Splenic ¹⁸F-FDG Uptake on Baseline PET/CT is Associated with Oncological Outcomes and Tumor Immune State in Uterine Cervical Cancer

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37 **Research Highlights**

- 38 ▪ Splenic metabolic activity was a prognostic factor for recurrence in cervical cancer.
- 39 ▪ Splenic metabolic activity was a predictive factor for pathological complete response in
- 40 cervical cancer.
- 41 ▪ Splenic metabolic activity was associated with the cervical tumor immune infiltrate.

Abstract

Objective

The spleen represents an important contributor to tumor immune escape, but the relevance of increased splenic metabolic activity remains to be fully elucidated.

Methods

We retrospectively measured the spleen-to-liver standard uptake value (SLR) on ^{18}F -FDG PET/CT examinations of 92 consecutive patients with FIGO stage IB1 to IVA cervical cancer and integrated the results with survival, response to treatment, tumor immune infiltrate, and baseline characteristics.

Results

$\text{SLR}_{\text{max}} > 0.92$ ($p = .026$) and $\text{SLR}_{\text{mean}} > 0.94$ ($p = .005$) were significantly associated with decreased DFS in univariable analysis. Multivariable models were built using best subset selection; $\Delta\text{SLR}_{\text{max}}$ and either SLR_{max} or SLR_{mean} were consistently selected, strongly reinforcing the association between SLR variables and DFS in relation to potential confounders (all models $p \leq .002$). Independent associations were found for SLR_{max} using multivariable Cox regression models for DFS (all $p \leq .003$). Further, uni- and multivariable analyses demonstrated the negative impact of higher SLR values on pathological complete response. A statistically significant higher proportion of patients with high SLR_{max} had a dense infiltrate of CD20^+ ($p = .036$) and CD68^+ ($p = .015$) immune cells, as well as PD-L1^+ tumor cells ($p = .019$) as compared to those with low SLR_{max} . Finally, high SLR_{max} status was neither associated with systemic inflammatory markers (except for an increased white blood cell count; $p = .038$), nor with clinically overt infection.

Conclusion

65 This hypothesis-generating study provides the first evidence that increased splenic metabolic
66 activity is a negative prognostic and predictive biomarker in locally advanced cervical cancer.
67 In addition, it might help to discriminate immunologically ‘hot’ from ‘cold’ cervical tumors.
68 **Keywords:** ¹⁸F-FDG PET; spleen; cervical cancer; immune infiltrate; biomarker;
69 chemoradiation

Background

Cervical cancer is the fourth most common cancer and the fourth leading cause of cancer-related death among women worldwide, with roughly 570,000 new cases and 310,000 deaths annually [1]. Among the most important negative prognostic factors in cervical cancer are advanced *Fédération Internationale de Gynécologie et d'Obstétrique* (FIGO) stage, large tumor size, positive lymph node (LN) status, and parametrial invasion [2]. These well-established factors, although helpful, are not sufficient to accurately predict prognosis nor biologic behavior. Despite the current standard of care, approximately 40% of patients with locally advanced cervical cancer (LACC) present with disease recurrence [3]. Additional studies to investigate new prognostic factors based on disease biology—rather than the present clinicopathological factors—to improve outcomes are warranted.

^{18}F -fluorodeoxyglucose (^{18}F -FDG) positron-emission tomography (PET)/computed tomography (CT) is a functional imaging modality widely used for assessing the extent of the primary tumor, LN status, and distant disease, as well as evaluating treatment response in patients with cervical cancer [2]. Additionally, ^{18}F -FDG PET enabled the identification of new prognostic factors directly linked to clinical outcome, including maximum standardized uptake value (SUV_{max}) [4, 5], metabolic tumor volume (MTV) [5-7], and total lesion glycolysis (TLG) of the primary cervical tumor [8, 9].

As the largest (secondary) lymphoid organ, the spleen is an important site for mediating the effects of both innate and adaptive immune responses by acting as a reservoir for various subsets of myeloid and lymphoid cells [10]. In addition, the spleen is being increasingly studied in the context of cancer for its role in peripheral tolerance to tumor antigens and tumor immune escape [11-13]. Interestingly, splenic ^{18}F -FDG uptake is associated with systemic inflammatory markers and has prognostic utility in patients with breast or gastrointestinal cancer [14-17].

The present study reports the value of splenic ^{18}F -FDG uptake (among other metabolic parameters) in predicting LACC prognosis and response to (chemo)radiation, as well as its relationship with patients' immune state (both at the local and systemic level).

Patients and Methods

Patient Population

This study was conducted on a retrospectively identified cohort of consecutive LACC patients planned for neo-adjuvant (chemo)radiation (NA-CRT) followed by surgical resection between January 2006 and April 2018. The patient selection criteria included: (i) histologically proven LACC; (ii) availability of pretreatment ^{18}F -FDG PET, clinicopathological characteristics, and survival outcome data; and (iii) no distant metastases other than LN metastases treated with curative intent. This study was approved by the institutional review board (B670201836989 and B670201732304) and the requirement to obtain informed consent was waived because of its retrospective nature.

Work-Up, Treatment, and Follow-Up

The baseline work-up consisted of pelvic and rectovaginal examination, tumor biopsy, routine laboratory testing, magnetic resonance imaging (MRI) of the pelvis, whole body ^{18}F -FDG PET/CT and, if necessary, recto-sigmoidoscopy and/or cystoscopy.

All patients were intended to receive NA-CRT followed by surgery. Additional details of treatment are provided in the Appendix. Patients underwent routine follow-up for recurrence surveillance; recurrence was confirmed histologically and/or clinically (progressive disease by serial imaging studies).

Clinicopathological Characteristics

Tumor histology and size, grade, parametrial involvement (as assessed by clinical examination and/or MRI), local and distant LN status, FIGO stage (2009 edition), TNM stage (UICC, 7th edition), age at diagnosis, and pathological and MRI response to NA-CRT were collected from

patients' records. White blood cell count, lymphocyte count, neutrophil count, thrombocyte count, and C-reactive protein (CRP) were collected from laboratory studies performed before initiation of therapy (within 1 month from pretreatment ^{18}F -FDG PET/CT examinations).

Tumor histology was categorized as either squamous cell carcinoma or adenocarcinoma according to the World Health Organization classification system. Complete absence of contrast enhancement in the original tumor bed and LNs on MRI after NA-CRT without the appearance of new (contrast-captating) lesions that are typical of cancer was defined as radiological complete response (rCR). Pathological complete response (pCR) was defined as lack of residual invasive or *in situ* cancer in both the uterus and LNs (ypT0 ypN0/x).

^{18}F -FDG PET/CT Imaging Procedures

Patients were instructed to fast for at least 4 hours prior to the administration of $(37+[3.7\times\text{body weight}])\text{MBq}$ of ^{18}F -FDG. Imaging was typically deferred if venous blood glucose concentration exceeded 200mg/dL. Patients were instructed to rest for approximately 60 minutes in a sitting position prior to scanning. Thereafter, patients were positioned in the scanner with their arms above their heads. Skull base to mid-thigh ^{18}F -FDG PET/CT scans were performed using a Gemini GS16Power (Philips Healthcare, Nashville, TN, the United States of America [USA]), Biograph mCT 20 Flow (Siemens Medical Solution, Malvern, PA, USA), or Discovery MI 3ring scanner (GE Healthcare, Waukesha, WI, USA). Daily quality checks were performed, and the technical standards were used as per the manufacturer's instructions. Calibration of each scanner against dose calibrators and well counters was routinely performed. ^{18}F -FDG PET images were reconstructed using an iterative algorithm including corrections for scatter and attenuation based on the CT image, and were corrected for decay.

Image Analysis

^{18}F -FDG PET/CT examinations were performed before the start of NA-CRT (=pretreatment) and 2-3 weeks after its completion (=posttreatment). All pretreatment and posttreatment ^{18}F -

144 FDG PET/CT examinations were retrospectively reviewed on a dedicated Oasis Nuclear
 145 Medicine Workstation (version 1.9.4.9.sp6; Agfa Healthcare and Segami corporation,
 146 Columbia, MD, USA) by two independent readers to whom clinicopathological characteristics
 147 and patient outcomes were masked. Transversal, coronal, and sagittal ^{18}F -FDG PET, CT, and
 148 fused ^{18}F -FDG PET/CT images were used to ensure accurate measurement. After independent
 149 data collection, interobserver agreement was calculated and interpreted using the κ coefficient,
 150 as reported by Landis and Koch [18].

151 All measured ^{18}F -FDG PET/CT parameters are summarized in Supplementary Table 1. On
 152 pretreatment examinations, volumes of interest (VOIs) covering the entire primary cervical
 153 tumor were delineated and the maximum SUV (SUV_{max}), mean SUV (SUV_{mean}), TLG, and
 154 MTV were measured; the latter using a fixed threshold value of 40% of SUV_{max} , as initially
 155 described by Miller and Grigsby [19]. When physiologic activity (e.g., ovary, bowel, or urine)
 156 was included, VOIs were manually adjusted to avoid spillover uptake. SUV was calculated
 157 using the following formula: $\text{SUV} = (\text{concentration of radioactivity within a given VOI} [\text{MBq/mL}]) \times (\text{total body weight/injected radioactivity} [\text{g/MBq}])$. SUV_{max} was the highest SUV
 158 of a voxel within a given VOI. SUV_{mean} was calculated as the mean SUV of all voxels within a
 159 given MTV. TLG was calculated according to the following formula: $\text{TLG} = \text{SUV}_{\text{mean}} \times \text{MTV}$.
 160 On both pre- and posttreatment examinations, spheroid VOIs of 30mm and 50mm in diameter
 161 were placed in the center of the spleen and the right hepatic lobe respectively, and SUV_{max} and
 162 SUV_{mean} of the spleen and liver were measured. The spleen-to-liver ratio (SLR) was calculated
 163 by dividing the spleen SUV by the liver SUV. ΔSLR was calculated according to the following
 164 formula: $\Delta\text{SLR} = \text{posttreatment SLR} - \text{pretreatment SLR}$. In summary, $(\Delta)\text{SLR}$ was calculated as
 165 both $(\Delta)\text{SLR}_{\text{max}}$ ($[\Delta]\text{SUV}_{\text{max}}$ spleen-to- SUV_{max} liver ratio) and $(\Delta)\text{SLR}_{\text{mean}}$ ($[\Delta]\text{SUV}_{\text{mean}}$ spleen-
 166 to- SUV_{mean} liver ratio) for each patient.

Metabolic complete response (mCR) was defined as the complete resolution of ^{18}F -FDG uptake within all lesions on posttreatment examination, making them indistinguishable from the surrounding background (without the appearance of new ^{18}F -FDG-avid lesions that are typical of cancer).

Immunohistochemical Analysis of the Tumor Immune Infiltrate

Formalin-fixed paraffin embedded tissue blocks obtained from diagnostic biopsies of the primary tumor were retrieved to map immune infiltrates and to study the relationship between tumor immune response and splenic metabolic activity. Newly cut, representative, 4- μm slides were stained for CD3, CD4, CD8, FoxP3, CD20, CD68, CD163, and programmed death-ligand 1 (PD-L1). The staining procedures were performed using an automated slide stainer (Benchmark, Ventana Medical Systems, Tucson, USA) according to the manufacturer's instructions. An experienced gynecologic pathologist blinded to the clinical, metabolic, and outcome data evaluated all sections. PD-L1 expression by immune cells (IC) and tumor cells (TC) was categorized into two groups according to the percentage of membranous PD-L1 expression on cells: 0-50% versus >50%. For CD3⁺, CD4⁺, CD8⁺, Foxp3⁺, CD20⁺, CD68⁺, and CD163⁺ immune cells, if the cell density was scored above 2, the sample was defined as high density for that cell type. Additional details of immunohistochemistry are provided in the Appendix.

Statistical Analysis

Disease-free survival (DFS) was defined as the time elapsed between treatment completion and the diagnosis of disease recurrence. Patients who were lost to follow-up or without recurrence/death were censored at their last date of follow-up. Events were censored after 3 years in order to reduce noise and potential bias in the survival analysis. Optimal cutoffs of DFS were assessed using maximally selected log-rank statistics. DFS probabilities were calculated with the Kaplan-Meier method, and survival curves were compared with the log-

rank test. For pCR, cutoffs were determined by the Youden index. Multivariable analyses were performed by using a best subset selection procedure based on the Akaike Information Criterion (AIC). The five best three-variable models were reported to explore which independent variables of interest (e.g., $[\Delta]$ SLR in particular) were consistently selected; and, if so, to quantify the strength of association between them and the outcome variable. Independent associations for DFS were determined using multivariable Cox regression models. Clinicopathological characteristics were compared between high and low SLR groups using the Wilcoxon Rank-Sum test and the Pearson's Chi-Squared test for continuous and categorical variables, respectively. Statistical analysis was conducted using R software (version 3.6.1).

Results

Patient Characteristics

Clinicopathological characteristics and survival outcome were obtained from 114 consecutive LACC patients who were referred for ^{18}F -FDG PET prior to treatment initiation. Patients were excluded if any of the following criteria were present: (i) pretreatment ^{18}F -FDG PET data loss (n=10), (ii) prior malignancy (n=7), (iii) acute or chronic infection, including HIV seropositivity (n=3), (iv) distant metastases not amenable to curative therapy (n=1), or (v) history of organ transplantation (n=1). The final cohort was comprised of 92 patients. The baseline characteristics of the cohort are summarized in Table 1. After a mean follow-up period of 46.3 months (range 5.2-134.4 months), 32 patients had tumor recurrence, 29 of which within 3 years of therapy completion. Mean SLR_{max} (range 0.56-1.60) and SLR_{mean} (range 0.44-1.38) were 0.86. The overall κ coefficients for interobserver agreement were 0.557 (standard error 0.092) and 0.655 (standard error 0.086) for SLR_{max} and SLR_{mean} , respectively.

SLR and Prediction of Recurrence

In the univariable analysis, more advanced FIGO stage (p=.012), larger tumor size (p=.044), larger MTV (p=.002), and higher TLG (p=.003) were significantly associated with a shorter

time to tumor recurrence (Table 2, disease-free survival). Based on the optimal log-rank statistic to determine optimal cutoff values for each variable, SLR_{max} of >0.92 ($p=.026$) and SLR_{mean} of >0.94 ($p=.005$) were significantly associated with a worse DFS; age ($>40y$; $p=.025$), FIGO stage ($>II$; $p=.004$), tumor size ($>3cm$; $p=.025$), thrombocyte count ($>306/nL$; $p=.013$), MTV (>75.14 ; $p<.001$), and TLG (>482.2 ; $p<.001$) were also associated with tumor recurrence. On the basis of the Kaplan-Meier survival analysis, patients with $SLR_{max}>0.92$ had a significantly lower 3-year DFS rate (72%, 95% confidence interval CI [CI] [58%-82%] vs 52%, 95% CI [33%-68%]) than those with $SLR_{max}\leq 0.92$ ($p=.047$) (Figure 1A); patients with $SLR_{mean}>0.94$ also had a significantly lower 3-year DFS rate than those with $SLR_{mean}\leq 0.94$ (data not shown). Multivariable analysis comprising all variables was subsequently performed. The five best three-variable models all selected ΔSLR_{max} and either SLR_{max} or SLR_{mean} (Table 3A). We therefore focused on $(\Delta)SLR_{max}$ as candidate biomarker for pragmatic and uniformity reasons. Multivariable Cox regression revealed that SLR_{max} was a significant independent prognostic factor for tumor recurrence during follow-up (all $p\leq .003$; Table 3B). The prognostic model corresponding to the lowest AIC included SLR_{max} , ΔSLR_{max} , and parametrial invasion. Kaplan-Meier survival analysis showed that patients with a high prognostic score had significantly shorter DFS (hazard ratio for disease progression or death [HR], 6.40; 95% CI [2.70-15.20]; $p<.001$) than those with a low prognostic score (Figure 1B).

SLR and Prediction of Response to NA-CRT

In the univariable analysis, of all investigated variables, squamous cell carcinoma histology, $SLR_{max}\leq 0.88$, and rCR (as assessed by MRI) were significantly predictive of a pCR (Table 2, pathological complete response). Multivariable analysis comprising all variables was subsequently performed, with early FIGO stage and squamous cell carcinoma histology being consistently selected across the five best models (Table 4). Either SLR_{max} or SLR_{mean} was selected in three out of the five best models, respectively.

SLR and Immune State

Baseline characteristics stratified by SLR_{max} status are summarized in Table 1. Both groups were well-balanced except for significantly more local LN involvement (65% vs 39%; $p=.039$) in patients with $SLR_{max}>0.92$.

Supplementary Table 2 shows SLR_{max} in relation to systemic inflammatory markers. A significantly higher proportion of patients with $SLR_{max}>0.92$ had an elevated white blood cell count (64% vs 40%; $p=.038$) compared to those with $SLR_{max}\leq 0.92$. CRP, neutrophil count, and lymphocyte count did not differ significantly among both groups in this study.

Thirty-one biopsies were retrieved for IHC. Figure 2 and Supplementary Table 3 show SLR_{max} in relation to cell densities in the primary tumor. A statistically significant higher proportion of patients with $SLR_{max}>0.92$ had a dense infiltrate of pretreatment $CD20^+$ (78% vs 36%; $p=.036$) and $CD68^+$ (89% vs 41%; $p=.015$) immune cells, as well as $PD-L1^+$ tumor cells (56% vs 14%; $p=.019$) in the primary tumor as compared to $SLR_{max}\leq 0.92$ patients.

Discussion

To our knowledge, this is the first report on the prognostic significance of splenic metabolic activity in patients with LACC. We also provide the first association between splenic metabolic activity and pathological response as well as tumor immune state. ^{18}F -FDG uptake in tissues can be influenced by various biological and technical factors which could confound interpretation of results [20]. Therefore, we calculated the spleen-to-liver ratio (SLR) by dividing the splenic SUV by the hepatic SUV, which is an excellent internal reference point commonly used in clinical diagnostics [20]. This methodology was shown to be straightforward to apply with moderate to substantial agreement between two independent operators (κ coefficients 0.557 and 0.655).

The principle finding of the present study was that SLR_{max} on pretreatment ^{18}F -FDG PET/CT was among the most powerful significant independent prognostic factors for predicting

recurrence in FIGO stage IB1 to IVA cervical cancer treated with NA-CRT plus surgery (Tables 2 and 3; Supplementary Discussion). Based on our results, either further escalation of therapy or close follow-up after therapy may be warranted in patients with high SLR_{max} irrespective of their FIGO stage and conventional clinicopathological characteristics. In addition, local LN involvement, an important prognostic factor in guiding clinical decision-making [2], was the only baseline characteristic that was associated with SLR_{max} ; therefore, another direction for further investigation may be to verify if high SLR_{max} could be a potential biomarker for LN metastasis.

In clinical routine, ^{18}F -FDG PET/CT represents the most widely used functional imaging modality in cancer patients [21]. However, data on the prognostic value of splenic ^{18}F -FDG uptake in cancer patients are very limited. Recently, four studies from the same research group have shown that SLR_{mean} measured before the initiation of oncological therapy had prognostic information on predicting recurrence and/or death in breast, bile duct, and rectal cancer, whereas SLR_{max} did so in gastric cancer [14-17]. As in the present study, higher splenic ^{18}F -FDG uptake was associated with a worse prognosis in all four patient cohorts, but the cutoff value in each study varied. This might in part be explained by the small number of study subjects, but it might also reflect between-study differences in the definition of regions of interests and the validity of using cutoffs of SUVs. Alternative but less relevant between-study differences (due to using the spleen-to-liver SUV ratio rather than splenic SUV alone) are those in the radiotracer uptake time, calibration of the PET scanner, and the calculation method acquiring attenuation-corrected images [22]. Prospective data produced in a large multicenter study using a standardized protocol for ^{18}F -FDG PET image acquisition and analysis could be used to solve this problem. Unfortunately, further comparison between our results and those obtained in these studies would be confounded by many factors, such as the already-mentioned variation in data acquisition and analysis, but also by selection bias (e.g., selective

administration of PET scans to either lower or higher risk patients), pharmacogenomic heterogeneity (e.g., Asian vs European population), and differences in tumor biology. Multiple reports have suggested pretreatment SUV_{max} of the primary tumor as an independent prognostic factor for many cancers [23, 24], as well as LACC treated with definitive CRT [25]. The Grigsby group, for instance, was among the first to show that the SUV_{max} of the cervical tumor is a sensitive biomarker for treatment response and overall survival in patients with cervical cancer [4, 26]. However, SUV_{max} was not a significant factor for recurrence in the present study. This can be explained by the fact that SUV_{max} is a single-voxel measurement that does not reflect spatial heterogeneity of the primary cervical tumor. Accordingly, a high MTV and/or TLG of the primary tumor have previously been shown to correlate with aggressive tumor behavior and poor prognosis in cervical cancer patients [5-8], consistent with the results of the present study. Despite extensive research showing promising results [5-8, 27], such volumetric parameters have not been accepted and incorporated into clinical practice yet, mainly because their values are significantly affected by the segmentation methodology (e.g., threshold-based vs algorithm-based) [28].

Prognostic ability aside, an ideal biomarker should also be predictive for response (pCR) to therapy. Unfortunately, an inevitable consequence of the current standard treatment for LACC is the lack of data on pathological response assessment. Since 1999, concurrent cisplatin-based chemoradiation therapy is the standard treatment of LACC [29]. Despite optimal treatment, tumor recurrence continues to be common for LACC, with a 5-year DFS rate of 58% in one meta-analysis [3]. In light of these findings, the current paradigm was challenged by introducing intensity-modulated arc therapy followed by C1 radical hysterectomy in the setting of clinical trials at our institution [30]. The present study thus represents a unique cohort of LACC patients in whom hysterectomy was performed, enabling pathological response assessment after NA-CRT. Consequently, results of this study provided some preliminary, yet valuable, information

regarding the predictive ability of metabolic variables in LACC patients. In the univariable analysis, similar AUCs (between 0.59 and 0.62) were found for the factors significantly associated with pCR: rCR as assessed by MRI, squamous cell carcinoma histology, and low SLR_{max} (Table 2). This finding is intriguing because MRI is commonly used to evaluate treatment response after CRT by assessing the reduction in tumor size and gadolinium contrast enhancement [31, 32], and, as such, is widely accepted as the imaging standard for response assessment in clinical practice. Our data thus suggest that SLR (preferably in conjunction with MRI) might help to identify patients (*i.e.*, those with residual tumor) who might require salvage surgery to improve survival and those who might not. Although it should be noted that early FIGO stage and squamous cell histology were the strongest predictors of pCR in multivariable analysis (Table 4). This corresponds well with results of earlier studies suggesting that cervical adenocarcinoma is less radiosensitive than squamous cell carcinoma [33], with significantly lower pCR rates reported [34].

In the present study, patients with an infection or inflammatory condition were excluded (n=3). This enabled us to more ascertain that SLR variability was caused by differences in tumor development and tumor-promoting inflammation rather than ‘benign’ inflammation. To this end, the tumor immune infiltrate and systemic inflammatory markers between groups with either low or high SLR_{max} were also compared. Intriguingly, a statistically significant higher proportion of patients with high SLR_{max} had a dense infiltrate of pretreatment CD20⁺ (78% *vs* 36%; *p*=.036) and CD68⁺ (89% *vs* 41%; *p*=.015) immune cells, as well as PD-L1⁺ tumor cells (56% *vs* 14%; *p*=.019) in the primary tumor as compared to those with low SLR_{max}. It is critical to note, however, that there was a numerically higher density of all other immune cell subsets studied (*i.e.*, CD3⁺, CD4⁺, CD8⁺, Foxp3⁺, CD163⁺, and PD-L1⁺) in the SLR_{max} high group; whilst statistically not significant, this may still be clinically meaningful. Moreover, similar observations were made for SLR_{mean} (data not shown; Supplementary Figure 2). These findings

are unique to our study, and, if validated, splenic ^{18}F -FDG uptake may be useful to discriminate immunologically ‘hot’ tumors from ‘cold’ tumors. Of interest, Wong *et al.* reported on the association between an elevated SLR_{max} and increased overall survival in metastatic melanoma patients treated with immune checkpoint inhibitors [35]. Concordant with this, we noted that patients with high SLR had a denser immune infiltrate in the primary tumor and were more likely to have disease progression and less likely to achieve pCR than those who did not, suggesting either the tumor immune infiltrate might be significantly suppressed, ineffective, or exhausted—a setting in which immunotherapy could potentially produce clinical benefit [36]. Although LACC patients with high SLR_{max} may thus seem a rational target population for immunotherapy, future research should first provide a more mechanistic evaluation of the relationship between increased splenic metabolic activity and tumor immune escape.

Although median pretreatment CRP value, neutrophil count, and lymphocyte count were similar between both groups, the high SLR_{max} group had significantly more elevated white blood cell counts compared to those patients with low SLR_{max} (64% vs 40%; $p=.038$). This observation does not necessarily imply that high SLR_{max} is associated with subclinical infection, since tumor-associated leukocytosis is not an uncommon (paraneoplastic) feature of patients with solid malignancies. Nonetheless, the mechanisms that mediate this phenomenon may play a significant role in the underlying pathophysiology of cancer progression, such as the increased secretion of granulocyte(-macrophage) colony-stimulating factor (G[M]-CSF), interleukin (IL)-1, IL-6, and tumor-necrosis factor (TNF) by tumors or their microenvironments [37].

The spleen is the largest lymphoid organ of the human body; it accounts for 25% of all immune cells [10]. Speculatively, the finding of increased splenic metabolic activity could be explained by the local accumulation of immune cells endowed with potent tumor-promoting activities (e.g., regulatory T cells or tumor-associated macrophages) or with impaired effector function

(e.g., exhausted or anergic CD8⁺ T cells) that have increased glycolytic activity and that are associated with a worse prognosis. Interestingly, splenic ¹⁸F-FDG uptake has been shown to be related to peripheral cytokine levels (including IL-1 β , IL-4, and IL-6) in cancer patients, possibly reflecting the activation of splenic humoral activity [38]. It is, however, not inconceivable, that other cell types, such as activated stromal cells, are possible contributors to increased splenic metabolic activity [39]. For instance, fibroblastic reticular cells constitute the most prevalent stromal cell type of the white pulp and do not only provide critical structural support to the spleen but can also regulate tumor immune escape, antigenic tolerance, and solute transport properties following exposure to tumor-derived factors [13]. Whatever the (interplay of) causes, whether immunologic or stromal, we surmise that the significance of SLR in patients with LACC should not be overlooked since there may exist a novel, untapped association between splenic metabolic activity, tumor-associated systemic inflammation, tumor immune landscape, and outcome. Similarly, in cardiology, the existence of a ‘cardiosplenic axis’ has been established, with splenic metabolic activity being linked to pro-inflammatory remodeling of the arterial wall [40].

The primary limitation of this study is that it was performed retrospectively at a single institution with a relatively small number of patients (n=92) over a 12-year time period, and thus, recall, selection, and information bias cannot be completely accounted for. Second, data from three PET scanners were pooled into a single analysis. Third, our findings may not be generalizable to all institutions, where practice patterns, ¹⁸F-FDG PET image acquisition, and interpretation procedures may vary. Fourth, although SUV_{max} is a convenient metabolic measure, it represents only a single voxel in the VOI which may not reflect the heterogeneous nature of both benign and malignant tissues and, as such, concerns about the influence of image noise and pixel size on SLR_{max} may arise. Due to this simplicity of measurement, SLR_{max} might, however, be considered of interest for use in daily clinical routine.

Conclusion

In summary, this hypothesis-generating study demonstrated that SLR allows a standardized interpretation of splenic ^{18}F -FDG uptake and could be used to improve prognostic and predictive stratification in LACC. These findings warrant further study of splenic metabolic activity as an ‘immune barometer’ in LACC and other tumor types in larger studies and they provide new potential directions for future investigations in the field of immuno-oncology.

399 **List of Abbreviations**

400	AC:	Adenocarcinoma
401	AIC:	Akaike information criterion
402	AUC:	Area under the curve
403	CD:	Cluster of differentiation
404	CI:	Confidence interval
405	CRP:	C-reactive protein
406	CSF:	Colony-stimulating factor
407	DFS:	Disease-free survival
408	¹⁸ F-FDG:	¹⁸ F-fludeoxyglucose
409	FIGO:	<i>Fédération Internationale de Gynécologie et d'Obstétrique</i>
410	G(M)-CSF :	Granulocyte(-macrophage) colony-stimulating factor
411	HR:	Hazard ratio
412	IC:	Immune cell
413	IL:	Interleukin
414	LACC:	Locally advanced cervical cancer
415	LN:	Lymph node
416	mCR:	Metabolic complete response
417	MRI:	Magnetic resonance imaging
418	MTV:	Metabolic tumor volume
419	(NA)-CRT:	(Neoadjuvant) chemoradiation
420	pCR:	Pathological complete response
421	PD-L1:	Programmed death-ligand 1
422	PET/CT:	Positron-emission tomography/computed tomography
423	rCR:	Radiological complete response
424	Sens:	Sensitivity
425	SCC:	Squamous cell carcinoma
426	SLR:	Spleen-to-liver ratio
427	Spec:	Specificity
428	SUV:	Standard uptake value

429	TC:	Tumor cell
430	TLG:	Total lesion glycolysis
431	TNF:	Tumor-necrosis factor
432	UICC:	Union for International Cancer Control
433	ULN:	Upper limit of normal
434	VOI:	Volume of interest

435 **Declarations**

436 - Ethics approval and consent to participate:

437 This study was approved by the institutional review board (B670201836989 and
438 B670201732304) and the requirement to obtain informed consent was waived because
439 of its retrospective nature.

440 - Availability of data and material:

441 The datasets used and/or analyzed during the current study are available from the
442 corresponding author on reasonable request.

443 - Conflict of interest statement:

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Acquisition of data: E.A.D.; F.L.; L.L.; M.V.; K.D.; H.G.D.; Katrien V.

Analysis and interpretation of data: E.A.D.; L.L.; H.G.D.; Katrien V.

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Review and/or revision of the manuscript: all authors

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483 **Table 1: Patient Characteristics**

Characteristic	All patients	SLR _{max}		p
		Low	High	
<i>n</i>	92	61	31	
Age, year; mean (range)	55 (24-80)	56 (27-80)	53 (24-79)	.374
Follow-up, month; mean (range)	46.3 (5.2-134.4)	46.9 (5.2-134.4)	45.3 (5.5-131.1)	.674
FIGO stage, n (%)				.380
I	12 (13)	6 (10)	6 (19)	
II	55 (60)	37 (61)	18 (58)	
III	17 (18)	11 (18)	6 (19)	
IV ^a	8 (9)	7 (11)	1 (3)	
TNM stage, n (%)				.124
I	3 (3)	2 (3)	1 (3)	
II	37 (40)	29 (48)	8 (26)	
III	38 (41)	20 (33)	18 (58)	
IV ^a	14 (15)	10 (16)	4 (13)	
Pelvic LN status, n (%)				.039
Negative	48 (52)	37 (61)	11 (35)	
Positive	44 (48)	24 (39)	20 (65)	
Distant LN status, n (%)				1
Negative	86 (93)	57 (93)	29 (94)	
Positive	6 (7)	4 (7)	2 (6)	
Tumor size, cm				.407
Mean (range)	5.0 (1.8-8.5)	4.9 (1.8-7.5)	5.3 (2.3-8.5)	
Unknown, n	1	1	0	
Tumor histology, n (%)				.950
Adenocarcinoma	16 (17)	10 (16)	6 (19)	
Spinoecellular carcinoma	76 (83)	51 (84)	25 (81)	
Tumor grade, n (%)				.575
Well	7 (10)	5 (11)	2 (8)	
Moderate	30 (44)	21 (48)	9 (38)	
Poor	31 (46)	18 (41)	13 (54)	
Unknown	24	17	7	
Parametrial invasion, n (%)				.888
Absent	11 (12)	8 (13)	3 (10)	
Present	81 (88)	53 (87)	28 (90)	
Concomitant chemotherapy, n (%)				.800
Cisplatin	84 (91)	55 (90)	29 (94)	
5-Fluorouracil	3 (3)	2 (3)	1 (3)	
None	5 (5)	4 (7)	1 (3)	
Brachytherapy, n (%)				1
Yes	5 (5)	3 (5)	2 (6)	
No	87 (95)	58 (95)	29 (94)	
Surgery, n (%)				.669
Yes	86 (93)	58 (95)	28 (90)	
No	6 (7)	3 (5)	3 (10)	
Response MRI, n (%)				.120
rCR	40 (46)	31 (53)	9 (32)	
No rCR	47 (54)	28 (47)	19 (68)	
Unknown	5	2	3	
Response PET/CT, n (%)				.843
mCR	37 (45)	25 (46)	12 (41)	
No mCR	46 (55)	29 (54)	17 (59)	
Unknown	9	7	2	
MTV, mL; mean (range)	37.6 (2.6-159.8)	35.3 (2.6-150.3)	42.0 (2.9-159.8)	.411
TLG, mean (range)	301.2 (11.1-2419.4)	287.5 (11.1-2419.4)	328.1 (21.8-1155.8)	.544
SUV _{max} , mean (range)	14.8 (3.1-103.4)	15.2 (3.1-103.4)	14.1 (3.2-38.4)	.911
SLR _{max} , mean (range)	0.86 (0.56-1.60)	—	—	—
SLR _{mean} , mean (range)	0.86 (0.44-1.38)	—	—	—
ASLR _{max} , mean (range)	-0.05 (-0.75 to 0.36)	—	—	—
ASLR _{mean} , mean (range)	-0.05 (-0.67 to 0.29)	—	—	—
Outcome status, n (%)				n/a
Alive	68 (74)	47 (77)	21 (68)	
Disease-related death	17 (18)	9 (15)	8 (26)	
Other cause of death	7 (8)	5 (8)	2 (6)	

FIGO: Fédération Internationale de Gynécologie et d'Obstétrique; LN: lymph node; mCR: metabolic complete response; MRI: magnetic resonance imaging; MTV: metabolic tumor volume; n: absolute amount; PET/CT: positron-emission tomography/computed tomography; rCR: radiological complete response; SLR: spleen-to-liver ratio; SUV: standard uptake value; TLG: total lesion glycolysis

^aPhysical examination and limited endoscopy or imaging studies diagnosed eight patients with distant metastases (six with involvement of bladder mucosa and two with involvement of inguinal LNs), whereas more advanced imaging examinations diagnosed an additional six patients with distant metastases (five with involvement of periaortic LNs and one with a retroperitoneal lesion).

Note: Percentages do not sum to 100% due to rounding.

491 Baseline characteristics of all patients and according to SLR_{max} status (cutoff ≤ 0.92 vs > 0.92).

492 **Table 2: Univariable Analysis**

Variable	Disease-free survival			Pathological complete response			
	Cutoff	HR ^a (95% CI)	p	Cutoff	AUC (95% CI)	Sens	Spec
Age, year	Continuous >40 vs ≤40	1.38 (0.76-2.52) 7.04 (0.96-51.74)	.293 .025	>51.5 vs ≤51.5	0.55 (0.43-0.67)	0.67	0.52
FIGO stage	I vs II vs III vs IV >II vs ≤II	— 2.81 (1.35-5.85)	.012 .004	>II vs ≤II	0.59 (0.47-0.69)	0.36	0.79
TNM stage	I vs II vs III vs IV >II vs ≤II	— 1.95 (0.89-4.28)	.167 .091	>II vs ≤II	0.56 (0.45-0.67)	0.64	0.48
Pelvic LN status	N1 vs N0	1.26 (0.61-2.62)	.527	N0 vs N1	0.52 (0.42-0.63)	0.55	0.50
Distant LN status	M1 vs M0	0.41 (0.06-3.00)	.363	M0 vs M1	0.53 (0.48-0.57)	0.97	0.09
Tumor size, cm	Continuous >3 vs ≤3	1.83 (1.01-3.30) _b	.044 .025	>4.55 vs ≤4.55	0.52 (0.40-0.65)	0.66	0.47
Thrombocytes, /nL	Continuous >306 vs ≤306	1.21 (0.83-1.77) 2.65 (1.19-5.91)	.312 .013	>456 vs ≤456	0.53 (0.40-0.65)	0.16	0.93
Tumor histology	SCC vs AC	0.81 (0.31-2.12)	.667	SCC vs AC	0.59 (0.52-0.66)	0.94	0.24
Tumor grade	G1 vs G2 vs G3 >G2 vs ≤G2	— 0.95 (0.40-2.24)	.981 .910	≤2 vs 3	0.55 (0.42-0.67)	0.62	0.51
Parametrial invasion	Present vs absent	4.21 (0.57-30.97)	.124	Present vs absent	0.52 (0.45-0.59)	0.91	0.14
MTV, mL	Continuous >75.14 vs ≤75.14	1.69 (1.21-2.34) 5.40 (2.37-12.29)	.002 <.001	≤10.22 vs >10.22	0.57 (0.44-0.69)	0.33	0.88
TLG, mL	Continuous >482.2 vs ≤482.2	1.63 (1.17-2.27) 4.55 (2.10-9.86)	.003 <.001	≤172.6 vs >172.6	0.55 (0.43-0.68)	0.61	0.57
SUV _{max}	Continuous >14.39 vs ≤14.39	1.13 (0.77-1.66) 1.70 (0.82-3.54)	.523 .151	>18.19 vs ≤18.19	0.54 (0.42-0.66)	0.85	0.31
SLR _{max}	Continuous >0.92 vs ≤0.92	1.32 (0.88-1.98) 2.25 (1.08-4.66)	.185 .026	≤0.88 vs >0.88 ^c	0.62 (0.51-0.73)	0.79	0.53
SLR _{mean}	Continuous >0.94 vs ≤0.94	1.39 (0.96-2.02) 2.79 (1.33-5.86)	.086 .005	≤0.94 vs >0.94 ^c	0.61 (0.49-0.72)	0.91	0.34
ASLR _{max}	Continuous >0.13 vs ≤0.13	1.07 (0.70-1.65) 2.20 (0.83-5.87)	.749 .105	>0.13 vs ≤0.13	0.54 (0.41-0.66)	0.23	0.94
ASLR _{mean}	Continuous >-0.10 vs ≤-0.10	0.91 (0.60-1.39) 0.48 (0.22-1.04)	.670 .057	>-0.23 vs ≤-0.23	0.50 (0.37-0.65)	1.00	0.14
rCR (MRI)	Present vs absent	1.03 (0.49-2.20)	.929	Present vs absent	0.62 (0.51-0.72)	0.61	0.62
mCR (PET/CT)	Present vs absent	0.94 (0.44-2.00)	.865	Present vs absent	0.55 (0.44-0.66)	0.50	0.60

493 AC: adenocarcinoma; AUC: area under the curve; CI: confidence interval; FIGO: Fédération Internationale de Gynécologie et d'Obstétrique;
494 HR: hazard ratio for disease progression or death; LN: lymph node; mCR: metabolic complete response; MRI: magnetic resonance imaging;
495 MTV: metabolic tumor volume; PET/CT: positron emission tomography/computed tomography; rCR: radiological complete response; SCC:
496 spinocellular carcinoma; Sens: sensitivity; SLR: spleen-to-liver ratio; Spec: specificity; SUV: standard uptake value; TLG: total lesion
497 glycolysis

498 ^aFor continuous variables, hazard ratios were calculated for a change from percentile 25 to 75 (interquartile range).

499 ^bUsing the cutoff, no events were observed in one group leading to a complete separation of events.

500 ^cThe Receiver Operating Characteristic (ROC) curves for the predictive value of SLR_{max} and SLR_{mean} for pCR are shown in Supplementary
501 Figure 1.

502 Univariable analysis of disease-free survival and pathological complete response.

Table 3: Multivariable Analysis (Disease-Free Survival)

A					B				
Model	Variables	Performance				Model	Variables	Performance	
		AIC	C-index	HR ^a (95% CI)	p			HR ^a (95% CI)	p
M1	<i>SLR_{mean} & ΔSLR_{max} & parametrial invasion</i>	204.3				M1	<i>SLR_{mean}</i>	3.40 (1.63-7.10)	.001
	Continuous		0.73	2.9 (1.62-5.23)	<0.001		<i>ΔSLR_{max}</i>	2.38 (1.28-4.42)	.006
	High vs low		0.68	4.6 (2.10-10.07)	<0.001		<i>Parametrial invasion</i>	7.35 (0.97-55.65)	.054
M2	<i>SLR_{max} & ΔSLR_{max} & parametrial invasion</i>	206.0				M2	<i>SLR_{max}</i>	3.94 (1.68-9.27)	.002
	Continuous		0.70	2.8 (1.58-5.14)	<0.001		<i>ΔSLR_{max}</i>	2.99 (1.38-6.50)	.006
	High vs low		0.62	6.4 (2.70-15.20)	<0.001		<i>Parametrial invasion</i>	4.54 (0.61-33.58)	.138
M3	<i>SLR_{max} & ΔSLR_{max} & ΔSLR_{mean}</i>	206.7				M3	<i>SLR_{max}</i>	4.87 (1.88-12.58)	.001
	Continuous		0.71	2.8 (1.54-5.27)	.001		<i>ΔSLR_{max}</i>	6.39 (1.87-21.85)	.003
	High vs low		0.68	5.1 (1.74-14.72)	.001		<i>ΔSLR_{mean}</i>	0.47 (0.20-1.11)	.086
M4	<i>SLR_{max} & ΔSLR_{max} & MTV</i>	206.9				M4	<i>SLR_{max}</i>	3.77 (1.56-9.15)	.003
	Continuous		0.70	3.1 (1.63-5.87)	<0.001		<i>ΔSLR_{max}</i>	2.99 (1.37-6.56)	.006
	High vs low		0.61	4.9 (2.01-11.85)	<0.001		<i>MTV</i>	1.42 (0.96-2.12)	.082
M5	<i>SLR_{mean} & ΔSLR_{max} & MTV</i>	207.0				M5	<i>SLR_{mean}</i>	2.73 (1.35-5.53)	.005
	Continuous		0.68	2.5 (1.49-4.23)	.001		<i>ΔSLR_{max}</i>	2.28 (1.20-4.32)	.012
	High vs low		0.64	3.1 (1.45-6.76)	.002		<i>MTV</i>	1.53 (1.05-2.24)	.026

AIC: Akaike information criterion; CI: confidence interval; HR: hazard ratio for disease progression or death; MTV: metabolic tumor volume; SLR: spleen-to-liver ratio

^aFor continuous variables, hazard ratios were calculated for a change from percentile 25 to 75 (interquartile range).

Multivariable analyses were performed by using a best subset selection procedure based on the AIC (A). Independent associations were found using multivariable Cox regression models for disease-free survival (B).

Table 4: Multivariable Analysis (Pathological Response)

Best five models	Performance			
	AIC	AUC (95% CI)	Sens	Spec
<i>SLR_{mean} & ΔSLR_{mean} & FIGO</i>	90.7	0.76 (0.63-0.87)	0.50	0.92
<i>TLG & FIGO & tumor histology</i>	90.8	0.79 (0.68-0.88)	0.73	0.76
<i>SLR_{mean} & FIGO & tumor histology</i>	91.0	0.76 (0.64-0.86)	0.81	0.59
<i>Response MRI & FIGO & tumor histology</i>	91.1	0.76 (0.63-0.86)	0.62	0.73
<i>SLR_{max} & FIGO & tumor histology</i>	91.5	0.75 (0.63-0.85)	0.85	0.59

AIC: Akaike information criterion; AUC: area under the curve; CI: confidence interval; MTV: metabolic tumor volume; Sens: sensitivity; SLR: spleen-to-liver ratio; Spec: specificity; TLG: total lesion glycolysis

Multivariable analyses were performed by using a best subset selection procedure based on the AIC.

Figure 1: Disease-Free Survival Stratified by Biomarker Status

Figure File 1

CI: confidence interval; DFS: disease-free survival; SLR: spleen-to-liver ratio

Three-year DFS stratified by (left panel) SLR_{max} status (72%, 95% CI [58%-82%] vs 52%, 95% CI [33%-68%]; p=.047) and (right panel) the selected multivariable model ('prognostic score') (SLR_{max} & ΔSLR_{max} & parametrial invasion) (72%, 95% CI [59%-81%] vs 20%, 95% CI [3%-47%]; p<.0001).

Figure 2: High SLR_{max} Status is Associated with a Dense Tumor Immune Landscape

Figure File 2

CD: cluster of differentiation; IC: immune cells; PD-L1: programmed death-ligand 1; SLR: spleen-to-liver ratio; TC: tumor cells; TILs: tumor-infiltrating lymphocytes

Tumor immune infiltrate according to SLR_{max} status (cutoff ≤0.92 vs >0.92). The dots indicate the percentage of patients with a high density of a given cell type. Significant differences between both groups are indicated with an asterisk (*).

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