Cyclo-oxygenase-2 Expression is Associated with Mean Standardised Uptake Value on 18F-Fluorodeoxyglucose Positron Emission Tomography in Oesophageal Adenocarcinoma

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Abstract

Objectives

This pilot study investigated the association of four PET image features and cyclo-oxygenase-2 (COX-2) expression in patients with oesophageal adenocarcinoma. The prognostic significance of these biomarkers was also assessed.

Methods

Fifty consecutive patients [median age=68 (range 47-84), males=45) with oesophageal adenocarcinoma had PET/CT staging between January 2011 and July 2015. The maximum and mean standardised uptake values (SUV_{max} and SUV_{mean}), metabolic tumour volume (MTV) and tumour lesion glycolysis (TLG) were calculated from the primary tumour. Their association with COX-2 status was assessed using Mann-Whitney U tests. Kaplan-Meier and Cox regression analysis tested their prognostic significance. A p-value <0.05 was considered statistically significant.

Results

Thirty-two tumours (64.0%) were COX-2 positive. There was a significant association between SUV_{mean} and COX-2 status (p=0.019). TLG (hazard ratio (HR) 1.001, 95% confidence intervals (CI) 1.000-1.002, p=0.018) was significantly associated with overall survival on multivariable analysis.

Conclusions

This study investigated the association between PET image features and COX-2 expression in oesophageal adenocarcinoma. The preliminary results signal that a combination of TLG (calculated as product of MTV and SUV_{mean}) and COX-2 status may be a strong and clinically important prognostic biomarker. Our research group are planning a prospective, multi-centre study to validate these findings.

Advances in knowledge

1. Mean standardised uptake value (SUV_{mean}) on PET imaging is associated with COX-2 expression in oesophageal adenocarcinoma.

Introduction

The prognosis of oesophageal cancer is poor and adenocarcinoma is the most common histological cell type in developed countries. [1] The development of oesophageal adenocarcinoma has been linked with prolonged mucosal inflammation causing progression through the metaplastic to dysplastic to adenocarcinoma sequence. [2]

Cyclo-oxygenase-2 (COX-2) is involved in promoting angiogenesis and is an enzyme that is activated in response to extra-cellular stimuli such as pro-inflammatory cytokines. [3] It is over-expressed in epithelial solid cancers associated with inflammation. Non-steroidal anti-inflammatory drugs (NSAIDS) may reduce the risk of cancer in the gastrointestinal (GI) tract and aspirin has become the focus of preventative clinical trials. [4] Importantly, COX-2 has been shown to have prognostic significance. [5]

Positron emission tomography (PET) combined with computed tomography (CT) using 18-F fluorodeoxyglucose (FDG) is now routinely used in the oesophageal cancer staging pathway. The focus of many PET research studies in oesophageal cancer is the identification of prognostic imaging biomarkers. Our research group have found that PET image features, maximum and mean standardised uptake value (SUV_{max} and SUV_{mean}), metabolic tumour volume (MTV) and tumour lesion glycolysis (TLG) have prognostic significance [6], with similar results confirmed in other studies. [7,8]

Prognostic biomarker studies that correlate immunohistochemical tumour marker expression with imaging biomarkers are currently lacking in oesophageal cancer research, however an association between the SUV_{max} and COX-2 has been found in lung adenocarcinoma. [9] The discovery of combined prognostic radiological and pathological biomarkers may improve patient risk stratification and treatment decision making. Improved patient

selection for more personalised treatment regimens may ultimately improve the currently low survival rates.

Therefore, the primary aim of this pilot study was to obtain preliminary data associating PET image features (SUV_{max}, SUV_{mean}, MTV and TLG) and COX-2 expression in oesophageal adenocarcinoma. The secondary aim was to assess the prognostic significance of these biomarkers.

Materials & Methods

Patient Selection

This retrospective pilot study considered all consecutive patients with biopsy-proven oesophageal and gastro-oesophageal junction adenocarcinoma in a single tertiary centre who had staging PET/CT between January 2011 and July 2015 (n=71). Patients were radiologically staged using the TNM 7th edition. [10] Given that consecutive patients were studied, the patients included in the study received a variety of surgical, oncological and palliative treatments. (Table 1) Ethical approval was granted to quantify COX-2 expression from archived tissue and correlate with routinely performed PET/CT (REC 14/WA/1208). The requirement for informed consent was waived.

Patients with insufficient archived tissue from the biopsy sample (n=17) and cases in which the primary tumour was non-avid on PET/CT (n=4) were excluded. Fifty patients were included in the study.

PET image features

The standard PET/CT protocol in our centre has previously been published in detail [6] and is included in Appendix 1. This identical protocol was used for each patient in this study. (Fig. 1) Data preparation was performed by a single researcher, a radiology resident with 5 years' experience of PET research who was blinded to COX-2 status. Primary oesophageal tumour delineation was

performed using the Automatic decision Tree-based Learning Algorithm for Advanced image Segmentation in positron emission tomography (ATLAAS) tool [11], which eliminates inter-observer variation in tumour outline. SUV_{max}, SUV_{mean}, MTV and TLG were automatically calculated from the primary tumour only using software developed and validated by our research group. Further details are included in Appendix 1. The SUV_{max} is the value extracted from the pixel with the highest uptake value in the ATLAAS-defined region of interest. The SUV_{mean} is calculated as the average uptake value across the MTV. TLG is calculated as the product of SUV_{mean} and MTV.

COX-2 Preparation

COX-2 analysis was performed from archived diagnostic biopsy tissue because this biomarker is not routinely tested during the staging pathway. The tissue was obtained prior to PET/CT and treatment initiation. COX-2 staining was performed on the Leica Bond III automated immunostaining platform and detection carried out using the Leica Bond Polymer Refine DAB system. Primary antibody COX-2 mouse monoclonal (Clone CX-294, (Dako, Ely, UK)) was applied.

COX-2 Quantification

COX-2 expression was quantified by a Consultant Upper GI Histopathologist and classified as a categorical variable based on the intensity of staining using the following grading: 0, 1+, 2+ and 3+. [12] (Fig. 2) A grading of 0/1+ was considered negative and 2+/3+ considered positive. A pre-constructed control tissue microarray (TMA) was added to the slides as a control with standardised 3+, 2+, 1+ and 0 scoring tumour for COX-2. Internal negative controls within non-tumour components of the biopsies were also tested.

Survival Data

The secondary outcome of the study was overall survival, defined in months from the data of diagnosis. No patient was lost to follow-up, with each patient

receiving clinical follow-up 3-monthly in the first year, then 6-monthly thereafter for the next 4 years, or until death.

Statistical analysis

Categorical variables were described as frequency (percent) and continuous variables as median (range). Mann-Whitney U tests were used to assess for significant differences between PET image features and COX-2. Chi-square tests assessed differences between TNM stage and COX-2 status. Kaplan-Meier analysis with log-rank test evaluated the association of TNM stage and COX-2 status with overall survival. Univariable and multivariable Cox regression models tested the association of continuous PET image features with overall survival. The multivariable model included TNM stage, SUV_{max}, SUV_{mean}, MTV, TLG and COX-2 status. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS v23.0 (IBM, Chicago, USA).

Results

Patient Cohort

Baseline characteristics of the patient cohort are detailed in Table 1. The median age of patients was 68.0 years (range 47–84). Thirty-two tumours were classified as positive COX-2 status (2+=18, 3+=14) and 18 tumours were classified as negative COX-2 status (0=4, 1+=14). Most patients (46%) were treated palliatively.

PET Image Features

Four PET image features were calculated from the ATLAAS-defined primary tumour. The median SUV_{max} was 13.49 (min 3.56, max 42.05, interquartile range (IQR) 11.85). The median SUV_{mean} was 7.16 (min 2.07, max 34.97, IQR 5.50). The median MTV was 19.09 mL (min 0.66, max 94.76, IQR 20.13). The

median TLG was 125.33 (min 11.25, max 1809.08, IQR 234.69). No significant difference in MTV or TLG was found between patients treated with radical intent compared with those treated palliatively (p=0.376 and p=0.224, respectively).

Association of TNM Stage and PET Image Features with COX-2 Status

SUV_{mean} (U=172.0, mean rank 31.9 vs 21.9, p=0.019) showed a significant association with COX-2 status. (Fig. 3) A higher grade of COX-2 expression correlated with a lower SUV_{mean} value. SUV_{max}, MTV and TLG did not reach statistical significance with COX-2 status (p=0.079, p=0.486 and p=0.203, respectively).

Prognostic Significance of TNM Stage, PET Image Features and COX-2 Status

MTV (hazard ratio (HR) 1.024, 95% confidence intervals (CI) 1.007-1.041, p=0.005) and TLG (HR 1.001, 95% CI 1.000-1.002, p=0.011) were both significantly associated with overall survival on univariate analysis. T-stage (X^2 24.998, df 5, p<0.001) and N-stage (X^2 12.201, df 3, p=0.007) were significantly associated with overall survival. As there were only 10 cases with M1 disease, M-stage had borderline prognostic significance ($X^2$3.151, df 1, p=0.076). SUV_{max} (p=0.803) and SUV_{mean} (p=0.838) were not associated with overall survival. COX-2 status was also not associated with overall survival (X^2 0.010, df 1, 0.921). (Fig. 4) After 36 months, the negative and positive status groups appeared to separate, but this was not statistically significant, possibly a reflection on the small sample size. On multivariable analysis, T-stage (p=0.008) and TLG (p=0.18) were independently and significantly associated with overall survival. (Table 2)

Discussion

This study presents preliminary data regarding COX-2 expression and PET image features in oesophageal adenocarcinoma. SUV_{mean} was associated with

COX-2 status, with positive COX-2 expression linked to lower FDG-uptake values. In addition, MTV on univariable analysis, and T-stage and TLG on multivariable analyses were significantly associated with overall survival in this patient cohort.

These findings suggest that metabolic activity on FDG-PET may be influenced by COX-2 expression. Variation in FDG-uptake is associated with underlying pathophysiological features such as angiogenesis, vascularity, perfusion, hypoxia and necrosis. [13] Furthermore, COX-2 has a role in angiogenesis and subsequently tumour vasculature. This pilot study demonstrated an unexpected inverse association between FDG-uptake and COX-2 status. Previous research has suggested that COX-2 may be over-expressed in more aggressive tumours which tend to be associated with higher FDG-uptake. [14] However, this inverse association has been found in other tumour sites including breast. [15]

The cause of this inverse association is not fully understood. CT perfusion studies in oesophageal and colorectal cancer have investigated tumour vasculature. [16] This dynamic technique quantifies tumour perfusion and can predict response to neo-adjuvant treatment. In oesophageal cancer, increased blood flow and reduced mean transit time are associated with tumour regression grade. To date, no in vivo studies have investigated COX-2 expression and tumour perfusion in oesophageal cancer, but increased COX-2 may promote angiogenesis, resulting in varied tumour blood flow affecting tumour growth and hence reduced metabolic activity. Alternatively, variation in angiogenesis may contribute to intra-tumoural necrosis and reduced FDG-uptake. These hypotheses may explain the inverse relationship identified here but the relationship between COX-2 expression and FDG-uptake remains controversial. [14]

COX-2 expression is prognostically significant. In one systematic review and meta-analysis in oesophageal adenocarcinoma, COX-2 had the largest survival effect (HR 2.47, 95% CI 1.15-3.79) out of 9 tumour markers including human

epidermal receptor growth factor 2 (HER-2) and Ki-67. [5] In this current study, MTV on univariable and TLG on multivariable analyses were significantly associated with overall survival. These results support other published research. [7] As expected, disease stage was also significantly associated with overall survival. Tumours with larger MTV and higher FDG-uptake are likely to indicate more advanced tumours and therefore be associated with poorer outcomes. Although COX-2 was not prognostically significant in this patient cohort, preliminary data was obtained from 50 consecutive patients only. This pilot study is under-powered to detect this difference and a larger cohort may have demonstrated statistical significance between COX-2 status groups. In addition, treatment regimens did not influence survival differences between these groups, with relatively equal proportions of patients receiving radical or palliative treatments.

Given the proven link of increased cancer risk with inflammation, the association with COX-2 status and FDG-uptake on PET could signal the potential for clinically important combined biomarkers. Aspirin has become the focus of preventative clinical trials given the link between non-steroidal anti-inflammatory drug (NSAID) use and inflammation in cancer. [4] COX-2 is thought to be over-expressed in areas of inflammation such as segments of Barrett's oesophagus. [2] Our research group is planning a prospective, multi-centre study to further investigate and validate the association of COX-2 status and PET image features in oesophageal adenocarcinoma. Such research should follow the key recommendations outlined in the imaging biomarker roadmap for cancer studies, which describes the necessary stages in the translational pathway of potential image biomarkers from discovery to validation and adoption into clinical practice. [17]

This pilot study had several strengths. All patients underwent PET/CT using an identical protocol and scanner. This sample of patients were treated as part of a large, experienced Regional Upper GI cancer MDT, serving a population of

over 1.4 million. All diagnostic biopsies and tumour markers were evaluated by a Consultant GI Histopathologist. No patients were lost to follow-up.

Limitations

This retrospective, single centre pilot study with a small patient cohort has a number of limitations which should be addressed in future studies. As for similar studies involving biopsy data, it is possible that results are affected by differences in tumour marker expression between the sample and the whole imaged tumour volume. Multiple populations of clonal cells are known to exist within tumours, therefore independent biopsies sampling different regions of malignant tissue may result in varying levels of tumour marker expression. In addition, tumour marker analysis was conducted from archived tissue. Older tissue may degrade affecting its quality and quantity [18] but cases in which there was insufficient tissue were excluded from this study. The oldest biopsy in our cohort was 4 years old.

Conclusion

This pilot study associated PET image features and COX-2 expression in oesophageal adenocarcinoma. This study provides preliminary evidence that SUV_{mean} is associated with COX-2 status and further evidence that MTV and TLG are prognostically significant. These findings suggest that further research is warranted and will inform a prospective, multi-centre study investigating PET image features and COX-2 expression.

References

[1] Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. Gut 2015;64:381–7. doi:10.1136/gutjnl-2014-308124.

[2] Fitzgerald RC. Molecular basis of Barrett's oesophagus and oesophageal adenocarcinoma. Gut 2006;55:1810–8. doi:10.1136/gut.2005.089144.

[3] Mobius C, Stein HJ, Spiess C, Becker I, Feith M, Theisen J, et al. COX2 expression, angiogenesis, proliferation and survival in Barrett's cancer. Eur J Surg Oncol 2005;31:755–9. doi:10.1016/j.ejso.2005.01.006.

[4] Coyle C, Cafferty FH, Rowley S, MacKenzie M, Berkman L, Gupta S, et al. ADD-ASPIRIN: A phase III, double-blind, placebo controlled, randomised trial assessing the effects of aspirin on disease recurrence and survival after primary therapy in common non-metastatic solid tumours. Contemp Clin Trials 2016;51:56–64. doi:10.1016/j.cct.2016.10.004.

[5] McCormick Matthews LH, Noble F, Tod J, Jaynes E, Harris S, Primrose JN, et al. Systematic review and meta-analysis of immunohistochemical prognostic biomarkers in resected oesophageal adenocarcinoma. Br J Cancer 2015;113:107–18. doi:10.1038/bjc.2015.179.

[6] Foley KG, Fielding P, Lewis WG, Karran A, Chan D, Blake P, et al. Prognostic significance of novel 18F-FDG PET/CT defined tumour variables in patients with oesophageal cancer. Eur J Radiol 2014;83:1069–73. doi:10.1016/j.ejrad.2014.03.031.

[7] Tamandl D, Ta J, Schmid R, Preusser M, Paireder M, Schoppmann SF, et al. Prognostic value of volumetric PET parameters in unresectable and metastatic esophageal cancer. Eur J Radiol 2016;85:540–5. doi:10.1016/j.ejrad.2016.01.002.

[8] Hatt M, Visvikis D, Albarghach NM, Tixier F, Pradier O, Cheze-le Rest C. Prognostic value of 18F-FDG PET image-based parameters in oesophageal cancer and impact of tumour delineation methodology. Eur J Nucl Med Mol Imaging 2011;38:1191–202. doi:10.1007/s00259-011-1755-7.

[9] Shimizu K, Hirami Y, Saisho S, Yukawa T, Maeda A, Yasuda K, et al. Maximal standardized uptake value on FDG-PET is correlated with cyclooxygenase-2 expression in patients with lung adenocarcinoma. Ann Thorac Surg 2012;93:398–403. doi:10.1016/j.athoracsur.2011.10.033.

[10] Sobin LH, Gospodarowicz MK, Wittekind CH. UICC TNM Classification of Malignant Tumours. 7th ed. New York: Wiley; 2009.

[11] Berthon B, Marshall C, Evans M, Spezi E. ATLAAS: an automatic decision tree-based learning algorithm for advanced image segmentation in positron emission tomography. Phys Med Biol 2016;61:4855–69. doi:10.1088/0031-9155/61/13/4855.

[12] Rakha EA, Pinder SE, Bartlett JM, Ibrahim M, Starczynski J, Carder PJ, et al. Updated UK Recommendations for HER2 assessment in breast cancer. J Clin Pathol 2015;68:93–9. doi:10.1136/jclinpath-2014-202571.

[13] Henriksson E, Kjellen E, Wahlberg P, Ohlsson T, Wennerberg J, Brun E.

2-Deoxy-2-[18F] fluoro-D-glucose uptake and correlation to intratumoral heterogeneity. Anticancer Res 2007;27:2155–9.
[14] Hu Z, Yang Y, Zhao Y, Huang Y. The prognostic value of cyclooxygenase-2 expression in patients with esophageal cancer: evidence from a meta-analysis. Onco Targets Ther 2017;10:2893–901. doi:10.2147/OTT.S134599.
[15] Groheux D, Majdoub M, Tixier F, Le Rest CC, Martineau A, Merlet P, et al. Do clinical, histological or immunohistochemical primary tumour characteristics translate into different (18)F-FDG PET/CT volumetric and heterogeneity features in stage II/III breast cancer? Eur J Nucl Med Mol Imaging 2015;42:1682–91. doi:10.1007/s00259-015-3110-x.
[16] Djuric-Stefanovic A, Micev M, Stojanovic-Rundic S, Pesko P, Saranovic D. Absolute CT perfusion parameter values after the neoadjuvant chemoradiotherapy of the squamous cell esophageal carcinoma correlate with the histopathologic tumor regression grade. Eur J Radiol 2015;84:2477–84. doi:10.1016/j.ejrad.2015.09.025.
[17] O'Connor JP, Aboagye EO, Adams JE, Aerts HJ, Barrington SF, Beer AJ, et al. Imaging biomarker roadmap for cancer studies. Nat Rev Clin Oncol 2017;14:169–86. doi:10.1038/nrclinonc.2016.162.
[18] Ravo M, Mutarelli M, Ferraro L, Grober OM, Paris O, Tarallo R, et al. Quantitative expression profiling of highly degraded RNA from formalin-fixed, paraffin-embedded breast tumor biopsies by oligonucleotide microarrays. Lab Invest 2008;88:430–40. doi:10.1038/labinvest.2008.11.

Tables

Table 1. Baseline Characteristics of the Patient Cohort

Characteristic	Frequency (%)
M: F Ratio	45 (90.0): 5 (10.0)
Tumour Location	
Oesophagus	23 (46.0)
GOJ	27 (54.0)
Adenocarcinoma Differentiation	
Well	2 (4.0)
Moderate	16 (32.0)
Poor	19 (38.0)
Gx	13 (26.0)
Radiological T-stage	
T1	2 (4.0)
T2	3 (6.0)
T3	34 (68.0)
T4a	7 (14.0)
T4b	2 (4.0)
Tx	2 (4.0)
Radiological N-stage	
N0	13 (26.0)
N1	14 (28.0)
N2	15 (30.0)
N3	8 (16.0)
Radiological M-stage	
M0	40 (80.0)
M1	10 (20.0)
Stage Groups	
Stage I	3 (6.0)
Stage II	10 (20.0)
Stage III	27 (54.0)
Stage IV	10 (20.0)
Treatment	
Palliative	23 (46.0)
NACT	14 (28.0)
dCRT	6 (12.0)
Surgery alone	5 (10.0)
NACRT	2 (4.0)

GOJ Gastro-oesophageal junction, Gx/Tx unable to be assessed, NACT neo-adjuvant chemotherapy, dCRT definitive chemoradiotherapy, NACRT neo-adjuvant chemoradiotherapy

Table 2. Results of Multivariable Analysis

Variable	p-value	Hazard Ratio	df	95% CI Lower	95% CI Upper
T-Stage	0.008		5		
T2	0.487	2.272	1	0.224	23.039
T3	0.878	0.853	1	0.112	6.506
T4a	0.705	1.548	1	0.162	14.821
T4b	0.216	4.861	1	0.398	59.406
Tx	0.019	25.017	1	1.694	369.534
TLG	0.018	1.001	1	1.000	1.002

Tx unable to be assessed, df degrees of freedom, CI confidence intervals, TLG Tumour Lesion Glycolysis

Figures

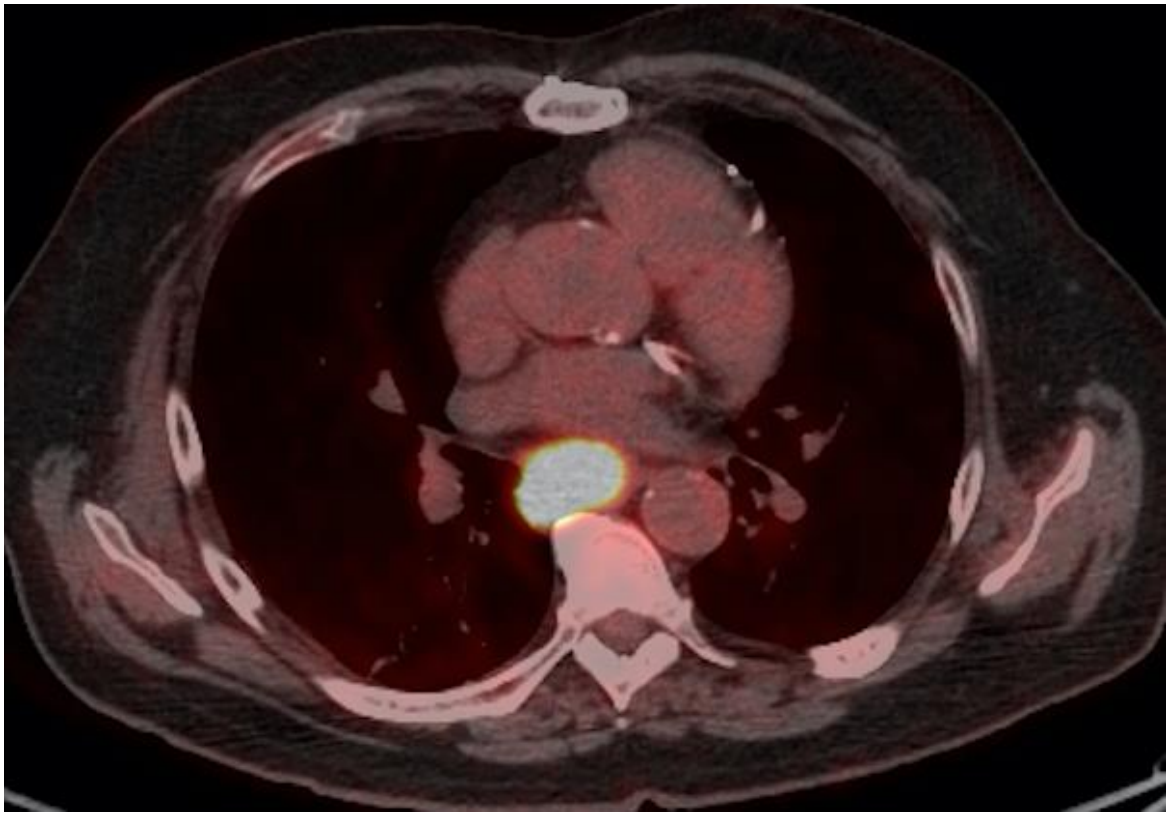

Figure 1. A selected fused PET/CT image showing a FDG-avid distal oesophageal adenocarcinoma.

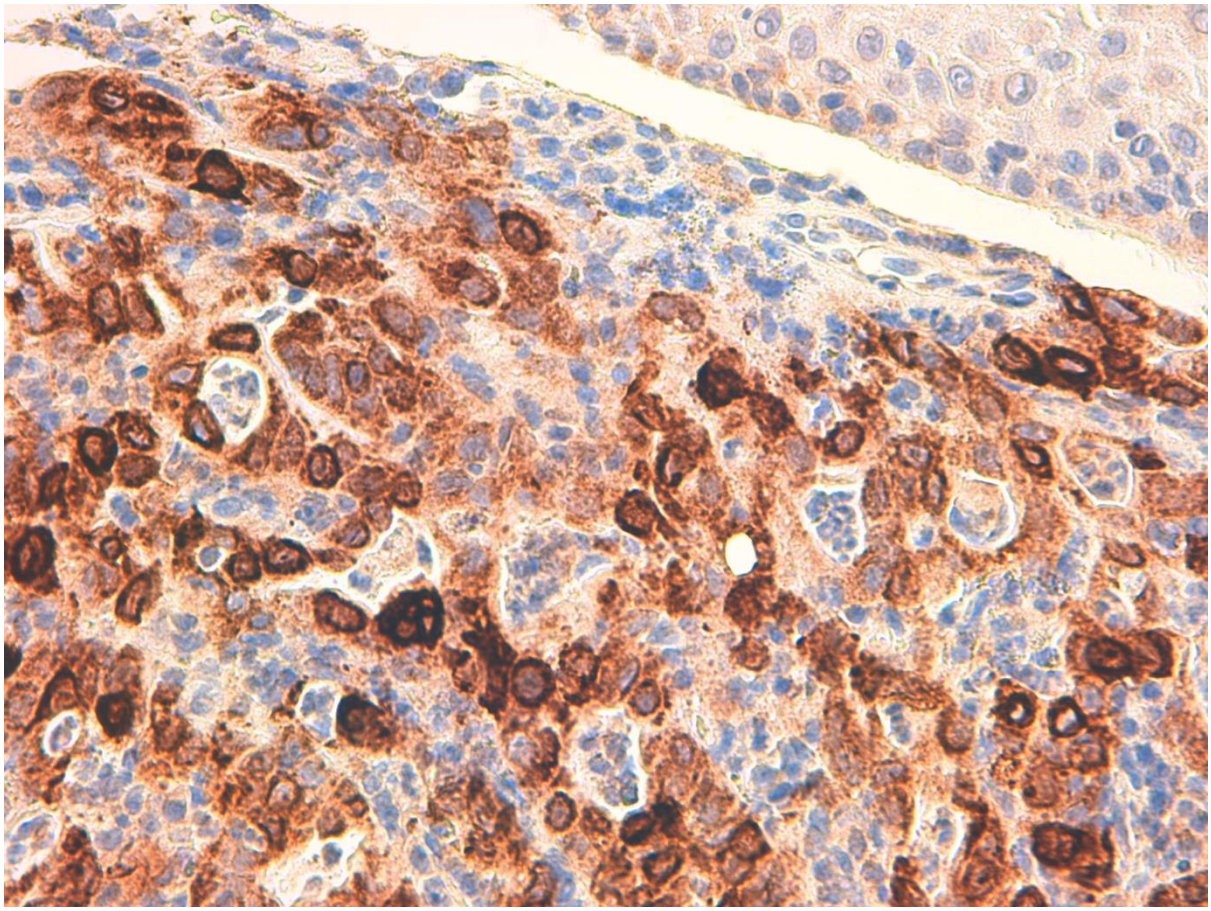

Figure 2. A high magnification image of an adenocarcinoma biopsy sample showing high COX-2 expression (brown cells).

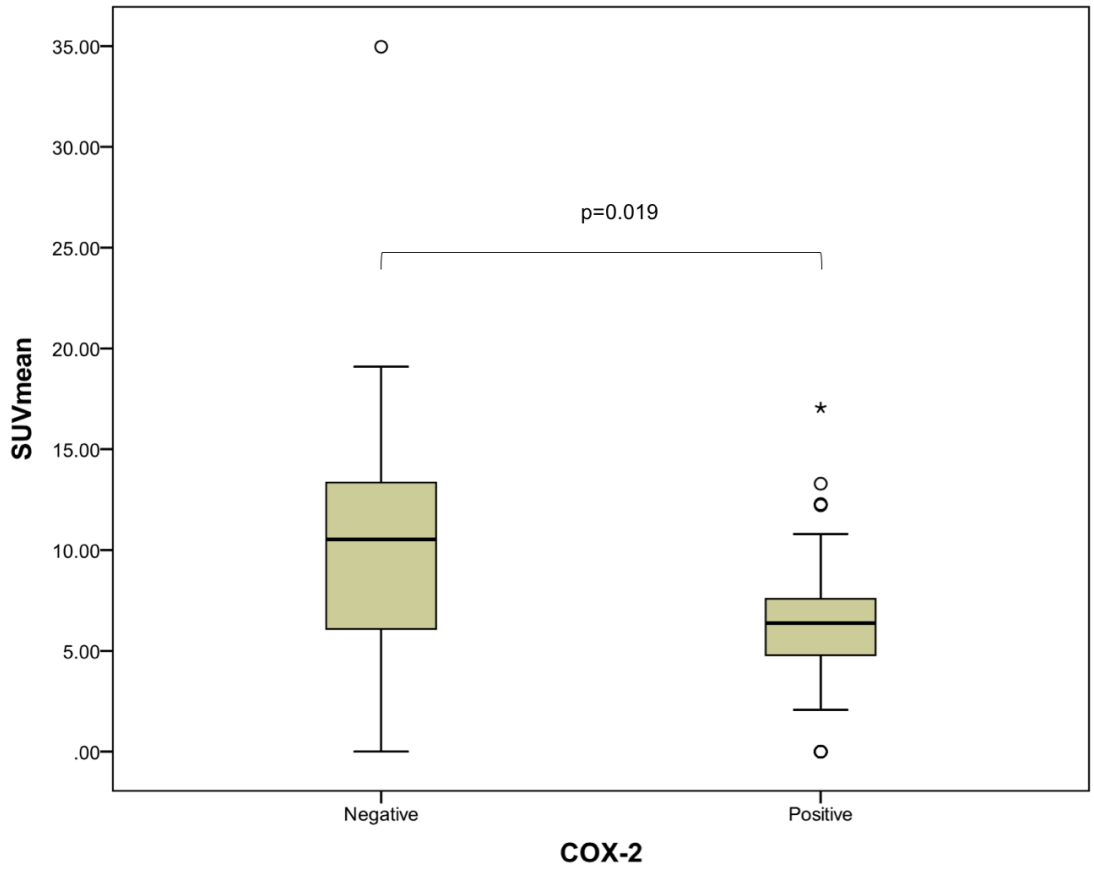

Figure 3. Box-plot representations of COX-2 status with SUV_{mean} (p=0.019).

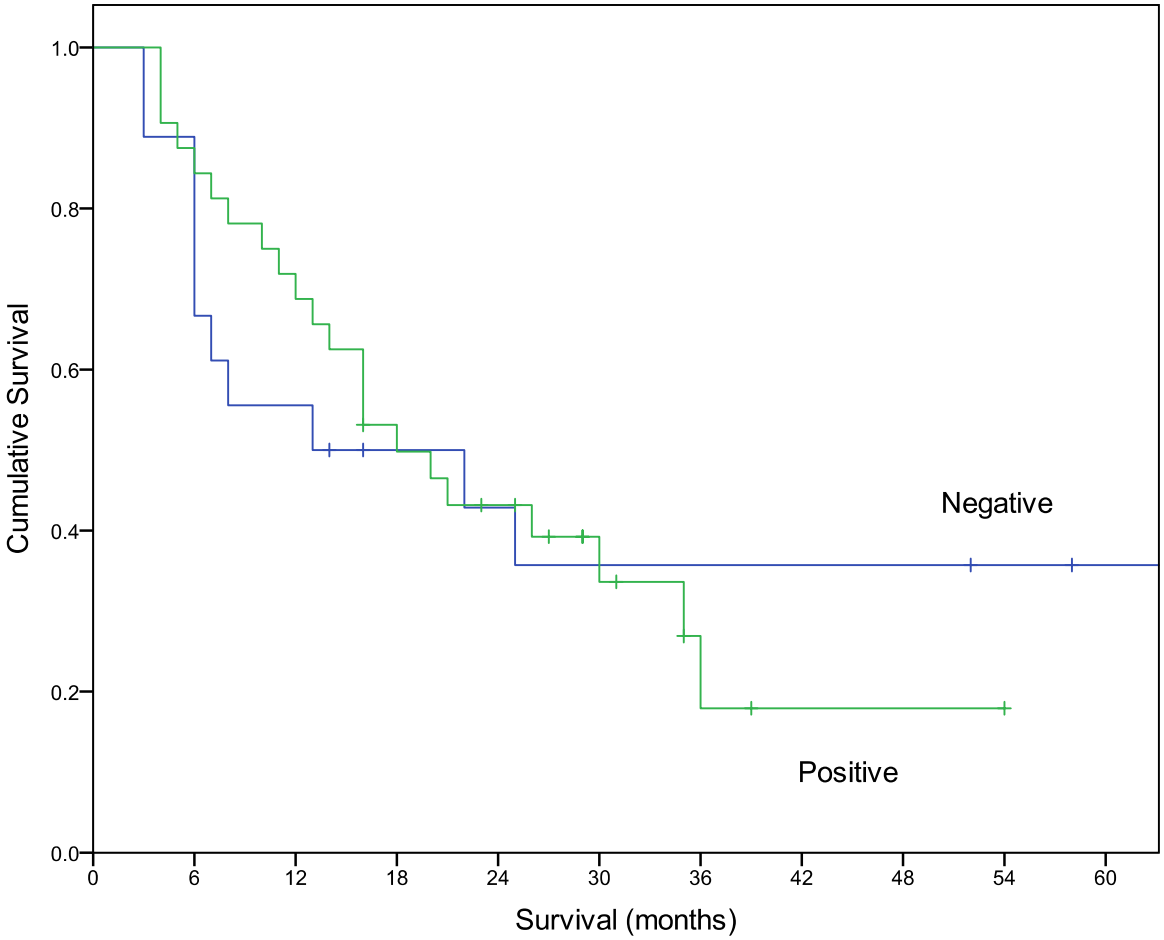

Figure 4. Cumulative survival of negative and positive COX-2 status groups (X^2 0.010, df 1, p=0.921).