

Hypoxia-inducible factor-1 α , adrenomedullin and Bcl-2 although expected are not related to increased uptake of fluorine-18-fluorodeoxyglucose in endometrial cancer

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Abstract

Objective: To study the relation between SUVmax, hypoxia inducible factor 1 α (HIF-1 α), angiogenetic factor adrenomedullin (AM) and antiapoptotic factor Bcl-2 in endometrial cancer. **Subjects and Methods:** Thirty eight patients who were diagnosed after a preoperative endometrial biopsy with endometrium cancer underwent pre-operative positron emission tomography/computed tomography (PET/CT) utilizing fluorine-18-fluorodeoxy glucose (¹⁸F-FDG). Maximum standardized uptake values (SUVmax) of the primary tumor were measured. After hysterectomy and bilateral salpingo-oophorectomy, microscopic slides of the 38 endometrial adenocarcinoma patients were evaluated by a surgical pathologist to confirm the diagnosis. Immunohistochemical staining for AM, Bcl-2 and HIF-1 α was studied. **Results:** In all patients, ¹⁸F-FDG uptake was detected. The mean SUVmax of the tumors was 11.8 \pm 5.9. Although SUVmax was higher in HIF-1 α positive tumors, this finding was not statistically important. No correlation was found between SUVmax and HIF-1 α positivity. Mean SUVmax was 6.4 \pm 3 and 12.3 \pm 1.4 in AM negative and AM positive patients, respectively. Mean SUVmax was 10.6 \pm 4.9 and 12.3 \pm 1.4 in Bcl-2 negative and Bcl-2 positive patients, respectively. We found no correlation between SUVmax, AM or Bcl-2 expression. All red scores were not related with SUVmax in regression analysis. **Conclusion:** Our study in a small number of patients is the first to show that SUVmax, although expected is not associated with HIF-1 α , AM or with Bcl-2 in endometrial cancer. Increased uptake of ¹⁸F-FDG in endometrial cancer seems to be independent of HIF-1 α and its downstream factors.

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Introduction

Positron emission tomography (PET), a revolutionary imaging technique in oncology, is based on altered functional and metabolic activity of tumor tissue. Unique features of cancer cells are: increased uptake of glucose and anaerobic metabolism which constitute the molecular basis for using PET as a diagnostic means. Otto Heinrich Warburg, claimed that energy metabolism of tumor cells and of normal cells is different. His study reported that cancer cells can grow under hypoxic conditions and consume larger amount of glucose by anaerobic glycolysis and was awarded the Nobel Prize in 1931 [1]. Hypoxia inducible factor (HIF-1 α) protein was shown to be related to Warburg effect [2] and regulate the genes responsible for increased utilization of glucose and energy metabolism.

Uncontrolled growth of cancer cells suppresses host tissue vascularization and causes in the host tissue inadequate blood supply, poor oxygenation and insufficient availability of glucose. Disturbed diffusion geometry, abnormal structure of tumor microvessels and microcirculation result in hypoxia [3]. However, cancer cells require a solid source of energy and nutrients [3, 4]. Adaptation and interaction of tumor with the altered microenvironment is necessary for tumor progression [5]. Tumor cells sense the limited availability of oxygen and glucose by oxygen sensitive transcription factor HIF-1 α [4]. Factor HIF-1 α induces a number of key downstream genes for survival and progression of tumor including adrenomedullin (AM), vascular endothelial growth factor (VEGF), glucose transporters and 6-phosphofructo kinase. Induction of (AM) by HIF-1 α provides neo-angiogenesis for nutrient supply, tumor progression [6] and induction of glucose transporters and glycolytic enzymes cause enhanced glucose availability and metabolism.

Adrenomedullin is a 52 amino acid peptide structurally and functionally related to cal-

citonin and to calcitonin gene-related peptide [7]. Adrenomedullin coordinates the growth of vessels and apoptosis [8], and upregulates Bcl-2 to resist hypoxic cells death [8]. Furthermore augments VEGF release from endometrial tumors [9].

Bcl-2 is an antiapoptotic protein which is upregulated in hypoxic conditions [10]. The role of Bcl-2 expression in the inhibition of apoptosis in endometrial carcinoma is well known and its expression may be associated with tumor differentiation and cell proliferation [6].

Fluorine-18-fluoro-glucose (^{18}F -FDG), is a glucose analog, most commonly utilized as a tracer that enters the cells via glut transporters and is metabolized by hexokinases. Maximum standardized uptake value (SUVmax) provides a semi-quantitative analysis of tumor glucose metabolism [11]. Correlation between SUVmax, HIF-1 α , GLUT, and hexokinases has been studied in many tumors [3, 11-13]. There has been one study on SUVmax and HIF-1A in endometrial cancer. Relation between SUVmax, HIF-1 α and AM has not yet been studied. Data on hypoxic proteins other than AM and SUVmax in endometrial cancer are also scarce. Research providing information on the scientific basis of PET/CT may help the clinician to assess the role and pitfalls of ^{18}F -FDG PET/CT in endometrial cancer and further develop the imaging modality. Besides, it may also have implementation in understanding of signal changes and responses to therapy at molecular level. We, therefore, have studied correlation between SUVmax, HIF-1 α , AM and Bcl-2 in endometrial cancer.

Subjects and Methods

Thirty eight patients with endometrial cancer were included to the study. All patients with a preoperative endometrial biopsy suggesting endometrium cancer had pre-operative PET/CT utilizing ^{18}F -FDG, and then surgical treatment: hysterectomy with bilateral salpingo-oophorectomy. Specimens were sent for pathological examination. Patients with endometrioid type tumors were included in the study. All other histological types of cancer were excluded. In order to exclude metabolic and systemic diseases causing hypoxia, patients with cardiovascular disease, pulmonary disease, malnutrition, hemoglobin less than 10g/dL, arterial O_2 partial pressure less than 90-100mmHg and albumin level less than 3g/dL were excluded.

PET/CT protocol and image analysis

For the whole body ^{18}F -FDG PET/CT images we used the full ring PET/CT scanner Philips Gemini TF (Ohio, Cleveland, USA), with dedicated lutetium yttrium oxyorthosilicate (LYSO) and a 64 slice CT. All patients were kept fasting for 6h prior to the intravenous (i.v.) injection of 3.7MBq/kg of ^{18}F -FDG. During the waiting period of 60min, all patients were orally hydrated with 1.5L of contrast solution. Sixty minutes later, after asking the patients to empty the bladder and injecting the i.v. contrast the combined examination started.

The CT scan was first acquired followed by the PET scan. Non-corrected and attenuation-corrected PET and CT images were evaluated in the rotating maximum-intensity projection and in the cross-sectional transverse-sagittal-coronal planes view. The ^{18}F -FDG uptake of the primary tumor was semi-quantified by the SUVmax value (Figure 1A-D).

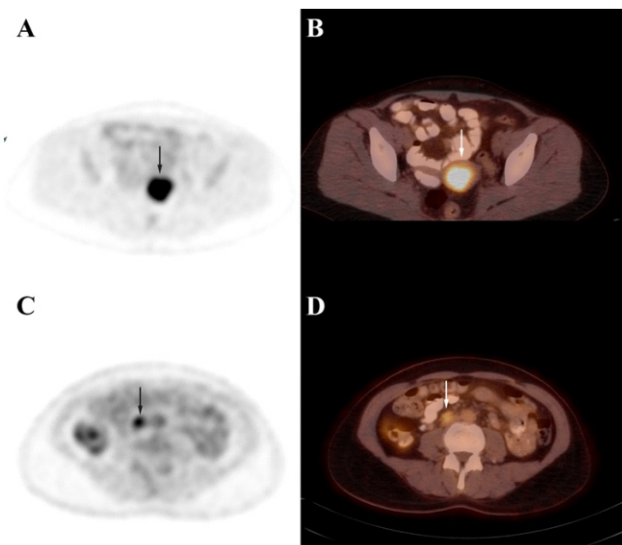


Figure 1A-D. A 45 years old female with endometrial cancer. ^{18}F -FDG-PET/CT images showed increased ^{18}F -FDG uptake in the tumor (A-B) and in the metastatic precaval lymph nodes (C-D).

Histopathological and immunohistochemical protocol

The microscopy slides of 38 patients with endometrioid adenocarcinoma were re-evaluated by a surgical pathologist to confirm the diagnosis. Tumors were graded according to the International Federation of Gynecology and Obstetrics (FIGO) Grading System [14]. Slides lacking necrosis from the most representative areas were selected for immunohistochemical staining with AM, Bcl-2 and HIF-1 α . Immunohistochemical staining was performed by using the avidin-biotin-peroxidase method. Immunohistochemical anti-AM (clone: HTA91a/G2, 1/100, Abcam, CA, USA), Bcl-2 (Bcl-2 alpha Ab-1, clone: 100/D5, 1/100, Thermo Scientific, Fremont, CA, USA) and Anti-Hif-1-alpha, (clone: EP1215Y, 1:100, Abcam, CA, USA) expressions were evaluated blindly. Negative controls for immunostaining were prepared by omitting the incubation step with primary antibody. Positive controls were adrenal medulla for AM, tonsil for Bcl-2 and human ovarian carcinoma for HIF-1 α . Cytoplasmic expression was considered as positive for AM and Bcl-2, while nuclear expression was considered as positive for HIF-1 α (Figure 2A-C).

Data analysis

Allred scoring system was used for the evaluation of immunohistochemical AM and Bcl-2 expressions [15]. For this purpose an intensity score (0; negative, 1; weak, 2; moderate, 3; strong), a proportional score (0; 0%, 1; $\leq 1\%$, 2; 1%-10%, 3; 11%-33%, 4; 34%-66%, 5; 67%-100%) and a total Allred score (between 0 and 8) were defined. Adrenomedullin and Bcl-2 expression less than 10% were considered negative. For the

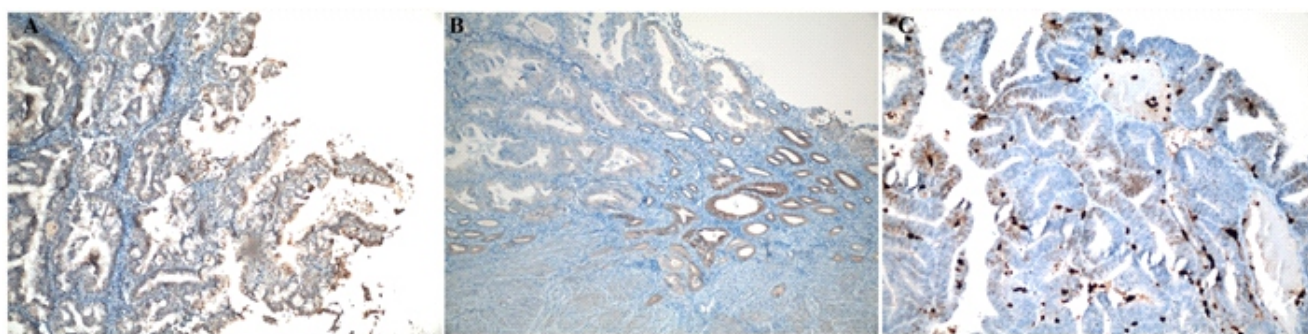


Figure 2A-C. Immunohistochemical cytoplasmic adrenomedullin expression (A; x200), Bcl-2 expression loss (B; x100) and nuclear HIF-1α positivity (C; x200) in endometrial adenocarcinoma.

HIF-1α immunohistochemistry two cut-off values were set for the statistical analysis ($\geq 1\%$ and $\geq 10\%$) [16].

Statistical analysis

Data were expressed as means \pm SD. Student t-test was used to compare means. Categorical data were analyzed by Chi-square test. Spearman and Pearson correlation tests were used to analyze the degree of association between SUVmax and biological variables. All statistical analyses were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium). A P value less than 0.05 was considered statistically significant.

Results

Patients and histopathological findings

Mean age of patients was 57.6 \pm 8.5. Tumor grade was grade 1, 2 and 3 in 47.4%, 42.1% and 10.5%, respectively ($P=0.10$). Mean tumor size was 4.5 \pm 2.9. There was no myometrial invasion in 21.1% of specimens. A total of 57.9% percent of patients had myometrial invasion in less than 50% of the myometrium and 21.1% had myometrial invasion in more than 50% of myometrium ($P=0.07$). Lymphovascular space invasion was negative in 94.7% and positive in 5.3% ($P=0.0002$).

PET/CT findings and correlation with hypoxia, adrenomedullin and apoptosis

In all patients ^{18}F -FDG uptake was detected. Mean SUVmax of the tumors was 11.8 \pm 5.9. SUVmax and histopathological relations are shown in Table 1.

Although SUVmax was higher in HIF-1α positive tumors (mean SUVmax: 9.4 \pm 4.8 vs 12.5 \pm 6.1), this finding was not statistically important ($P=0.77$). No correlation was found between SUVmax and HIF-1α positivity. Mean SUVmax was 6.4 \pm 3 and 12.3 \pm 5.7 in AM negative and AM positive patients ($P=0.17$). Mean SUVmax was 10.6 \pm 4.9 and 12.3 \pm 5.7 in Bcl-2 negative and Bcl-2 positive patients ($P=0.19$).

We found no correlation between SUVmax, adrenomedullin or Bcl-2 expression. AM and Bcl-2 Allred scores were not related with SUVmax in regression analyses.

Immunohistochemical findings

Adrenomedullin expression was negative in 10.5% of patients and positive in 94.7% ($P=0.001$). Adrenomedullin intensity was negative, weak, intermediate and strong in 15.8%, 15.8%, 52.6% and 15.8%, respectively ($P=0.05$). Median Allred score of AM was 7 (range: 0-8).

Table 1. SUVmax and immunohistopathological findings.

	SUVmax	P value
HIF-1α		
HIF-1α<1%	9.4 \pm 4.8	0.36
HIF-1α>%1	12.5 \pm 6.1	
HIF-1α<10%	12 \pm 6.3	0.90
HIF-1α>10%	11.6 \pm 5.0	
Bcl-2 expression		
Negative	10.6 \pm 4.9	0.19
Positive	12.3 \pm 5.7	
AM expression		
Negative	6.4 \pm 3	0.17
Positive	12.5 \pm 1.4	

Bcl-2 expression was negative in 68.4% and positive in 31.6% ($P=0.16$). Bcl-2 intensity was negative, weak, intermediate and strong in 31.6%, 47.4%, 21.1% and 0%, respectively ($P=0.36$). Median Allred score of Bcl-2 was 3 (range: 0-5).

Due to cut-off value of 1%, HIF-1α expression was negative in 21.1% of specimens and positive in 78.9% of specimens ($P=0.02$). With a cut-off level of 10%, HIF-1α expression was negative in 73.6% of specimens, and positive in 26.3% of specimens ($P=0.06$).

Discussion

We have found no relation between SUVmax and HIF-1 α , AM, Bcl-2. There has been one study on SUVmax and HIF-1 α in endometrial cancer and we were unable to find any study between SUVmax and AM, Bcl-2 in endometrial cancer findings [17].

HIF-1 α is an essential component of adaptation of tumor cells, carcinogenesis, and progression [18]. Expression of HIF-1 α is shown to be increased from inactive endometrium through endometrial hyperplasia to endometrial cancer [19]. Other studies reported that overexpression of HIF-1 α is associated with poor prognosis and recurrence [18-20]. However, contradictory results of HIF-1 α in endometrial cancer have also been reported [18, 20-22]. A cut-off value for HIF-1 α positivity is not universally accepted, and variable cut-off values have been used [18]. Therefore, we have studied a 1% and a 10% cut-off value. HIF-1 α expression was negative in 21.1% of specimens and positive in 78.9% of specimens ($P=0.02$) with a cut-off level at 1% and negative in 73.6%, and positive in 26.3% of specimens ($P=0.06$) with a cut-off of 10%. These findings were in accordance with others reporting a positivity rate of 26-49% for HIF-1 α [17, 18, 20].

We have found no correlation between SUVmax and HIF-1 α . The only other study in the literature evaluating SUVmax and HIF-1 α positivity also found such no relation [17]. However, ^{18}F -FDG uptake is shown to be associated with the presence of HIF-1 α in other malignancies including cervix, cancer of the brain, the oral cavity and breast [23-25]. A significant correlation among the rate of expression of GLUT, hexokinase and HIF-1 α reported in these studies indicated that GLUT and hexokinases were induced by HIF-1 α . Controversially, it is reported that ^{18}F -FDG uptake by increased GLUT may also be independent to HIF-1 α [17, 25, 26].

Adrenomedullin is mainly under the control of HIF-1 α and belongs to a family of proteins secreted in response to hypoxia [27]. Adrenomedullin is a critical factor for the growth of neoplasia, helps the tumor evade the immune system [10, 27] and promotes cell survival via Bcl-2 in endometrial cancer [10]. It has been shown that AM is increased in a stepwise manner from normal, simple hyperplasia, complex hyperplasia to endometrium cancer [10]. A varied expression of AM in different tumor grades has been reported [28]. Together with VEGF, AM acts synergistically for angiogenesis [27,28]. Vascular morphologic changes induced by AM do not require the VEGF and blocking only VEGF does not interrupt formation of capillaries [27]. In a single study evaluating SUVmax and VEGF relation in endometrial cancer it was reported that there is no relation between SUVmax and VEGF [17]. Therefore, we have studied AM, Bcl-2 and SUVmax. Adrenomedullin expression was negative in 10.5% of patients and positive in 94.7%. Bcl-2 expression was negative in 68.4% and positive in 31.6%. We have found no association between SUVmax and AM, Bcl-2.

In conclusion, our study in a small number of patients is the first to show that SUVmax, although expected is not associated with HIF-1 α , AM or with Bcl-2 in endometrial can-

cer. Increased uptake of ^{18}F -FDG in endometrial cancer seems to be independent of HIF-1 α and its downstream factors.

The authors declare that they have no conflicts of interest

Bibliography

1. http://www.nobelprize.org/nobel_prizes/medicine/lau-reates/1931/
2. Denko NC. Hypoxia, HIF1 α and glucose metabolism in the solid tumor. *Nat Rev Cancer* 2008; 8: 705-13.
3. Höckel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 2001; 93: 266-76.
4. Airley RE, Mobasheri A. Hypoxic regulation of glucose transport, anaerobic metabolism and angiogenesis in cancer: novel pathways and targets for anticancer therapeutics. *Chemotherapy* 2007; 53: 233-56.
5. DeVita VT, Lawrence TS, Rosenberg SA. Hallmarks of Cancer: An Organizing Principle for Cancer Medicine. In: Hanahan D and Weinberg RA. Eds. *Cancer: Principles & Practice of Oncology*. 10th edn. Wolters Kluwer, Philadelphia. 2015; 164-210.
6. Oehler MK, Norbury C, Hague S et al. Adrenomedullin inhibits hypoxic cell death by upregulation of Bcl-2 in endometrial cancer cells: a possible promotion mechanism for tumour growth. *Oncogene* 2001; 20: 2937-45.
7. Kitamura K, Kangawa K, Kawamoto M et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993; 192: 553.
8. Zudaire E, Martinez A, Cuttitta F. Adrenomedullin and cancer. *Regul Pept* 2003; 112: 175-83.
9. Evans JJ, Chitcholtan K, Dann JM et al. Adrenomedullin interacts with VEGF in endometrial cancer and has varied modulation in tumours of different grades. *Gynecol Oncol* 2012; 125: 214-9.
10. Nunobiki O, Nakamura B, Taniguchi E et al. Adrenomedullin, Bcl-2 and microvessel density in normal, hyperplastic and neoplastic endometrium. *Pathol Int* 2009; 59: 530-6.
11. Smith TA, Zanda M, Fleming IN. Hypoxia stimulates ^{18}F -fluorodeoxyglucose uptake in breast cancer cells via hypoxia inducible factor-1 and AMP-activated protein kinase. *Nucl Med Biol* 2013; 40: 858-64. doi: 10.1016/j.nucmedbio.2013.05.006.
12. Hu SL, Yang ZY, Zhou ZR et al. Role of SUV(max) obtained by ^{18}F -FDG PET/CT in patients with a solitary pancreatic lesion: predicting malignant potential and proliferation. *Nucl Med Commun* 2013; 34: 533-9.
13. Kim SJ, Hwang SH, Kim IJ et al. The association of ^{18}F -deoxyglucose (FDG) uptake of PET with polymorphisms in the glucose transporter gene (SLC2A1) and hypoxia-related genes (HIF1A, VEGFA, AP-EX1) in non-small cell lung cancer. SLC2A1 polymorphisms and FDG-PET in NSCLC patients. *J Exp Clin Cancer Res* 2010; 29: 69.
14. Creasman W, Odicino F, Maisonneuve P et al. Carcinoma of the corpus uteri: FIGO Annual Report. *J Epidemiol Biostat* 2001; 6: 45-86.
15. Allred DC, Harvey JM, Berardo M et al. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998; 11: 155-68.
16. Dales JP, Garcia S, Meunier-Carpentier S et al. Overexpression of hypoxia inducible factor HIF-1 α predicts early relapse in breast cancer: Retrospective study in a series of 745 patients. *Int J Cancer* 2005; 116: 734-9.
17. Jo MS, Choi OH, Suh DS et al. Correlation between expression of biological markers and ^{18}F fluorodeoxyglucose uptake in endometrial cancer. *Oncol Res Treat* 2014; 37: 30-4.
18. Seeber LM, Horrée N, Van Der Groep P et al. Necrosis related HIF-1 α expression predicts prognosis in patients with endometrioid endometrial carcinoma. *BMC Cancer* 2010; 10: 307.
19. Horree N, Van Diest PJ, Van Der Groep P et al. Hypoxia and angio-

- genesis in endometrioid endometrial carcinogenesis. *Cell Oncol* 2007; 29: 219-27.
20. Sivridis E, Giatromanolaki A, Gatter KC et al. Association of hypoxia-inducible factors 1alpha and 2alpha with activated angiogenic pathways and prognosis in patients with endometrial carcinoma. *Cancer* 2002; 95: 1055-63.
 21. Dousias V, Vrekoussis T, Navrozoglou I et al. Hypoxia-induced factor-1α in endometrial carcinoma: a mini-review of current evidence. *Histol Histopathol* 2012; 27: 1247-53.
 22. Pijnenborg JM, Wijnakker M, Hagelstein J et al. Hypoxia contributes to development of recurrent endometrial carcinoma. *Int J Gynecol Cancer* 2007; 17: 897-904.
 23. Yamada T, Uchida M, Kwang-Lee K et al. Correlation of metabolism/hypoxia markers and fluorodeoxyglucose uptake in oral squamous cell carcinomas. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012; 113: 464-71.
 24. Park SI, Suh DS, Kim SJ et al. Correlation between biological marker expression and F-fluorodeoxyglucose uptake in cervical cancer measured by positron emission tomography. *Onkologie* 2013; 36: 169-74.
 25. Bos R, Van Der Hoeven JJ, Van Der Wall E et al. Biologic correlates of (18)fluorodeoxyglucose uptake in human breast cancer measured by positron emission tomography. *J Clin Oncol* 2002; 20: 379-87.
 26. Robey IF, Stephen RM, Brown KS et al. Regulation of the Warburg effect in early-passage breast cancer cells. *Neoplasia* 2008; 10: 74-5-56.
 27. Deville JL, Salas S, Figarella-Branger D et al. Adrenomedullin as the-rapeutic target in angiogenesis. *Expert Opin Ther Targets* 2010; 14: 1059-72.
 28. Evans JJ, Chitcholtan K, Dann JM et al. Adrenomedullin interacts with VEGF in endometrial cancer and has varied modulation in tumours of different grades. *Gynecol Oncol* 2012; 125: 214-9.



David Teniers the Younger, 'Surgical Operation' 17th Century. Oil on wood, 38x61cm, Prado, Madrid.