

Additional Prognostic Value of SUV_{max} Measured by F-18 FDG PET/CT over Biological Marker Expressions in Surgically Resected Cervical Cancer Patients

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Keywords

Cervical cancer · SUV_{max} · Disease-free survival · Biological marker expression

Summary

Purpose: We compared the prognostic ability of the maximum standardized uptake value (SUV_{max}) and various biological marker expressions to predict recurrence in patients with surgically resected cervical cancer. **Methods:** A retrospective review identified 60 patients with cervical cancer who received [¹⁸F]fluorodeoxyglucose positron emission tomography/computed tomography (F-18 FDG PET/CT) at the time of the diagnosis of cancer. The SUV_{max} , expressions of carbonic anhydrase-IX (CA-IX), glucose transporter 1 (GLUT-1), and vascular endothelial growth factor (VEGF), and known prognostic factors were investigated. **Results:** The median follow-up time was 22.2 months (range 3.4–43.1 months). Using univariate analyses, the stage (stage II, $p = 0.0066$), SUV_{max} (> 6 , $p = 0.027$), parametrial involvement ($p < 0.0001$), and positivity for CA-IX ($p = 0.0191$) were associated with recurrences of cervical cancer. With the Cox proportional hazard regression model, the SUV_{max} was a potent predictor for disease-free survival (DFS). **Conclusion:** Although CA-IX expression was related to DFS in the current study, the potent predictor for DFS was SUV_{max} . Therefore, SUV_{max} is of greater prognostic value than biological marker expression in patients with surgically resected cervical cancer.

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Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in females worldwide, accounting for 9% (529,800) of the total new cancer cases and 8% (275,100) of the total cancer deaths among females in 2008 [1]. According to the International Federation of Gynecology and Obstetrics (FIGO), cervical cancer has a 5-year recurrence rate and a 5-year overall mortality rate of 28% and 27.8%, respectively [2]. Furthermore, despite optimal treatment, about 1/3 of patients with disease stage IB or higher experience recurrence [3].

The traditional prognostic factors, such as pelvic lymph node (LN) metastasis, parametrial invasion, positive tumor margins, a large tumor size, deep stromal invasion and lymphovascular invasion (LVI) are currently considered important prognostic factors for survival and provide some useful clinical and pathological information [4]. However, with the exception of the presence of LN metastasis, the accuracy of these traditional prognostic factors continues to be debated for patients with cervical cancer [5]. Recently, some studies have shown that the expression of various biological marker, including carbonic anhydrase-IX (CA-IX), glucose transporter GLUT-1, and angiogenetic factor, in cervical cancer patients was related to poor prognosis [6–10].

Positron emission tomography (PET) using 2-deoxy-2-[¹⁸F] fluoro-D-glucose (FDG) (F-18 FDG PET) has been reported to be a valuable functional imaging modality in various cancers [11–15]. Also, several studies have suggested the prognostic role of F-18 FDG PET in patients with cervical cancer [16–18].

In the current study, we compared the prognostic ability of the maximum standardized uptake value (SUV_{max}) of FDG and various biological marker expressions to predict recurrence in patients with surgically resected cervical cancer.

Materials and Methods

Patient Eligibility

This study was approved by our institutional review board and written informed consent was obtained from the patients. We undertook a retrospective review of the Gynecologic Cancer registry at our institution and identified patients suffering from cervical cancer between 2011 and 2014. Patients were excluded from the current study if they had a history of another previous cancer or multiple primary cancer. Patients were required to have undergone computed tomography (CT) and F-18 FDG PET/CT as part of the process of establishing their pathological diagnosis. Further criteria were that the patients had no treatment before diagnosis and had at least 3 months of clinical follow-up. 60 patients met these inclusion and exclusion criteria. The median interval from F-18 FDG PET/CT to surgery was 39 days (range 15–77 days).

Clinical Follow-Up

All the patients were treated with radical hysterectomy and pelvic LN dissection for invasive cervical cancer. After treatment each patient was monitored regularly. During the follow-up, complete physical examination, chest CT, abdomen and pelvic CT, F-18 FDG PET/CT, and routine laboratory test were performed every 3 months. Recurrence was defined as any appearance of a new cancer focus in a disease-free patient in imaging modality or an elevation of tumor marker. The time interval between the surgery and cancer recurrence were determined. The duration of disease-free survival (DFS) was calculated as the time interval between the surgery date and date of the cancer recurrence.

F-18 FDG PET/CT

F-18 FDG PET/CT image was done with a dedicated PET/CT scanner (Gemini, Philips, Milpitas, CA, USA), consisting of a dedicated germanium oxorthosilicate full-ring PET scanner and a dual slice helical CT scanner. Standard patient preparation included at least 8 h of fasting and a serum glucose level of less than 120 mg/dl before F-18 FDG administration. PET/CT imaging was performed 60 min after injection of F-18 FDG, and low-dose CT (30 mA, 120 kV) covering the area from the base of the skull to the proximal thighs was performed for attenuation correction and precise anatomical localization. Thereafter, the emission scan was performed in the 3-dimensional mode. The emission scan time per bed position was 3 min; 9 bed positions were acquired. PET data were obtained using a high-resolution whole-body scanner with an axial field of view of 18 cm. The average axial resolution varied between 4.2 mm full width at half maximum (FWHM) in the center and 5.6 mm at 10 cm. The average total PET/CT examination time was 30 min. After scatter and decay correction, the PET data were reconstructed iteratively with attenuation correction and reoriented in axial, sagittal, and coronal slices. The row action maximum-likelihood algorithm was used for 3-dimensional reconstruction. The SUV_{max} was obtained using the following formula: $SUV_{max} = \text{maximum activity in the region of interest (MBq/g)} / (\text{injected dose (MBq)} / \text{body weight (g)})$.

Immunohistochemical Analysis of Biological Marker Expressions

Surgically resected specimens were immediately fixed in 10% buffered formalin (pH 7.0). Sections containing both tumor and surrounding cervix tissue were embedded in paraffin, and serial sections (4 mm) were cut from selected, paraffin-embedded tissue blocks. For pathological diagnosis, 1 section was stained with hematoxylin and eosin. Other sections were used for immunohistochemistry. These sections were transferred to poly-L-lysine-coated glass slides and air-dried overnight at 37.8°C. The glass slides were dewaxed in xylene (3 changes), rehydrated in a graded series of decreasing ethanol concentrations, and then rinsed in Tris-buffered saline (50 mM Tris/HCl pH 7.4 containing 100 mM NaCl). Endogenous peroxidase activity was inactivated with 5% hydrogen peroxide in methanol for 15 min at 37.8°C. After an appropriate antigen retrieval procedure, primary antibodies were applied to the sections. Antibodies against GLUT-1 (rabbit polyclonal antibody; Dako) were incubated with the tissue sections at room temperature for 1 h. Antibodies against vascular endothelial growth factor (VEGF; rabbit polyclonal antibody; Santa Cruz Biotechnology) and CA-IX (rabbit polyclonal antibody; Novus) were incubated with the sections overnight at 48°C. Immunohistochemical procedures were per-

Table 1. Clinical characteristics of the patients

Characteristics		
Median age at diagnosis, years (range)	49.6 (33–75)	
Median SUV_{max} (range)	6 (2.3–26)	
	n	%
Total number of patients	60	100
FIGO stage		
IA	13	21.7
IB	32	53.3
IIA	15	25
Pathology		
Squamous cell carcinoma	13	21.7
Adenocarcinoma	47	78.3
Pelvic LN (+)	13	21.7
Lymphovascular invasion (+)	24	40
Paracervical involvement (+)	8	13.3
Parametrial involvement (+)	6	10
CA-IX expression (+)	24	40
GLUT-1 expression (+)	37	61.7
VEGF expression (+)	27	45

SUV_{max} = maximum standardized uptake value, FIGO = International Federation of Gynecology and Obstetrics, LN = lymph node, CA-IX = carbonic anhydrase-9, GLUT-1 = glucose transporter-1, VEGF = vascular endothelial growth factor.

formed using a Biotin-free Catalyzed Amplification System II (K1497; Dako). The reaction products were visualized by exposing sections to 3,3'-diaminobenzidine. Nuclei were lightly counterstained for about 20 s with Mayer's hematoxylin. Sections were then mounted in diluted malinol after application of Universal Mount (Dako). Appropriate positive control specimens were used in each staining batch.

Scoring of Immunohistochemistry

Using light microscopy, all the tissue sections were scored semiquantitatively considering the proportion of the cells showing immunoreactivity among whole tumor cells. In each analysis, the percentages of strongly immunoreactive tumor cells in total tumor cells were visually analyzed in several low-power fields (original magnification, 10 × 10) covering the entire specimen, and the average percentage was calculated and scored positive (> 10%) or negative (< 10%) expression of each biological marker.

Statistical Analysis

All numerical data were expressed as medians. Survival analysis was conducted using Kaplan-Meier analysis, and survival curves stratified by age, histological type, differentiation of primary tumor, pelvic LN involvement, lymphovascular invasion, parametrial involvement, stage, and biological marker expressions (GLUT-1, CA-IX, and VEGF), and SUV_{max} were generated. Log rank test was used to compare the DFS. The DFS was measured as the time from surgery to the first recurrence of cervical cancer. Independent predictive factors for DFS were determined using the Cox proportional hazard model. Data analyses were conducted with MedCalc. Statistical significance was defined as $p < 0.05$.

Results

Patient Characteristics and Follow-Up

The characteristics of the patients are given in table 1. The median age at the time of diagnosis was 49.6 years (range 33–75 years).

Table 2. Univariate analysis for disease-free survival

	Disease-free survival		
	HR	95% CI of HR	p value
Age (> 55 years)	0.61	0.16–2.25	0.5243
Stage (stage II)	7.38	0.96–16.8	0.0066
SUV _{max} (> 6)	4.65	1.56–13.8	0.027
Pathology (squamous cell carcinoma)	0.65	0.17–2.4	0.4742
Differentiation (well differentiated)	0.28	0.07–1.02	0.1856
Lymphovascular invasion (+)	1.07	0.35–3.19	0.9015
Paracervical involvement (+)	5.06	1.07–23.7	0.0012
Parametrial involvement (+)	11.9	0.64–224.1	< 0.0001
Pelvic LN (+)	2.44	0.63–9.4	0.1044
CA-IX expression (+)	3.72	0.93–14.7	0.0191
GLUT-1 expression (+)	1.08	0.34–3.47	0.8864
VEGF expression (+)	1.28	0.38–4.29	0.6804

HR = hazard ratio, CI = confidence interval.

Table 3. Multivariate analysis for disease-free survival

	Disease-free survival		
	HR	95% CI of HR	p value
Stage (stage II)	1.22	0.23–6.83	0.7760
SUV _{max} (> 6)	22.3	1.78–279.3	0.0165
Paracervical involvement (+)	41.3	2.44–698.7	0.0103
Parametrial involvement (+)	1.74	0.16–19	0.6486
CA-IX expression (+)	1.41	0.21–9.45	0.7231

13 patients had adenocarcinoma and 47 patients had squamous cell carcinoma. The median follow-up time was 22.2 months (range 3.4–43.1 months).

Factors Associated with DFS in Univariate Analysis

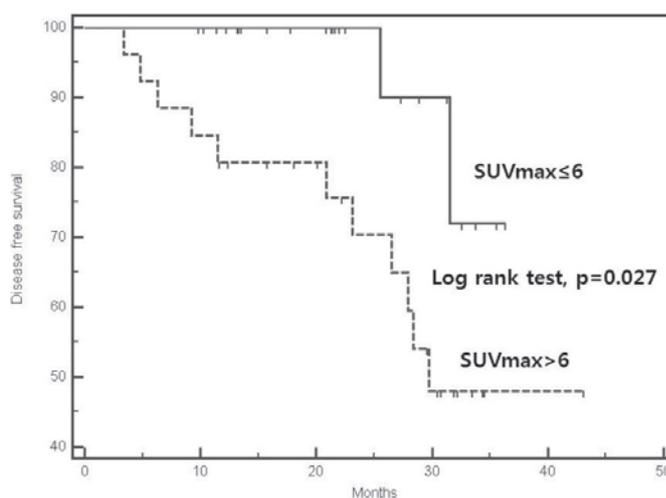
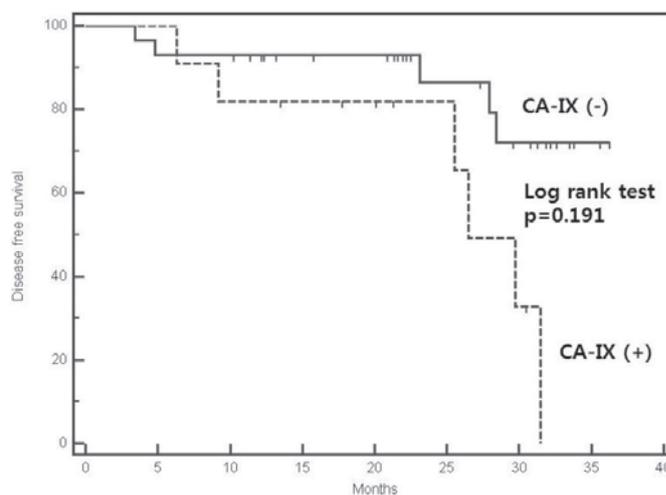
Table 2 demonstrates factors associated with overall survival (OS) and DFS in univariate analysis. In the univariate analysis, stage, SUV_{max}, parametrial involvement, and CA-IX expression were associated with DFS (fig. 1 and 2).

SUV_{max} as Prognostic Factor

Figure 1 demonstrates the Kaplan-Meier survival curves according to the SUV_{max}. 12 patients (44.4%) with a tumor SUV_{max} of > 6 and 2 patients (6%) with tumor SUV_{max} of ≤ 6 had a recurrence of disease during the follow-up period (Log rank test, $X^2 = 4.89$, $p = 0.027$).

Prediction of DFS

In order to define the prognostic factors for DFS, multivariate survival analysis was performed. The Cox proportional hazard regression analysis revealed that the SUV_{max} was a potent predictor for DFS (table 3).

**Fig. 1.** Kaplan-Meier curve stratified by maximum standardized uptake value (SUV_{max}) of [¹⁸F]fluorodeoxyglucose (F-18 FDG) positron emission tomography/computed tomography (PET/CT) for disease-free survival (DFS).**Fig. 2.** Survival curve for the prediction of DFS by carbonic anhydrase-IX (CA-IX) expression in cervical cancer patients.

Discussion

Hypoxia is known to be an important regulator of tumor angiogenesis and growth. The presence of hypoxic conditions within solid tumors is 1 of the most important factors affecting treatment outcomes. The tissue hypoxia can be measured by endogenous hypoxia markers such as HIF1a, HIF2a, GLUT-1, GLUT-3, and CA-IX [19, 20].

In the current study, we investigated whether the SUV_{max} measured by F-18 FDG PET/CT has an additional prognostic value over endogenous hypoxia markers in patients with surgically resected cervical cancer. Similar to previous studies, the current study showed that positive CA-IX expression was related to frequent recurrence of cervical cancer. Other studies reported that positive CA-IX expression is strongly associated with DFS and metastasis-free survival in cervical cancer patients [7, 8, 21]. However, the

prognostic implication of CA-IX expression in cervical cancer is controversial as there have been different clinical outcomes. Contrary to the current findings, a recent study did not find an association between CA-IX expression and patient outcome in locally advanced cervical cancer [22].

Overexpression of GLUT-1 is found in various cancers including cervical cancer [23]. The prognostic implication of overexpression of GLUT-1 has been reported to be associated with a poor prognosis in several cancers including cervical cancer [9, 24–27]. However, in the current study, we did not find any prognostic ability of GLUT-1 in cervical cancer patients. By contrast, OS and recurrence-free survival were found to be significantly shorter for cervical cancer patients with expression of GLUT-1 [28]. That we did not find a prognostic role of GLUT-1 in the current study may be due to the early stage of cervical cancer in the patients in our study.

VEGF is a potent stimulator of angiogenesis. VEGF mRNA and tumor microvessel density were shown to be highest in invasive carcinoma sections in cervical cancer [29]. Another study showed that the highest levels of VEGF mRNA expression were observed in early cervical cancers, suggesting a possible role for VEGF in early invasion in cervical cancer [30].

Recently, volumetric parameters of F-18 FDG PET/CT have been used for prediction of survival in cervical cancer patients [31]. It was demonstrated that quantitative parameters derived from the pre-treatment diffusion-weighted magnetic resonance imaging (mean apparent diffusion coefficient, ADC_{mean}) and from F-18 FDG PET/CT (metabolic tumor volume, MTV, and total lesion glycolysis, TLG) were associated with high-risk features and may serve as prognostic biomarkers of survival in patients with cervical cancer [31]. Further studies should be conducted to determine the prognosis for cancer patients using these volumetric parameters of F-18 FDG PET/CT.

In conclusion, although CA-IX expression is related with DFS in the current study, the potent predictor for DFS was SUV_{max} . The SUV_{max} measured by F-18 FDG PET/CT provided additional prognostic value over biological marker expression in patients with cervical cancer.

Disclosure Statement

The authors declare that there are no conflicts of interest.

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