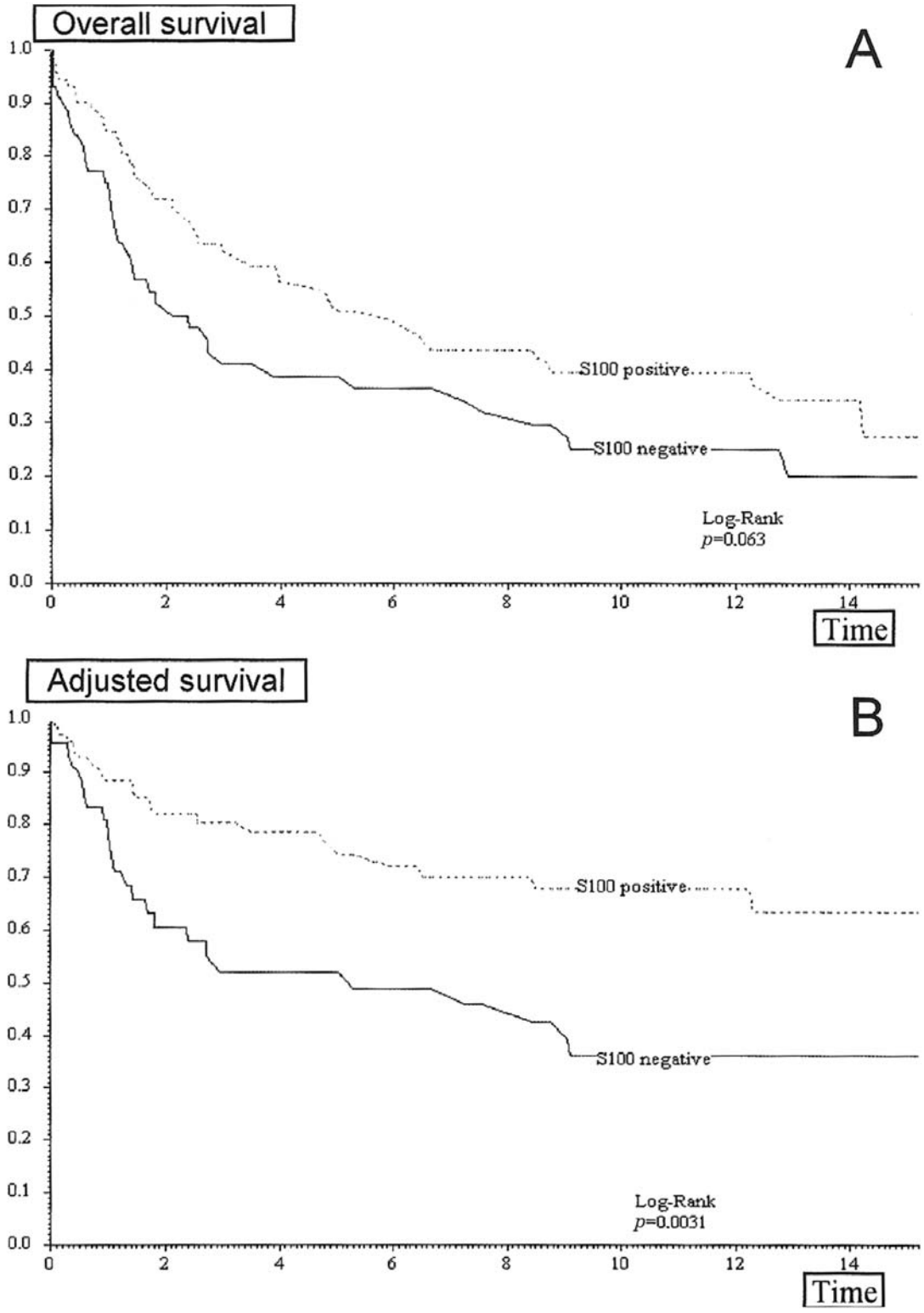

Figure 2. *Kaplan-Meier plots of survival rate. A: A higher (not significant, p=0.063) overall survival rate for patients with endometrial carcinoma containing more than 10 S100$^+$ DC per mm^2 is demonstrated. X-axes: time (years). B: The adjusted survival rate for patients with endometrial carcinoma containing more than 10 S100$^+$ DC per mm^2 was significantly higher (p= 0.0031) than of those patients whose carcinomas contained less than 10 DC per mm^2. X-axes: time (years).*

13 Lespagnard L, Gancberg D, Rouas G, Leclercq G, De Saint-Aubain Somerhausen N, Di Leo A, Piccart M, Verhest A and Larsimont D: Tumor infiltrating dendritic cells in adenocarcinomas of the breast: a study of 143 neoplasms with a correlation to usual prognostic factors and to clinical outcome. Int J Cancer (Pred Oncol) *84*: 309-314, 1999.
14 Banchereau J and Steinman RM: Dendritic cells and the control of immunity. Nature Review *392(6673)*: 245-252, 1998.
15 Wright-Browne V, McClain KL, Talpaz M, Ordonez N and Estrov Z: Physiology and pathophysiology of dendritic cells. Hum Pathol *28(5)*: 563-579, 1997.
16 Nestle FO, Burg G, Fah J, Wrone-Smith T and Nickoloff BJ: Human sunlight-induced basal-cell-carcinoma-associated dendritic cells are deficient in T cell co-stimulatory molecules and are impaired as antigen-presenting cells. Am J Pathol *150(2)*: 641-651, 1997.
17 Enk AH, Jonuleit H, Saloga J and Knop J: Dendritic cells as mediators of tumor-induced tolerance in metastatic melanoma. Int J Cancer *73(3)*: 309-316, 1997.
18 Guermonprez P, Valladeau J, Zitvogel L, Thery C and Amigorena S: Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol *20*: 621-667, 2002.
19 Bethwaite PB, Holloway LJ, Thornton A and Delahunt B: Infiltration by immunocompetent cells in early stage invasive carcinoma of the uterine cervix: a prognostic study. Pathology *28(4)*: 321-327, 1996.
20 Okuyama T, Maehara Y, Kakeji Y, Tsuijitani S, Korenaga D and Sugimachi K: Interrelation between tumor-associated cell surface glycoprotein and host immune response in gastric carcinoma patients. Cancer *82(8)*: 1468-1475, 1998.
21 Maehara Y, Tomisaki S, Oda S, Kakeji Y, Tsujitani S, Ichiyoshi Y, Akazawa K and Sugimachi K: Lymph node metastasis and relation to tumor growth potential and local immune response in advanced gastric cancer. Int J Cancer *74(2)*: 224-228, 1997.
22 Furukawa T, Watanabe S, Kodama T, Sato Y, Shimosato Y and Suemasu K: T-zone histiocytes in adenocarcinoma of the lung in relation to postoperative prognosis. Cancer *56(11)*: 2651-2656, 1985
23 Matsuda H, Mori M, Tsujitani S, Ohno S, Kuwano H and Sugimachi K: Immunohistochemical evaluation of squamous cell carcinoma antigen and S-100 protein-positive cells in human malignant esophageal tissues. Cancer *65(10)*: 2261-2265, 1990.

Received August 2, 2004
Accepted February 28, 2005