L1 cell adhesion molecule (L1CAM) in stage IB cervical cancer: distinct expression in squamous cell carcinomas and adenocarcinomas

Joao Paulo Mancusi de Carvalho,[1] Rafael C Salim,[2] Filomena Marino Carvalho ,[2] Maria Luiza Nogueira Dias Genta,[1] Edmund Chada Baracat,[1] Jesus Paula Carvalho[1]

[1]Gynecology, Universidade de Sao Paulo, Sao Paulo, São Paulo, Brazil
[2]Pathology, Universidade de Sao Paulo, Sao Paulo, São Paulo, Brazil

Correspondence to
Professor Filomena Marino Carvalho, Pathology, Universidade de Sao Paulo, Sao Paulo, São Paulo, Brazil; filomena@usp.br

Received 10 February 2020
Revised 22 March 2020
Accepted 4 April 2020
Published Online First 4 May 2020

ABSTRACT

Aims L1 cell adhesion molecule (L1CAM) has been shown to be correlated with tumour progression, attributed to its possible association with epithelial-mesenchymal transition (EMT), characterised by the expression of vimentin and loss of e-cadherin. Herein, we investigate the associations between L1CAM and clinicopathological parameters, as well as the expression of vimentin and e-cadherin, in carcinomas restricted to the cervix.

Methods The study was retrospective observational and included 45 squamous cell carcinomas (63.4%) and 26 adenocarcinomas (36.6%) submitted to primary surgical treatment. Patient age, FIGO (International Federation of Gynecology and Obstetrics) stage, tumour size and follow-up were obtained from the medical records. All the slides were revised to evaluate histological differentiation, lymphovascular space invasion, depth of infiltration, disease-free cervical wall thickness, pattern of invasion front, Silva pattern (for adenocarcinomas) and the percentage of tumour-infiltrating lymphocytes. Tissue microarrays were constructed for immunohistochemical staining for L1CAM, e-cadherin and vimentin.

Results Adenocarcinomas were associated with lower disease-free and overall survival. L1CAM and vimentin expressions were more frequent among adenocarcinomas, although loss of e-cadherin expression was more common among squamous carcinomas. L1CAM expression was associated with larger tumours, vimentin expression and lower disease-free survival. No association was observed between the expression of either L1CAM or vimentin and loss of e-cadherin. High levels of tumour-infiltrating lymphocytes were more frequent in squamous cell carcinoma, high-grade tumours, destructive pattern at front of invasion and loss of e-cadherin expression.

Conclusions Our results confirm the prognostic role of L1CAM in cervical carcinomas, but suggest a role for mechanisms other than EMT.

To cite: Mancusi de Carvalho JP, Salim RC, Carvalho FM, *et al*. *J Clin Pathol* 2020;**73**:748–753.

INTRODUCTION

Cervical cancer remains the most prevalent gynaecological tumour in the world. According to Globocan data, the age-standardised rate was estimated as 13.1 per 100 000 in 2018; the number of deaths was estimated as 6.9 per 100 000.[1] The incidence and mortality of cervical cancer are directly associated with the Human Development Index (HDI). The cumulative risk of mortality in low-HDI countries is 2.68%, while the cumulative risk of mortality in countries with very high HDI is 0.32%.[1] In Brazil, in 2018/2019, the National Institute of Cancer (INCA - Instituto Nacional de Cancer) estimated 16 370 new cases of cervical cancer each year, representing an estimated risk of 15.43 per 100 000 women.[2]

Cervical cancer is clearly related to persistent human papillomavirus (HPV) infection, although the association varies according to histological type, with squamous cell carcinoma (SCC) being the most common subtype related to the virus. Adenocarcinomas are more heterogeneous. The two categories proposed by the International Endocervical Adenocarcinoma Criteria and Classification (IECC) system are based on morphological features linked to HPV infection.[3] Tumours are categorised as HPV-associated (HPVA) or non-HPV-associated (NHPVA). The most frequent subtype of HPVA adenocarcinoma is the usual endocervical, accounting for 80% of all adenocarcinomas.[4]

Epithelial-mesenchymal transition (EMT) is a biological process involved in embryogenesis and pathological conditions that represents one of the main modes of carcinoma invasion and metastasis. EMT is a complex mechanism by which epithelial cells lose their capacity for adhesion and obtain mesenchymal phenotypes to gain advantages in dissemination. EMT plays an important role in the progression of various tumour types, including cervical carcinoma.[5–8] In the context of cancer, EMT is characterised by decreased expression of genes involved in cellular adhesion (eg, e-cadherin) and the overexpression of mesenchymal genes (eg, vimentin).[8] Protein expression of vimentin and e-cadherin has been used as a marker for EMT and for predicting prognosis in patients with various types of tumours.[7 9 10] L1 cell adhesion molecule (L1CAM) has been shown to be a prognostic marker in various cancer types and has been suggested to play a role in EMT.[11 12]

The aim of the present study was to determine the protein expression of L1CAM and its association with the classic markers for EMT (vimentin and e-cadherin) and with clinical parameters in a cohort of patients with squamous cell and the usual endocervical adenocarcinoma FIGO (International Federation of Gynecology and Obstetrics) 2018 stage IB cervical carcinoma submitted to primary surgical treatment.

METHODS

Case selection

A total of 1445 patients diagnosed with invasive cervical carcinoma were treated at the Hospital das Clinicas HCFMUSP (Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo) (1999–2007) and Instituto do Cancer do Estado de Sao Paulo (ICESP) (2008–2017) in Brazil. Both institutions are public academic tertiary hospitals associated with Universidade de Sao Paulo. The same gynaecological oncology team performs the procedures at both institutions.

This study included 45 patients with histologically confirmed SCC (63.4%) and 26 patients with usual endocervical adenocarcinoma (36.6%). All patients had undergone primary surgical treatment and were reclassified as stage IB cervical carcinoma according to the FIGO 2018 criteria.[13] The entire cohort (71 cases) corresponded to all cases of cervical cancer in the study period (1999–2007) that met the inclusion criteria (FIGO 2018 IB and primary surgical treatment).

Clinical and follow-up data were collected from the hospitals' medical electronic records. Patients were treated with primary radical hysterectomy plus pelvic lymph node dissection. Adjuvant pelvic radiotherapy was indicated according to the criteria proposed by Sedlis *et al*.[14]

Informed patient consent was waived as the study was retrospective, with the use of data from medical records and minimum tissue from paraffin blocks, with no risk or benefit arising from the results, and with study participants guaranteed anonymity.

Pathological study

All specimens were fixed in a 10% buffered formaldehyde solution and embedded in paraffin. Samples were processed according to the recommendations of the College of American Pathologists for cancer reporting.[15] All slides stained with H&E were re-evaluated by one of the authors (RCS), a senior gynaecological pathologist who is also a member of the ICESP pathology team. Discordances were analysed by a third senior gynaecological pathologist (FMC) to reach consensus. Histological type was determined according to the 2014 WHO classification of tumours.[4] To restrict the study to HPV-related neoplasms, we included only samples of SCC and samples of usual endocervical adenocarcinoma. Information pertaining to tumour size was obtained from the pathological report and expressed in millimetres. For each sample, we analysed histological differentiation (grades G1–G3: well, moderately or poorly differentiated), depth of infiltration (mm), disease-free cervical wall thickness (mm), pattern at front of invasion (pushing, expansive, with a well-delineated infiltrating border vs infiltrative/destructive), the percentage of tumour-infiltrating lymphocytes (TILs) and lymphovascular space invasion (LVSI) (present or absent). TILs were assessed according to the recommendations of the International Immuno-Oncology Biomarkers Working Group.[16] A cut-off of 30% was used to categorise tumours with low versus high TIL levels. Silva pattern was determined for each adenocarcinoma sample.[17] The Silva system stratifies the usual adenocarcinoma into three patterns of invasion. Pattern A tumours are characterised by well-demarcated glands that frequently form clusters or groups with relative lobular architecture; these tumours lack destructive stromal invasion and LVSI. Pattern B tumours present destructive invasion (small clusters or individual tumour cells within desmoplastic stroma). Pattern C tumours are diffusely infiltrative and present a desmoplastic response.

To obtain samples for tissue microarrays (TMA), we selected two areas from the invasion front, two areas from the centre of the tumour and additional samples from any phenotypically distinct area.

Table 1 Antibodies used for immunohistochemistry

Primary antibody	Manufacturer	Clone	Dilution	Antigen retrieval time (min)	Staining pattern
Vimentin	Dako (Glostrup, Denmark)	V9	Predilute	20	Cytoplasmic
E-cadherin	Cell Marque (Rocklin, California, USA)	EP700Y	1:100	40	Membranous
L1CAM	Covance (San Diego, USA)	14.10	1:300	20	Membranous

L1CAM, L1 cell adhesion molecule.

TMA construction

One cylinder of the material (2.0 mm in diameter) was punched from each of the selected areas and mounted into recipient paraffin blocks at 2 mm intervals using a precision microarray instrument (Beecher Instruments, Silver Spring, Maryland, USA). A grid system was established such that each core had an x and y coordinate reference for sample identification. The blocks were sealed at 60°C. Sections (3 µm) from each TMA block were prepared using standard techniques and were mounted on Starfrost slides. The first histological sections cut were stained with H&E to ensure that the appropriate sections of the tumour had been sampled.

Immunohistochemistry and scoring

After sectioning, slides were dried at 60°C for 90–120 min, then submitted to pretreatment using Agilent Dako PT Link platform (Agilent Technologies, Carpinteria, California) at low pH (L1CAM, vimentin) or high pH (e-cadherin). Staining was then performed with the EnVision FLEX detection kit (Agilent Technologies). The details pertaining to the primary antibodies used, including manufacturer, clone, dilution, antigen retrieval time and cell localisation, are summarised in table 1.

Diaminobenzidine-tetrahydrochloride (Dako) was used as chromogen. Sections were counterstained with Mayer's haematoxylin.

Vimentin staining was considered positive if more than 1% of stained neoplastic cells were present in any of the TMA cores. E-cadherin staining was evaluated based on staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) and the proportion of stained cells. The proportion score was defined as follows: 0 (negative), 1 (≤10%), 2 (11%–50%), 3 (51%–80%) or 4 (>80%). The immunoreactive score was determined as intensity score × proportion score and ranged from 0 to 12, as reported previously.[9 10] For this study, we defined loss of expression as a score below 8 and positive expression as a score ≥8. L1CAM staining was considered positive if observed in ≥10% of tumour cells.

Statistical analysis

Associations between categorical parameters were analysed using the χ^2 test or Fisher's exact test. Means were compared using the Mann-Whitney U test. The Spearman rank correlation test was used to analyse two quantitative variables. Overall survival (OS) and disease-free survival (DFS) rates were estimated using the Kaplan-Meier method and compared with the log-rank test. Statistical analyses were performed using MedCalc Software

V.19.1.3 (BVBA, Ostend, Belgium). P values less than 0.05 were considered significant.

RESULTS

For this study, we obtained 71 cases: 45 (63.4%) SCC and 26 (36.6%) adenocarcinomas. The duration of follow-up ranged from 6.2 to 210.5 months (mean 79.2; 95% CI 68.4 to 89.9), with a median of 66.2 months. Ten (14.08%) patients recurred and six (8.45%) died of the disease. The FIGO 2018 stage was distributed as follows: IB1, 28 (39.4%); IB2, 30 (42.3%); and IB3, 13 (18.3%). Tumour size correlated with the depth of infiltration (r=0.351, p=0.003). Patients with adenocarcinoma tended to be younger (median 47 vs 54 years), but the difference was not significant. A comparison of the clinicopathological characteristics in both groups is presented in table 2.

Adenocarcinomas were associated with lower DFS and OS (figure 1A,B). No adenocarcinoma was of high grade. Although tumour size and FIGO stage tended to be greater for adenocarcinoma than for SCC, depth of wall invasion (depth of infiltration and disease-free cervical wall thickness) was smaller in adenocarcinoma, compared with SCC. LVSI was more frequent in SCC (44.4% vs 23.1%).

Adenocarcinomas were more likely than SCC to be L1CAM-positive (50% vs 15.6%, p=0.002) and vimentin-positive (53.8% vs 28.9%, p=0.038). However, loss of e-cadherin expression was more common in SCC, compared with adenocarcinoma (31.1% vs 7.7%, p=0.037). Immune reaction in the tumour stroma, as determined by the proportion of TILs, was lower in adenocarcinoma than in SCC (38.5% vs 71.1%, p=0.007).

L1CAM was associated with vimentin expression, but not with loss of e-cadherin expression. In this series, loss of e-cadherin expression was not associated with vimentin expression. Sixteen tumours presented with loss of e-cadherin expression: 7 (25.9%) were vimentin-positive and 9 (20.5%) were vimentin-negative. Only 2 (7.7%) cases of adenocarcinomas showed a loss of e-cadherin expression. Fourteen (31.1%) cases of SCC showed loss of e-cadherin expression; among these, 5 (35.7%) had vimentin expression. Neither vimentin expression nor loss of cadherin was associated with survival or recurrence.

L1CAM-positive tumours were associated with worse DFS but not with significant change in OS (figure 1C,D). The mean DFS interval among L1CAM-positive and L1CAM-negative tumours was, respectively, 137.1±17.4 months (95% CI 102.9 to 171.3) and 184.8±12.6 months (95% CI 160.1 to 209.5) (p=0.032). OS interval was 150.6±16.7 (95% CI 117.9 to 183.4) and 196.5±8.0 (95% CI 180.8 to 212.2) in L1CAM-positive and L1CAM-negative groups, respectively (p=0.22). L1CAM-positive tumours tended to be bigger: 40% of L1CAM-positive tumours were FIGO stage IB3, while only 9.8% of negative tumours were FIGO stage IB3 (p=0.005). No association was found with destructive pattern of invasion, infiltration depth, disease-free cervical wall thickness or LVSI (except in the adenocarcinoma subgroup). L1CAM expression did not correlate with TIL level.

The clinicopathological features of the patients included in the study, stratified by L1CAM expression, are presented in table 3. The influence of tumour size, although not significant, tended to be more important in the group of adenocarcinomas (FIGO 2018 IB3 46.2% vs 15.4%; median tumour size, 32 mm vs 28 mm). No other variable differed significantly between L1CAM-positive and L1CAM-negative SCC or adenocarcinomas.

Loss of e-cadherin expression was associated with high TILs (table 4). Tumours with high TIL levels were more frequently poorly differentiated, with destructive pattern of infiltration. The characteristics of tumours according to TILs are summarised in table 4.

Multivariate analysis was not performed due to the small number of events (6 deaths and 10 recurrences).

Table 2 Clinicopathological characteristics of squamous cell carcinoma and HPV-associated adenocarcinoma in 71 cases of FIGO 2018 stage IB disease

	Squamous cell carcinoma	Adenocarcinoma	P value
Number of patients	45 (63.4%)	26 (36.6%)	
Age (years)			
Range (median; 95% CI)	28–70 (54; 48.9 to 58.5)	29–77 (47; 42.5 to 56.5)	NS
FIGO 2018 stage			
IB1	20 (44.4%)	8 (30.8%)	NS
IB2	20 (44.4%)	10 (38.5%)	
IB3	5 (11.1%)	8 (30.8%)	
Tumour size (mm)			
Range (median; 95% CI)	5–70 (22.0; 16.5 to 28)	8.5–55.0 (29; 20 to 38.8)	NS
Depth of infiltration (mm)			
Range (median; 95% CI)	4–20 (9; 7.6 to 11)	1–18 (7.9; 5.4 to 9.7)	0.077
Disease-free cervical wall thickness			
Range (median; 95% CI)	1–20 (4.5; 3 to 8)	1–14 (7; 5 to 8.5)	NS
Histological grade			
1	1 (2.2%)	10 (38.5%)	<0.0001
2	28 (62.2%)	16 (61.5%)	
3	16 (35.6%)	0	
Pattern at front of invasion			
Pushing, expansive	3 (6.8%)	5 (19.2%)	NS
Infiltrative, destructive	41 (93.2%)	21 (80.8%)	
Tumour-infiltrating lymphocytes			
<30%	13 (28.9%)	16 (61.5%)	0.007
≥30%	32 (71.1%)	10 (38.5%)	
Lymphovascular space invasion			
Yes	20 (44.4%)	6 (23.1%)	0.074
No	25 (55.6%)	20 (76.9%)	
L1CAM			
Positive	7 (15.6%)	13 (50%)	
Negative	38 (84.4%)	13 (50%)	
Vimentin			
Positive	13 (28.9%)	14 (53.8%)	0.038
Negative	32 (71.1%)	12 (46.2%)	
Loss of e-cadherin			
Yes	14 (31.1%)	2 (7.7%)	0.024
No	31 (68.9%)	24 (92.3%)	
Number of recurrence	4 (8.9%)	6 (23.1%)	NS
Number of deaths	1 (2.2%)	5 (19.2%)	0.022
DFS interval (months)			
Mean±SD (95% CI)	191.2±11.7 (168.4 to 214.1)	136.6±15.7 (105.8 to 167.5)	0.025
OS interval (months)			
Mean±SD (95% CI)	206.3±4.16 (198.1 to 214.4)	145.2±15.5 (114.8 to 175.6)	0.015

DFS, disease-free survival; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; L1CAM, L1 cell adhesion molecule; OS, overall survival.

DISCUSSION

L1CAM is a 200–220 kDa transmembrane adhesion glycoprotein with intracellular and extracellular domains that interact with both cytoskeletal proteins and other molecules in the microenvironment, with numerous effects on cancer progression.[18]

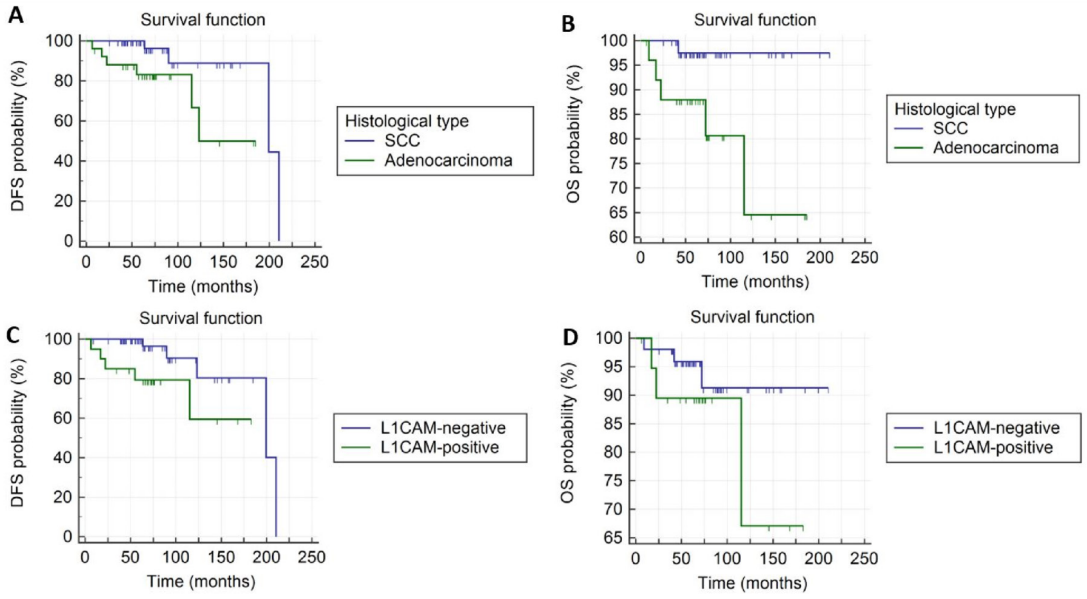

Figure 1 Survival curves for 71 patients with FIGO (International Federation of Gynecology and Obstetrics) 2018 stage IB cervical carcinoma. (A) DFS according to histological type (log-rank test, p=0.025). (B) OS according to histological type (log-rank test, p=0.015). (C) DFS according to L1CAM expression (log-rank test, p=0.031). (D) OS according to L1CAM expression (log-rank test, p=0.22). DFS, disease-free survival; L1CAM, L1 cell adhesion molecule; OS, overall survival SCC, squamous cell carcinoma.

Table 3 L1CAM expression and clinicopathological characteristics of 71 cases of FIGO 2018 stage IB cervical carcinoma

	Total			SCC			Adenocarcinomas		
	L1CAM+	L1CAM−	P value	L1CAM+	L1CAM−	P value	L1CAM+	L1CAM−	P value
n	20 (28.2%)	51 (71.8%)		7 (15.6%)	38 (84.4%)		13 (50%)	13 (50%)	
Age (years)									
Range (median)	29–77 (44.5)	28–71 (54)	NS	32–69 (50.57±15.35) (47)	28–70 (53.37±12.16) (53)	NS	29–77 (46.31±14.40) (43)	36–71 (51.08±10.87) (52)	NS
FIGO 2018 stage									
IB1	8 (40%)	20 (39.2%)	0.005	3 (42.5%)	17 (44.7%)	NS	5 (38.5%)	3 (23.1%)	0.047
IB2	4 (20%)	26 (51%)		2 (28.6%)	18 (47.4%)		2 (15.4%)	8 (61.5%)	
IB3	8 (40%)	5 (9.8%)		2 (28.6%)	3 (7.9%)		6 (46.2%)	2 (15.4%)	
Histological grade									
1	5 (25%)	6 (11.8%)	NS	0 (0%)	1 (2.6%)	NS	5 (50%)	5 (50%)	NS
2	12 (60%)	32 (62.7%)		4 (57.1%)	24 (63.2%)		8 (50%	8 (50%)	
3	3 (15%)	13 (25.5%)		3 (42.9%)	13 (34.2%)				
Tumour size (mm)									
Range (median)	12–60 (27.5)	5–70 (24.5)	0.06	12–60 (29.64±16.96) (25)	5–70 (23.68±13.46) (22)	NS	19–55 (33.46±13.73) (32)	8.5–42 (27±10.70) (28)	NS
Depth of infiltration (mm)									
Range (median)	3.9–17 (8)	1–20 (9)	NS	5–12 (8)	4–20 (10)	NS	3.9–17 (8)	1–18 (5.7)	NS
Disease-free cervical wall thickness (mm)									
Range (median)	1–20 (5)	1–16 (7)	NS	1–20 (6)	1–16 (4.5)	NS	1–14 (5)	1–14 (8)	NS
Pattern at front of invasion									
Pushing, expansive	1 (5.3%)	7 (13.7%)	NS	0 (0%)	3 (7.9%)	NS	1 (7.7%)	4 (30.8%)	NS
Infiltrative, destructive	18 (94.7%)	44 (86.3%)		6 (100%)	35 (92.1%)		12 (92.3%)	9 (69.2%)	
LVSI									
Yes	9 (45%)	17 (33.3%)	NS	3 (42.9%)	17 (44.7%)	NS	6 (46.2%)	0 (0%)	0.015
No	11 (55%)	34 (66.7%)		4 (57.1%)	21 (55.3%)		7 (53.8%)	13 (100%)	
Vimentin									
Positive	13 (65%)	14 (27.5%)	0.004	4 (57.1%)	9 (23.7%)	0.168	9 (69.2%)	5 (38.5%)	NS
Negative	7 (35%)	37 (72.5%)		3 (42.9%)	29 (76.3%)		4 (30.8%)	8 (61.5%)	
Loss of e-cadherin									
Yes	4 (20%)	12 (23.5%)	NS	3 (42.9%)	11 (28.9%)	0.659	1 (7.7%)	1 (7.7%)	NS
No	16 (80%)	39 (76.5%)		4 (57.1%)	27 (71.1%)		12 (92.3%)	12 (92.3%)	

FIGO, International Federation of Gynecology and Obstetrics; L1CAM, L1 cell adhesion molecule; LVSI, lymphovascular space invasion; SCC, squamous cell carcinoma.

Table 4 Clinicopathological characteristics of 71 cases of FIGO 2018 stage IB cervical cancer according to TIL level

	High TILs (≥30%)	Low TILs (<30)	P value
n	42	29	
Age (years)			
Range (median)	28–69 (53)	30–77 (52)	NS
FIGO 2018 stage			
IB1	18 (42.9%)	10 (34.5%)	NS
IB2	15 (35.7%)	15 (51.7%)	
IB3	9 (21.4%)	4 (13.8%)	
Grade			
1	4 (9.5%)	7 (24.1%)	0.017
2	25 (59.5%)	19 (65.5%)	
3	13 (31%)	3 (10.3%)	
Tumour size (mm)			
Range (median)	5–70 (25)	8.5–47 (24)	NS
Depth of infiltration (mm)			
Range (median)	4–20 (9)	1–18 (8)	NS
Disease-free cervical wall thickness (mm)			
Range (median)	1–20 (5.5)	1–11 (7)	NS
Pattern at front of invasion			
Pushing, expansive	2 (4.8%)	6 (21.4%)	0.033
Infiltrative, destructive	40 (95.2%)	22 (78.6%)	
LVSI			
Yes	15 (35.7%)	11 (37.9%)	NS
No	27 (64.3%)	18 (62.1%)	
L1CAM			
Positive	10 (23.8%)	10 (34.5%)	NS
Negative	32 (76.2%)	19 (65.5%)	
Vimentin			
Positive	14 (33.3%)	13 (44.8%)	NS
Negative	28 (66.7%)	16 (44.8%)	
Loss of e-cadherin			
Yes	13 (31%)	3 (10.3%)	0.042
No	29 (69%)	26 (89.7%)	
Number of recurrence	5 (11.9%)	5 (17.2%)	NS
Number of deaths	5 (11.9%)	1 (3.4%)	NS
DFS interval (months)			
Mean±SD (95% CI)	184.2±14.4 (155.9 to 212.5)	165.7±16.9 (132.5 to 198.8)	NS
OS interval (months)			
Mean±SD (95% CI)	179.1±13.5 (152.7 to 205.5)	193.4±6.0 (181.7 to 205.2)	NS

DFS, disease-free survival; FIGO, International Federation of Gynecology and Obstetrics; L1CAM, L1 cell adhesion molecule; LVSI, lymphovascular space invasion; OS, overall survival; TILs, tumour-infiltrating lymphocytes.

L1CAM expression has been related to the worsening of prognosis and treatment resistance in various tumours. These effects are thought to reflect the association of L1CAM expression with EMT.[11 12 19–22]

Through its intracellular domain, L1CAM inhibits the formation of cadherin-based adherens junctions and promotes the motility of epithelial cells, acting as a trigger of EMT.[23] This function results in the inverse relationship between L1CAM expression and e-cadherin expression observed in various tumours.[24 25]

In our series, which included FIGO 2018 stage IB samples of early cervical cancer, we did not find any association between L1CAM and e-cadherin. Only vimentin expression was related to L1CAM expression. E-cadherin is an epithelial marker of adhesion and reflects the intracellular environment. Vimentin is a mesenchymal marker of mobility. There is therefore no expectation of a direct or indirect association between e-cadherin and vimentin expression levels.[8 9] Different combinations of these molecules reflect various patterns of invasion and prognosis. In cervical cancer, the downregulation of e-cadherin is associated with poor prognosis.[9 26] In a meta-analysis conducted by Peng *et al*,[26] the loss of e-cadherin expression was associated with clinical stage, deep stromal invasion, lymph node involvement and distant metastasis. None of these conditions was present in our series, which included only early cervical cancer, restricted to cervix. In this study, L1CAM expression was more frequent among adenocarcinomas, the subgroup with worse prognosis, increased tumour size and lower DFS.

The role of L1CAM as a prognostic and predictive factor in cancer goes far beyond its involvement with EMT activation. Versluis *et al*[27] presented 90 cases of uterine carcinosarcoma, a model for studying EMT. The authors found L1CAM expression only in the epithelial component and reported no association between L1CAM expression and prognosis.[27] The L1CAM molecule has multiple cleavage sites. Biological effects are mediated by the full-length form as well as the cleavage products.[18] L1CAM exerts intracellular and extracellular effects on various molecular pathways. Although the overexpression of L1CAM in cancer is usually associated with aggressive biology, a better understanding of the pathways involved could be useful for the development of targeted therapies.

One special characteristic of our series is the selection of histological types associated with HPV. Although HPV infection is a necessary condition for the initiation of cervical cancer, other events are required for progression. Various HPV types are activated via distinct molecular pathways.[28 29] Of cervical cancers, 92% are HPV active, that is, they express HPV oncogenes E6 and E7, suggesting that HPV is necessary to maintain cellular proliferation.[29] However, some tumours, although initiated by HPV infection, become HPV independent and express different driver genes. These tumours occur in older women, are more aggressive and are more frequent in adenocarcinomas than in SCC (18% vs 4.7%).[29] Recently, the IECC proposed the classification of adenocarcinomas into HPVA and NHPVA types.[3] The major HPVA subtypes are the usual type, including villoglandular and micropapillary variants, and the mucinous type.[3] In this way, we selected tumours almost sure to be related to HPV. The subgroup of adenocarcinomas in our series presented worse DFS and OS, compared with SCC. Although the expression of L1CAM and vimentin was higher in adenocarcinomas than in SCC, high TILs and loss of e-cadherin expression were less common. These findings suggest that, in spite of both being associated with HPV, adenocarcinomas and SCC are biologically and molecularly distinct. In our opinion, adenocarcinomas arising in young women, candidate for conservative approach, require caution, even in early stage, until we have more robust biological indicators for behaviour.

The lack of an inverse relationship between L1CAM/vimentin expression and loss of e-cadherin expression leads us to question the role of EMT in early cervical cancer. Not only this finding but other results were intriguing as well. For example, the presence of high levels of TILs was associated with poor differentiation, destructive pattern of stroma infiltration and loss of e-cadherin expression. Between-group differences in DFS and OS did not reach the level of significance, but our comparison of tumours with high versus low TIL counts indicated lower OS in the former. Our series, although very homogeneous in terms of patient age, tumour stage and HPV infection, failed to clarify the roles of several other important factors, such as TIL type. In a study with samples from patients with pancreatic ductal adenocarcinoma, regulatory T cells (T-reg) enrichment in the microenvironment was associated with poor outcome.[30] T-regs mediate tumour immune escape. In a study by Grage-Griebenow *et al*,[30]

L1CAM was suggested to contribute to immune evasion. Other aspects of the immune reaction merit investigation, such as immune checkpoint molecules, tumour mutational burden and cytotoxic lymphocytes. It is quite possible that L1CAM modulates the quality of the immune response.

This study has several strengths. It included an unselected cohort of patients, all at the same FIGO stage, who were treated at a single cancer centre, by a single surgical and pathological team, the members of which followed rigid guidelines. This study not only explored the expression of L1CAM in endocervical adenocarcinoma, an original approach, but also compared expression levels with those observed in SCC. The roles of L1CAM, EMT and the microenvironment require additional research for elucidation.

The small number of cases is a weakness of this study, but the series included only tumours restricted to the cervix, without lymph node involvement, corresponding to a poorly studied, homogeneous group for which robust prognostic factors are lacking. The small number of events and the varied follow-up period may influence the survival analysis. Despite the small sample size, this study yielded interesting results that will be very useful in outlining future studies.

Take home messages

- L1 cell adhesion molecule (L1CAM) expression is more frequent in adenocarcinomas and it is associated with larger tumours and lower disease-free survival.
- L1CAM is directly associated with vimentin expression, but not with loss of e-cadherin, indicating a prognostic role other than epithelial-mesenchymal transition.
- Tumour-infiltrating lymphocytes are associated with loss of e-cadherin, high-grade tumours and destructive pattern of invasion front.

Handling editor Mona El-Bahrawy.

Contributors JPMC: data curation, writing–original draft. RCS: methodology. FMC: conceptualisation, formal analysis, writing–review and editing. MLNDG: data curation, methodology. ECB: supervision. JPC: conceptualisation, writing, supervision. All authors have access to the final version of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was approved by the Scientific Committee of Faculdade de Medicina da Universidade de Sao Paulo and by the Ethics Committee for Research Projects of the Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo (Comissao de Etica para Analise de Pesquisa - CAPPesq) (1.294.094) and Plataforma Brasil (CAAE 03350012.2.0000.0065). The study complies with the ethical precepts proposed by the legislation in force in Brazil (R466/2012).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. All data relevant to the study are included in the article. More details about the patients are contained in the institution's medical records and are protected by guaranteed anonymity.

ORCID iD

Filomena Marino Carvalho http://orcid.org/0000-0002-5838-3636

REFERENCES

1 GLOBOCAN. Incidence, mortality and prevalence by cancer site, 2018. Available: http://gco.iarc.fr/today/data/factsheets/populations/76-brazil-fact-sheets.pdf
2 Estimativa. Incidência de Câncer no Brasil, 2018. Available: http://www.inca.gov.br
3 Stolnicu S, Barsan I, Hoang L, *et al*. International endocervical adenocarcinoma criteria and classification (IECC): a new pathogenetic classification for invasive adenocarcinomas of the endocervix. *Am J Surg Pathol* 2018;42:214–26.
4 Kurman RJ CM, Herrington CS, Young RH. *Who classification of tumours of female reproductive organs*. 6th edn. IARC: Lyon, 2014.
5 Jiang J, Li X, Yin X, *et al*. Association of low expression of E-cadherin and β-catenin with the progression of early stage human squamous cervical cancer. *Oncol Lett* 2019;17:5729–39.
6 Stewart CJR, Crook ML, Little L, *et al*. Correlation between invasive pattern and immunophenotypic alterations in endocervical adenocarcinoma. *Histopathology* 2011;58:720–8.
7 Chen T, You Y, Jiang H, *et al*. Epithelial-Mesenchymal transition (EMT): a biological process in the development, stem cell differentiation, and tumorigenesis. *J Cell Physiol* 2017;232:3261–72.
8 Lu W, Kang Y. Epithelial-Mesenchymal plasticity in cancer progression and metastasis. *Dev Cell* 2019;49:361–74.
9 Cheng Y, Zhou Y, Jiang W, *et al*. Significance of E-cadherin, β-catenin, and vimentin expression as postoperative prognosis indicators in cervical squamous cell carcinoma. *Hum Pathol* 2012;43:1213–20.
10 Liu L-K, Jiang X-Y, Zhou X-X, *et al*. Upregulation of vimentin and aberrant expression of E-cadherin/beta-catenin complex in oral squamous cell carcinomas: correlation with the clinicopathological features and patient outcome. *Mod Pathol* 2010;23:213–24.
11 Schrevel M, Corver WE, Vegter ME, *et al*. L1 cell adhesion molecule (L1CAM) is a strong predictor for locoregional recurrences in cervical cancer. *Oncotarget* 2017;8:87568–81.
12 Chen J, Gao F, Liu N. L1Cam promotes epithelial to mesenchymal transition and formation of cancer initiating cells in human endometrial cancer. *Exp Ther Med* 2018;15:2792–7.
13 Bhatla N, Berek JS, Cuello Fredes M, *et al*. Revised FIGO staging for carcinoma of the cervix uteri. *Int J Gynaecol Obstet* 2019;145:129–35.
14 Sedlis A, Bundy BN, Rotman MZ, *et al*. A randomized trial of pelvic radiation therapy versus no further therapy in selected patients with stage Ib carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: a gynecologic Oncology Group study. *Gynecol Oncol* 1999;73:177–83.
15 Cancer protocol templates. Available: https://www.cap.org/protocols-and-guidelines/cancer-reporting-tools/cancer-protocol-templates
16 Salgado R, Denkert C, Demaria S, *et al*. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an international TILs Working group 2014. *Ann Oncol* 2015;26:259–71.
17 Diaz De Vivar A, Roma AA, Park KJ, *et al*. Invasive endocervical adenocarcinoma: proposal for a new pattern-based classification system with significant clinical implications: a multi-institutional study. *Int J Gynecol Pathol* 2013;32:592–601.
18 Maten Mvander, Reijnen C, Pijnenborg JMA, *et al*. L1 cell adhesion molecule in cancer, a systematic review on domain-specific functions. *Int J Mol Sci* 2019;20:1–3.
19 Kiefel H, Bondong S, Hazin J, *et al*. L1Cam: a major driver for tumor cell invasion and motility. *Cell Adh Migr* 2012;6:374–84.
20 Klat J, Mladenka A, Dvorackova J, *et al*. L1Cam as a negative prognostic factor in endometrioid endometrial adenocarcinoma FIGO stage IA-IB. *Anticancer Res* 2019;39:421–4.
21 Ichikawa T, Okugawa Y, Toiyama Y, *et al*. Clinical significance and biological role of L1 cell adhesion molecule in gastric cancer. *Br J Cancer* 2019;121:1058–68.
22 Tampakis A, Tampaki EC, Nonni A, *et al*. L1Cam expression in colorectal cancer identifies a high-risk group of patients with dismal prognosis already in early-stage disease. *Acta Oncol* 2020;59:55–9.
23 Shtutman M, Levina E, Ohouo P, *et al*. Cell adhesion molecule L1 disrupts E-cadherin-containing adherens junctions and increases scattering and motility of MCF7 breast carcinoma cells. *Cancer Res* 2006;66:11370–80.
24 Huszar M, Pfeifer M, Schirmer U, *et al*. Up-Regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. *J Pathol* 2010;220:551–61.
25 Tischler V, Pfeifer M, Hausladen S, *et al*. L1Cam protein expression is associated with poor prognosis in non-small cell lung cancer. *Mol Cancer* 2011;10:127.
26 Peng J, Qi S, Wang P, *et al*. Meta-Analysis of downregulated E-cadherin as a poor prognostic biomarker for cervical cancer. *Future Oncol* 2016;12:715–26.
27 Versluis M, Plat A, de Bruyn M, *et al*. L1Cam expression in uterine carcinosarcoma is limited to the epithelial component and may be involved in epithelial-mesenchymal transition. *Virchows Arch* 2018;473:591–8.
28 Cancer Genome Atlas Research Network, Albert Einstein College of Medicine, Analytical Biological Services, *et al*. Integrated genomic and molecular characterization of cervical cancer. *Nature* 2017;543:378–84.
29 Banister CE, Liu C, Pirisi L, *et al*. Identification and characterization of HPV-independent cervical cancers. *Oncotarget* 2017;8:13375–86.
30 Grage-Griebenow E, Jerg E, Gorys A, *et al*. L1Cam promotes enrichment of immunosuppressive T cells in human pancreatic cancer correlating with malignant progression. *Mol Oncol* 2014;8:982–97.