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Angiolymphatic invasion and absence of koilocytosis predict lymph node metastasis in penile cancer patients and might justify prophylactic lymphadenectomy

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Abstract

To analyze possible clinical–pathological parameters and predictors of lymph node metastasis and evaluate the impact of lymphadenectomy in the survival of these patients.

A retrospective study of patients diagnosed with penile cancer and submitted to regional lymphadenectomy at two reference hospitals in Maranhão, Northeast, Brazil, an area where the disease has a high incidence. We described here clinical and histopathological characteristics of patients diagnosed between January 2009 and September 2017.

Fifty-five patients with an average age of 55.4 years (range: 25–84 years) were analyzed, with 24.4 months being the average time between the onset of symptoms and start of treatment. Among patients without palpable lymph nodes at the first examination, 51% were affected by inguinal metastasis. In the multivariate analysis, the presence of angiolymphatic invasion ($P = .029$) and absence of koilocytosis ($P = .001$) were found to be predictive factors for lymph node metastasis. Patients submitted to prophylactic lymphadenectomy presented with a disease-free period of 25.4 months (±5.81), whereas those who underwent therapeutic lymphadenectomy presented with a disease-free period of 19.9 months (±3.12).

Angiolymphatic invasion and absence of koilocytosis appeared to be predictive factors for lymph node metastasis. Therefore, the submission of patients with metastatic risk to prophylactic lymphadenectomy may improve their survival. Thus, prophylactic lymphadenectomy in patients at risk for inguinal metastasis may create a positive impact in survival rates.

Abbreviations: AJCC = American Joint Committee on Cancer, EAU = European Association of Urology (EAU), HPV = Human Papilomavírus, PC = Penile cancer.

Keywords: lymphadenectomy, metastasis, penile cancer, predictor, survival

1. Introduction

Penile cancer (PC) is diagnosed at different rates worldwide, and affects men with a frequency of 1/100,000 in the United States and 0.6/100,000 in the United Kingdom. In developing countries, Brazil presents high rates of PC, with a distribution of 2.9 to 6.8/100,000 inhabitants.[1–3] Squamous cell carcinoma (SCC) is the most common histological type of PC, accounting for 95% of all cases. Despite the etiology being unclear, some risk factors such as phimosis history, human papillomavirus (HPV) infection, tobacco, low socioeconomic conditions that culminate in precarious hygiene habits, and repeated penial excoriations can be highlighted.[3,4]

Clinically, the disease has a slow onset, presenting superficial injuries that deepen into the penile tissue and invade the chorion, spongy tissue and corpora cavernosa. Lymph node involvement is the most important prognostic factor, given that the main route of tumor cells dissemination is the lymphatic vessel. However, there are still no parameters that can safely predict the lymph node status and ganglionic involvement.[5]

The presence of palpable lymph nodes in the clinical evaluation of patients varies between 20% and 96%. However, the presence of lymph node hyperplasia and infections is very common, and can mislead the lymph node evaluation and clinical diagnosis of

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metastasis in these sites.[5,6] Thus, 20% of clinically negative lymph nodes present micro-metastasis in anatomopathological diagnosis, and 50% of clinically positive lymph nodes present no metastasis diagnosis in histological analysis.[7] Thus, a major controversy reported in the literature is related to the best time to approach the inguinal lymph nodes in PC. This type of procedure has often been performed prophylactically despite being associated with high morbidity of patients.[8]

In regions of low socioeconomic status such as the state of Maranhão, prophylactic lymphadenectomy is justified by the fact that many patients, who live in rural zones distant from the reference hospitals settings often abandon treatment.[3] Based on these data, this study sought to analyze the clinicopathological parameters predictive of lymph node metastasis and to evaluate the impact of lymphadenectomy on the survival of these patients.

2. Materials and methods

We used data from patients diagnosed with PC who were treated at the Aldenora Bello Maranhense Institute of Oncology and Presidente Dutra University Hospital in the city of São Luís, Maranhão, Brazil, between January 2009 and September 2017 and who were submitted to lymphadenectomy. Patients who presented incomplete data regarding the lymphadenectomy procedure and histological characteristics were not included in the study. After patient selection was completed the anatomopathological material was submitted to a review by a uropathologist (GEBS).

2.1. Determination of variables

We considered age, lesion topography, the presence of infected penile lesion, palpable lymph nodes, time elapsed since lesion onset and health treatment, the type of lymphadenectomy performed (prophylactic or therapeutic), and the presence of lymph node metastasis after histological evaluation. Importantly, during the gross analysis of the lymphadenectomy material, all lymph nodes found were included in the analysis.

For the histological determination of subtypes, we adopted the World Health Organization (WHO) classification. Histological grading was determined by using the Broder classification according to the Royal College of Pathologists, and tumors were restaged according to the staging agreement of the 8th edition of the American Joint Committee on Cancer (AJCC), Cancer Staging Manual.[9,10]

Selection criteria for patients submitted to prophylactic or therapeutic lymphadenectomy followed the European Association of Urology (EAU) guidelines.[11] According to the EAU guidelines, patients with low-risk tumors (pTis, pTa G1, pT1a, and G1) should be under surveillance, and those with staged tumors in ≥pT1b and/or ≥G2 without palpable lymph nodes should undergo prophylactic lymphadenectomy. In addition, patient's whit palpable lymph nodes underwent therapeutic lymphadenectomy, and those who presented clinical signs of infection at physical examination underwent antibiotic therapy prior to lymphadenectomy. It is worth mentioning that in the hospitals in which this study was conducted, therapeutic procedures are not part of the institutional protocols. Patient's approach to prophylactic or therapeutic lymphadenectomy was defined in accordance with the individual recommendation of each urologist.

2.2. HPV histological identification

Histological diagnosis of HPV was conducted according to the three mandatory criteria for the presence of koilocytosis, namely,

1. perinuclear halo,
2. nuclear atypia; and
3. binucleation.

2.3. Follow-up evaluation

To determine the follow-up, patients who underwent lymphadenectomy were divided into two groups: those with and those without metastasis. After the final stage of follow-up, patients were classified into those alive without cancer and those alive with cancer, those who had cancer-related deaths, and those who missed follow-up.

2.4. Statistical analysis

Data analysis was performed using the SPSS 23.0 program (SPSS Inc., Chicago, IL). Association between the presence or absence of lymph node metastasis and clinical and histopathological data was determined using the chi-square test. The survival analysis was performed using the Kaplan–Meier method to determine the disease-free survival and its relation to early or late lymphadenectomy. The log-rank test was used to compare the survival curves. For all tests performed, we considered $P \leq .05$ as statistically significant.

2.5. Ethical aspects

This research follows the precepts and norms of the Brazilian National Health Council, Resolution 510/16, and is approved by the COMIC-HUUFMA scientific commission with the approval report number 1.093.435 and CAAE: 43774315.7.0000.5086.

3. Results

From January 2009 to September 2017, 55 patients who underwent lymphadenectomy and met the selection criteria were evaluated. Patients' ages ranged from 25 to 84 years, with an average of 55.4 years. The site that was the most affected by lesions was the glans (55%), and partial penectomy was the most common type of surgery performed (65%).

The tumor was staged in 51% of the cases between pT3 and pT4, and 62% od the cases were histological grades 1 to 2. Regarding tumor size, 51% presented lesion dimensions between 2.1 and 5.0 cm, and perineural and angiovascular invasions were absent in most cases, with percentages of 60% and 71%, respectively.

A considerable number of patients (40%) waited for >12 months after the symptom onset to seek their first examination. Most patients (53%) had no palpable lymph nodes at the initial examination, 67% underwent prophylactic lymphadenectomy and 58% had lymph node metastasis in the histological examination. Full description of data is presented in Table 1.

Table 2 presents possible factors considered as predisposing or not for the presence of inguinal metastasis. The absence of angiolymphatic invasion and presence of koilocytosis showed significant values of $P = .0037$ and $P = .001$, respectively, and were associated with the absence of lymph node metastasis.

Table 1

Clinical-pathological characteristics and lymph node status of the 55 PC patients who underwent lymphadenectomy.

Characteristics	Variables	N	%
Tumor subtype	Usual	22	40.0
	Warty	11	20.0
	Basaloid	4	7.3
	Mixed**	9	16.4
	Verrucous	1	1.8
	Warty-Basaloid	3	5.4
	NI*	5	9.1
	Glans	30	55
Topography	Glans and prepuce	6	11
	Glans, prepuce, and penile body	6	11
	Glans and penile body	2	4
	Prepuce and penile body	6	11
	NI*	5	8
Type of surgery	Partial penectomy	36	65
	Total penectomy	17	31
	NI*	2	4
Tumor/pathologic stage	pT1b–pT2	26	47
	pT3–pT4	29	53
Histological Grade	1–2	34	62
	3	21	38
Perineural invasion	Present	22	40
	Absent	33	60
Angiolymphatic invasion	Present	16	29
	Absent	39	71
Palpable lymph nodes at the first examination	Present	26	47
	Absent	29	53
Koilocytosis	Present	34	62
	Absent	17	31
	NI*	4	7
Lymphadenectomy	Prophylactic	32	58
	Therapeutic	23	42
Metastasis	Present	28	51
	Absent	27	49
Symptoms onset and first examination (elapsed time).	≤11 months	14	22
	≥12 months	22	40
	Could not be referred	19	38
Size of the tumor	2.1–5.0	28	51
	≥5.1 cm	24	44
	NI*	3	5

N = absolute frequency, % = relative frequency, *NI = not informed.
** Mixed: usual + warty or usual + basaloid

In the multivariate model with variables selected and described in Table 3, the presence of angiolymphatic invasion ($P = .029$) and absence of koilocytosis ($P = .001$) were found to be predictive factors for lymph node metastasis. Patients who were affected by angiolymphatic invasion had a seven-fold increased risk of developing metastasis compared to other variables.

Survival analysis using the long-rank test showed that patients who underwent early lymphadenectomy presented a disease-free survival period of 25.4 months (±5.81), while those who underwent late lymphadenectomy presented a disease-free survival period of 19.9 months (±3.18), although the difference was not significant ($P = .81$) (see Fig. 1).

4. Discussion

The epidemiological profile the patients in this study was formed by men who worked as farmers, had up to a primary level of education, and who took longer to seek health treatment. Data described are in accordance with literature findings regarding these socioeconomic conditions.[12–14] Most patients presented with advanced lesions and underwent partial penectomy. Some studies have shown that delayed onset of treatment implies advanced local disease and that, consequently, partial or total amputations are required.[15,16] Due to the patients' socioeconomic profiles in our study, they tended to not receive adequate monitoring, and the surgeons opted for a prophylactic lymphadenectomy. Unfortunately, currently, there is no reliable parameter as a risk marker for lymph node metastasis in PC, and the impact of prophylactic lymphadenectomy on survival is of patients with PC is not well established.

In our multivariate analysis, the presence of angiolymphatic invasion and absence of koilocytosis were shown to be the only predictors with significance for inguinal metastasis. Koilocytosis is demonstrated as a possible predictor for low metastatic risk (OR = 0.088). In the literature, angiovascular invasion was more commonly studied than koilocytosis as a factor associated with lymph node metastasis in PC.[17–19]

Table 2

Association between clinical and histological variables with presence or absence of lymph node metastasis.

Characteristics	LN+	LN−	P*
Tumor subtype			
HPV-related	14	10	.15
Non-HPV-related	9	17	
Tumor size			
2.1–5.0−cm	15	13	.15
≥ 5.1cm	10	14	
Histological grade			
I–II	17	17	1.0
III	11	10	
Tumor/pathologic stage			
pT1–pT2	11	15	.174
pT3–pT4	17	12	
Perineural invasion			
Presence	10	12	.58
Absence	18	15	
Angiolymphatic invasion			
Presence	12	4	.0037
Absence	16	23	
Lymphadenectomy			
Therapeutic	11	12	.78
Prophylactic	17	15	
Koilocytosis			
Presence	11	23	.001
Absence	14	03	

* Degree of significance of the associations performed by Chi-square test.

Table 3

Logistic regression between lymph node metastasis (dependent variable) and histopathological variables.

Variable	P	OR	95% CI*
Histological grade	.288	2.333	0.101–2.232
Palpable lymph nodes	.888	1.106	0.023–0.821
Angiolymphatic invasion	.029	7.224	0.831–22.730
Perineural invasion	.099	0.24	0.126–2.488
Tumor/pathologic stage	.649	1.389	0.124–2.017
Koilocytosis	.001	0.088	2.628–50.718

* CI = confidence interval, OR = odds ratio.

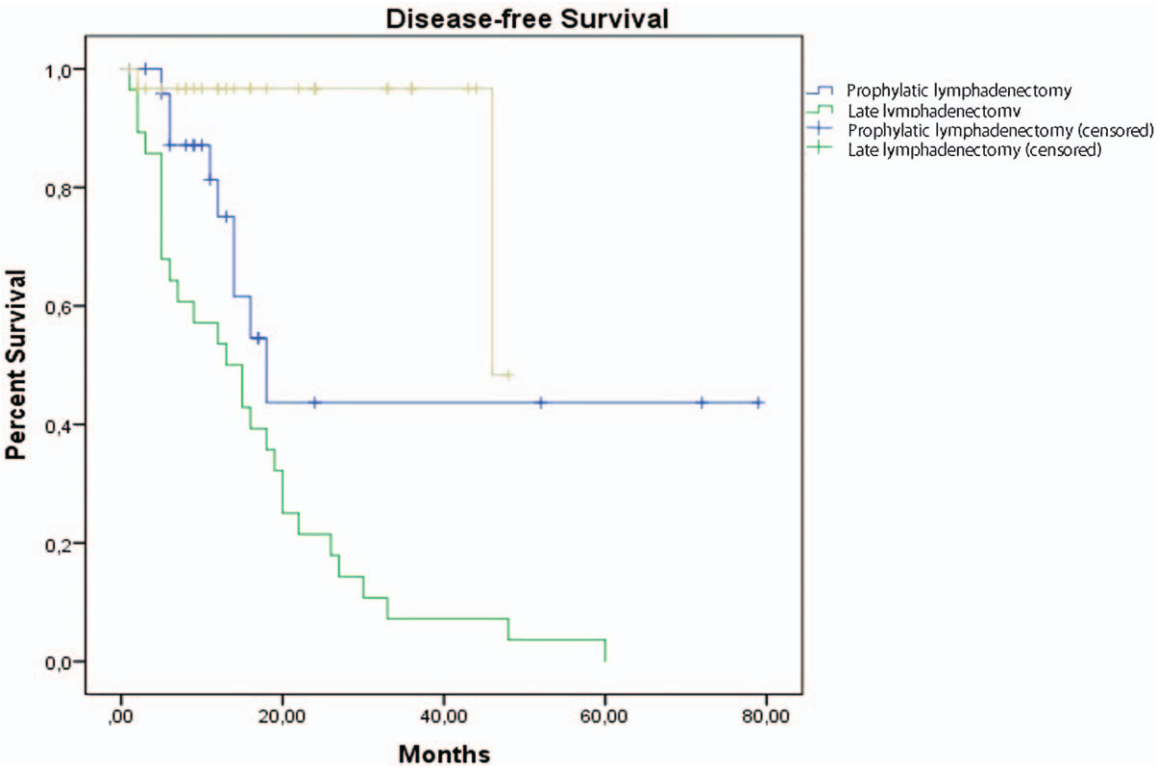

Figure 1. Survival analysis using Long-Rank test.

The literature diverges when discussing angiolymphatic embolization as a predictive factor for metastasis. Some studies have mentioned this variable as a major factor and predictor for metastasis[20,21] whereas others have described that angiolymphatic invasion is not characterized as a factor for lymph node metastasis.[19] Perineural invasion is also considered as a predictor for metastasis in many studies.[22,23] However, no significant relationship was found in our study.

Although the presence of HPV infection in tumors of the cervix, head, and neck has been studied exhaustively over the last years, only a few studies regarding the prognostic influence of HPV infection in PC exist. Some studies have described the presence of HPV infection in head and neck cancers as a factor related to better survival.[24] A study on PC conducted by Bezerra et al found no prognostic correlation between the presence of HPV infection and this neoplasia.[25] However, our research group observed that the presence of HPV infection was associated with a greater disease-free survival.[26]

Koilocytosis still is an understudied variable, and only one study has demonstrated the relationship between the presence of angiolymphatic embolization and absence of koilocytosis as prognostic factor for metastatic risk.[17] De Paula et al[19] found the absence of koilocytosis as an independent factor for the metastatic risk in their study. Nevertheless, this factor requires more studies to establish the role of koilocytosis as a predictor for lymph node metastasis.

New management strategies for lymph node involvement in PC seek to conduct an appropriate risk stratification of patients, are useful for therapeutic planning and optimize oncological outcomes through the evaluation of patients' prognostic factors.[6] As for the clinical examination of lymph nodes, the literature mentions that only 20% of non-palpable lymph nodes present as metastasis.[8] Contrary to the literature, our study showed that 51% of patients without palpable lymph nodes on admission were affected by lymph node metastasis, demonstrating a false-negative rate that is higher than that in previous findings. Such findings may be related to patients' delays in seeking health treatment, a factor which can later influence the clinical decisions of patients N0.

Some authors have recommended prophylactic lymphadenectomy if the patient is in a risk group for metastasis.[7] Li et al[27] have also described benefits for patients undergoing prophylactic lymphadenectomy. Some PC referral services adopt this approach based on studies that reviewed patients who were submitted to surveillance and later to therapeutic lymphadenectomy. Findings have demonstrated that several of these patients could no longer undergo rescue surgery due to critically advanced and unresectable disease.[27,28]

In this context, there is evidence that supports prophylactic lymphadenectomy for patients with primary PC with metastatic risk. Recent studies have stated that lymphadenectomy a positively impact in the survival of patients with PC[28,29] Although it was not statistically significant, our study corroborates these findings, as patients who underwent prophylactic lymphadenectomy, had a 5.5-months longer disease-free survival period than those who underwent therapeutic lymphadenectomy.

5. Limitations

Due to the storage conditions in the penectomy blocks, which prevented a more adequate paraffin block revision and incomplete data in the patients' medical records, we could not

perform the analysis of the 189 original cases, leaving only 55 cases that fit our methodological criteria. For the same reason, the evaluation of tumor subtype, size, and koilocytosis was reliable in all 55 cases.

6. Conclusion

Our study demonstrated, through multivariate analysis, that the presence of angiolymphatic invasion and absence of koilocytosis were predictors of lymph node metastasis in patients with PC. Although without statistical significance, patients who underwent prophylactic lymphadenectomy presented a longer disease-free survival period than those who underwent therapeutic lymphadenectomy.

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