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Review

A systematic review of non-HPV prognostic biomarkers used in penile squamous cell carcinoma

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ABSTRACT

The presence of lymph node metastasis is the most important prognostic indicator for patients with penile cancer. However, determining if clinically node negative patients may be harbouring lymph node micrometastasis undetected by conventional imaging modalities remains difficult. The aim of this systematic review is to provide an overview of biomarkers p53, Ki-67, and SCCAg in predicting lymph node metastasis (LNM) and cancer-specific survival (CSS) in penile squamous cell carcinoma (SCC). MEDLINE, EMBASE, Cochrane Library, Scopus, and ClinicalTrials.gov were searched from inception until 15 October 2020. Eligible studies were identified by three independent reviewers. Outcome measures included the presence of penile LNM and CSS. Extracted data were narratively synthesized with GRADE criteria utilized to evaluate the quality of evidence. In total, 999 articles were screened with 20 selected for inclusion. Studies reporting the use of p53 to predict LNM and CSS were rated as having the highest quality of evidence using the GRADE criteria, and the majority showed a positive association between p53 expression and LNM and CSS. All biomarkers and outcome combinations had at least one study showing a significant effect on predicting the outcome. However, studies were heterogeneous, and many reported nonsignificant effects. Identifying p53 overexpression may help one to identify patients at higher risks of LNM to be considered for early inguinal lymphadenectomy. There is contradictory and unreliable evidence for the prognostic value of Ki-67 and SCCAg in penile SCC for LNM and CSS. Larger studies are required with more rigorous methods and reports to improve the evidence base.

Keywords: Biomarkers; lymph node excision; lymphatic metastasis; penile neoplasms; prognosis; survival.

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Introduction

Although penile cancer is a rare male genital malignancy, it has significant adverse psychological and functional outcomes in affected men.[1] Penile squamous cell carcinoma (SCC) is the most common penile malignancy[2] with inguinal lymph node metastasis (LNM), one of the most important prognostic indicators in these patients[3] with a difference of 33% in 5-year cancer-specific survival (CSS) between patients with node positivity and those without.[4]

The detection of metastatic inguinal lymph nodes requires surgical resection by inguinal lymphadenectomy to limit the spread of disease. However, up to 25% of cN0 patients can still have microscopic LNM,[5] which cannot be palpated and are difficult to detect by conventional imaging modalities.[6] A proposed solution to avoid leaving behind lymph nodes with micrometastatic disease in this group is to offer prophylactic inguinal lymphadenectomy to all but the lowest risk groups (patients with penile cancer classified as ≥pT1G2) with cN0 disease.[7] The inherent problem with this approach is the significant overtreatment[8] of patients who do not harbor pathological lymph nodes which results in unwanted morbidity related to wound breakdown, lymphoceles, and problematic genital and lower limb

lymphoedema.[9] Therefore, there is a need to identify other biomarkers which could be used to risk stratify patients and avoid overtreatment of patients who would ultimately not benefit from an inguinal lymphadenectomy due to the absence of micrometastatic disease.

A number of tumor markers have been assessed as possible prognostic indicators in penile cancer. The molecular pathogenesis of penile cancer can be attributed to both HPV-mediated and non-HPV-mediated pathways. The HPV-mediated pathway occurs due to persistent epithelial infection with HPV, leading to integration of HPV DNA into the host cell genome, and subsequent malignant transformation due to the overexpression of viral oncoproteins E6 and E7. The mechanism behind non-HPV mediated penile SCC is less well understood, but it is thought to be related to chronic inflammation, somatic genetic alterations, and mutations of proteins such as p53, genomic copy number variations, and aberrant microRNA expression.[10] Human papilloma virus (HPV) infection is thought to be present in 30-50% of penile cancer patients,[11] and its use as a biomarker, along with its surrogate biomarker, P16INK4A, has been extensively studied in previous research.[12–14] However, other lesser researched biomarkers are available, including p53, Ki-67, and squamous cell carcinoma antigen (SCCAg).[15] Each of these proteins act at different stages of the cell cycle. p53 is a tumor suppressor protein that can be silenced in normal cells by HPV infection, leading to disordered cell growth and malignant transformation.[16] Ki-67 is a protein associated exclusively with cell proliferation, present at all stages of the cell cycle except G0 (resting cells), and higher concentrations of ki-67 positive cells has been correlated with poorer survival and tumor recurrence.[17] SCCAg is a subfraction of TA-4, a tumor associated antigen, and has been correlated with lymph node involvement and response to treatment in cervical cancer.[18]

While current research suggests that high expressions of these in penile cancer patients may be associated with, and predict LNM and a poorer CSS, conflicting results are currently available about their prognostic value.[15,19] An improved understanding of this could help with clinical decision making as increased expression and detection of these biomarkers in the primary lesion may indicate a need for more aggressive intervention such as inguinal lymphadenectomy. Therefore, this systematic review aims to evaluate the value of p53, Ki-67, and SCCAg biomarker expression in predicting LNM and CSS.

Main Points

- p53 is the most promising biomarker in the identification of patients with penile squamous cell carcinoma at higher risk of lymph node metastasis.
- p53 can predict both lymph node metastasis and survival in this group of patients.
- Further research is required into the prognostic usefulness of the biomarkers Ki-67 and SCCAg in patients with penile squamous cell carcinoma.

Method

This systematic review was performed following PRISMA guidelines[20] although a meta-analysis was unable to be performed due to large amount of heterogeneity in reporting of results and limited statistical data. This review also follows the Synthesis Without Meta-analysis (SWiM) reporting guidelines.[21] This review has been prospectively registered on PROSPERO[22] (CRD42020165625).

Study Eligibility Criteria

Eligible articles were peer-reviewed original studies investigating human adult males diagnosed with histologically confirmed penile SCC. The minimum sample size required was at least ten unique tissue samples/patients. Study types included were observational cohort or case-cohort studies. All studies had to identify the presence of p53 and/or Ki-67 and/or SCCAg biomarkers in penile SCC. Furthermore, they also had to statistically analyze (with univariate or multivariate tests resulting in a p-value and/or 95% confidence interval) the relationship between the expression(s) or overexpression(s) of any of the aforementioned biomarkers and LNM and/or CSS in penile SCC.

Studies that did not identify the expression of any of the included biomarkers or which did not statistically analyze the relationship between the expression of any included biomarker and LNM or CSS in penile SCC specifically were excluded. Additionally, animal studies, case studies, letters to editors, literature reviews, conference abstracts, and studies not available in the English language were excluded.

Information Sources and Search

MEDLINE, EMBASE, and Cochrane library databases were searched for all relevant articles from inception until 15 October 2020. The search terms used were prognostic biomarker, predictive biomarker, biological marker, marker, squamous cell carcinoma antigen, scc ag, scc ag, p53, Ki-67, and Ki67 with regard to biomarker and penile cancer, penile carcinoma, penile neoplasm, penile squamous cell cancer, penile squamous cell carcinoma, penile scc, and penis cancer with regard to carcinoma. Gray literature was searched using abstracts via Scopus, and ongoing relevant clinical trials were identified

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through ClinicalTrials.gov with authors of any potentially relevant trials contacted for any available preliminary data. A reference review was also conducted on all included studies with backward and forward citation tracking to identify any other pertinent literature.

Study Selection

All results were screened independently by three individuals (JB, SS, and CO'H) who were blinded to each other's decisions. Results were initially title screened, followed by abstract and full paper screening against the eligibility criteria. Any discrepancies were resolved by discussion until a complete consensus was reached. Specific reasons for exclusion were noted for all papers eliminated during full paper screening.

Data Collection and Data Items

Data from all included studies were extracted by two individuals (JB, SS) onto a predefined extraction datasheet. Data collected for study characteristics included authors, year, study design, sample size, age of participants, and length of follow-up. Data on outcome measures were collected, including biomarkers investigated, method of biomarker expression, cutoffs to define expression and overexpression, statistical method used, number of patients with LNM, CSS at time points dependent on paper, and univariate and multivariate analysis of the relationship between biomarker expression and outcomes.

Data were extracted for the entire relevant population, ie, all penile SCC patients studied in each article. Subgroup data for this population were not extracted. As meta-analysis was not possible due to heterogeneity between studies and lack of effect estimates in many studies, data synthesis was conducted through vote counting based on the direction of effect to determine effect sizes, following the SWiM reporting guidelines.[21].

Risk of Bias Assessment

The Newcastle-Ottawa Quality Assessment Scale for Cohort Studies[23] was used to evaluate risk of bias in each included study. A risk of bias assessment was performed by two individuals (JB, SS) using a semiquantitative approach. Studies were given stars across the three domains of selection, comparability, and outcome/exposure, and then given an AHRQ standard rating of good, fair, or poor. Due to the paucity of studies available, studies with a poor AHRQ standard rating were included in this review. Risk of bias across studies was also evaluated using the Grading of Recommendations Assessment, Development and Evaluation approach GRADE[24] approach for assessment of evidence about prognosis on an individual outcome level (LNM and CSS) for each biomarker. This was further used to produce GRADE evidence profile tables for the use of each biomarker.

Results

Study Selection

A total of 999 articles were identified for screening through database searching, in which 758 records were eliminated by removal of duplicates and title and abstract screening. Finally, 23 full-text articles were assessed for eligibility, and 20 were selected for inclusion [Figure 1].

Study Characteristics

A total of 1,534 histologically confirmed penile SCC patients were investigated across all included studies[14,25–43] between 2002 and 2019. Three were prospective cohort studies,[30,31,42] while 17 were retrospective cohort studies.[14,25–29,32–41,43]

Ten studies investigated p53 expression, eight investigated Ki-67 expression, and five investigated SCCAg expression as exposures. Sixteen studies investigated LNM and 12 CSS as outcome measures. Median follow-up ranged between 20 and 108 months. An overview of the included studies' characteristics is provided in supplementary table 1.

p53 Expression

Pathological Lymph Node Metastasis

Six studies[14,34–36,42,43] investigated the relationship between increased p53 expression and LNM in 1,534 penile SCC patients who had undergone surgery (supplementary table 2). All studies considered that lymph node metastasis can spread to any pelvic or inguinal lymph node.

All were retrospective cohort studies that used immunohistochemical staining to visualize p53 expression in primary penile tumor tissue samples under light microscopy. Four studies used the same anti-p53 monoclonal antibody for staining. Cutoffs used to define increased p53 expression were similar for the four studies[34,35,42,43] in which all used a threshold of 20% positively stained nuclei to determine increased p53 expression. Other studies used cutoffs >10% positive staining[36] and a scoring system synthesizing extent and strength of staining.[14]

Through vote counting, 100% of studies showed evidence of an effect of p53 on LNM. The majority of studies reported a positive association at some level between increased p53 expression and LNM with four studies reporting a statistically significant relationship between the two[34–36,43] with relative risks ranging from 1.04 to 266.4. Zargar-Shostari[14] found a positive association between positive p53 status and LNM (OR

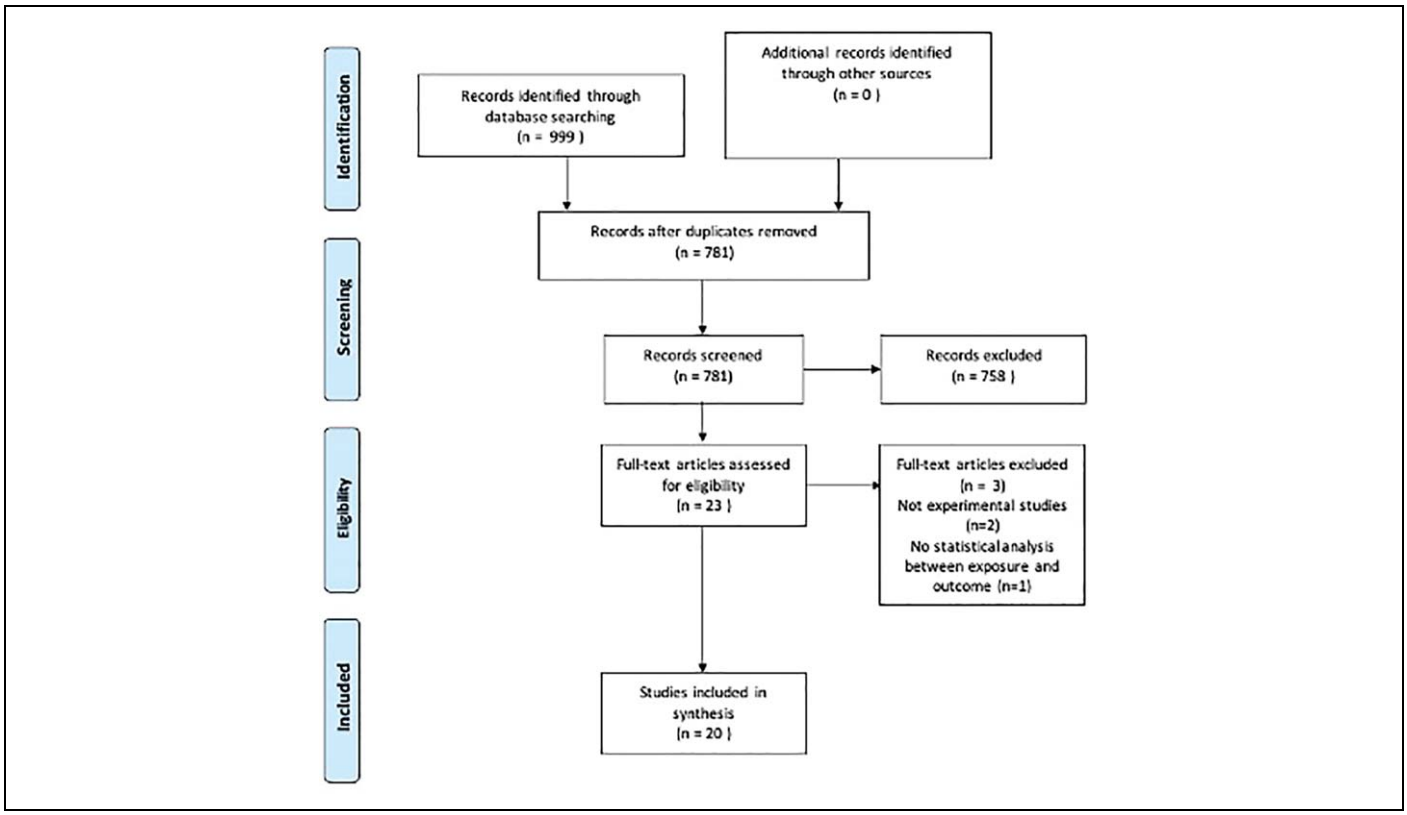

Figure 1. PRISMA flow chart illustrating article selection.

4.4; 95% CI 1.04-18.6) but only in patients who were also p-16 negative, while in the multivariate analysis carried out by Zhu et al.,[42] the relationship between p53 expression and LNM was not significant, despite a significant relationship in their previous study findings.[43]

Cancer-Specific Survival

Eight studies,[14,26,27,29,35,36,38,43] which included 485 penile SCC patients, investigated the relationship between increased p53 expression and CSS (supplementary table 2). All were retrospective cohort studies that used immunohistochemical staining to visualize p53 expression in malignant tissue samples under light microscopy. Different thresholds were used to categorize p53 positivity, ranging from >5% staining used by Bethune et al.[26] to >60% staining used by Prapiska et al.[38]

Through vote counting, 75% of studies showed evidence of an association between p53 expression and CSS. Six studies reported a significant relationship between p53 expression and CSS[27,29,35,36,38,43] with the association being shown on both univariate and multivariate analysis and the hazard ratios increased with increased p53 expression ranging from 3.20 (95% CI 1.05-9.76)[29] to 15.28.[27] Two studies showed no significant relationship between p53 and CSS.[14,26]

Ki-67 Expression

Pathological Lymph Node Metastasis

Seven studies,[25,28,32,37,39,40,43] which included 630 patients, investigated the relationship between increased Ki-67 expression and LNM in penile SCC patients who had received surgical treatment (supplementary table 3). All were retrospective cohort studies that used immunohistochemical staining to visualize Ki-67 expression in primary penile tumor tissue samples under light microscopy. Thresholds used to categorize Ki-67 expression varied greatly: studies either used a staining threshold above which defined positivity for Ki-67,[25,28,32,40,43] while others classed samples into groups based on percentage of positively stained nuclei[39] or depth of staining.[37]

Through vote counting, 71% of studies showed evidence of an association between Ki-67 expression and LNM. Four studies showed a significant positive relationship between high Ki-67 expression and LNM[25,28,37,39] with only Guimares et al.[28] calculating a risk ratio that was 3.73 (95% CI 1.4-9.7), while other studies calculated the difference in percentage of patients with LNM whose samples were either positive or negative for Ki-

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67. Three studies showed no significant relationship between elevated Ki-67 and LNM.[32,40,43] Guimares et al.[28] was the only study to report a statistically significant relationship between increased Ki-67 and LNM on multivariate analysis.

Cancer-Specific Survival

Seven studies[26,28,32,37,39,40,43] investigated the relationship between increased Ki-67 expression and CSS in 629 penile SCC patients (supplementary table 3). All were retrospective cohort studies that used immunohistochemical staining to visualize Ki-67 expression in malignant tissue samples under light microscopy. Different thresholds were used to categorize Ki-67 staining in all the included studies. Most studies[26,28,32,40,43] used percentages of positively stained nuclei as thresholds for increased Ki-67 expression in survival analysis. These ranged from >10%[26] to >48.1%.[32] May et al.[37] organized tissues into three categories based on the depth of staining.

Through vote counting, 57% of studies showed evidence of an association between Ki-67 expression and CSS. Three studies reported a significant positive association between high Ki-67 expression and poorer CSS.[37,39,43] May et al.[37] performed both univariate and multivariate analyses, and only reported significance on the univariate analysis ($P = .047$), while Protzel et al.[39] also reported a significant relationship on univariate analysis (log rank test = .0098). All other studies reported no statistically significant relationship between Ki-67 and poorer CSS on both univariate[26,28,32,40] and multivariate[28,43] analyses.

Squamous Cell Carcinoma Antigen Expression

Pathological Lymph Node Metastasis

Five studies[30,31,33,34,41] investigated the relationship between increased SCCAg expression and LNM in 382 penile SCC patients who had undergone surgical treatment (supplementary table 4). Two were prospective cohort studies,[30,31] whereas the other three[33,34,41] were retrospective cohort studies. SCCAg was measured in the sera of penile SCC patients using a variety of methods. Cutoffs used for increased expression were >1400 ng L^{-1} by Li et al.,[33] >1500 ng L^{-1} in three studies[31,34,41] and >2000 ng L^{-1} by Hungerhuber et al.[30] SCCAg measurements taken Pre-treatment SCCAg levels were included for statistical analysis in two studies,[33,41] while post-treatment SCCAg levels were included for statistical analysis in one study.[31] Two studies did not specify the timing of the SCCAg measurements used.[30,34]

Through vote counting, 100% of studies showed evidence of an association between SCCAg expression and LNM. Only Zhu et al.[41] reported a statistically significant positive relationship between increased SCCAg levels and LNM on univariate analysis, finding SCCAg >1500 ng L^{-1} had 34.3% sensitivity and 89.3% specificity for predicting LNM ($P = .05$). The four other studies[30,31,33,34] found no statistical significance for the relationship between increased SCCAg levels and LNM with Hungerhuber et al.[30] and Liu et al.[34] showing this lack of association on multivariate analysis.

Cancer-Specific Survival

Two studies[33,41] investigated the relationship between increased SCCAg expression and CSS in 171 penile SCC patients who had undergone surgical treatment (supplementary table 4). Both were retrospective cohort studies measured pre-treatment SCCAg levels in the sera of penile SCC patients with Li[33] using a value of >1400 ng L^{-1} and Zhu et al.[41] >1500 ng L^{-1} to define increased expression of SCCAg.

Through vote counting, 100% of studies showed evidence of an association between SCCAg expression and CSS. Both univariate and multivariate statistical analyses were performed to assess the relationship between increased SCCAg expression and CSS. Univariate analysis by Li et al.[33] revealed a statistically significant lower 3-year CSS difference of 28% between patients with increased SCCAg expression and patients with normal SCCAg expression, while multivariate analysis showed a statistically insignificant hazard ratio for 3-year CSS with increased SCCAg expression (HR 4.564, 95% CI 0.583-35.7, $P = .148$). In comparison, multivariate analysis by Zhu et al.[41] found that increased SCCAg was an independent prognostic factor of disease free survival but only for patients with LNM (OR 0.13, 95% CI 0.032-0.55).[41]

Risk of Bias (Quality) Assessment of Articles

Evaluation of risk of bias across studies was carried out using the GRADE approach for prognostic studies.[24] This showed the overall quality of evidence for use of p53 as a biomarker for LNM and CSS to be moderate and high, respectively. For the use of SCCAg as a biomarker for LNM, the quality of evidence was rated as being very low, while for CSS, the quality rating was moderate. For the use of Ki-67 as a biomarker for both LNM and CSS, quality of evidence was also rated as low (supplementary tables 5-7).

Individual risk of bias assessment using the Newcastle-Ottawa Quality Assessment Scale for Cohort Studies[23] resulted in seven studies[25,27,31,32,38,39,41] receiving an Agency for Healthcare Research and Quality (AHRQ)[44] standard quality rating of POOR. Reasons for this included not matching exposed and nonexposed individuals for possible confounders such as age, medical history, tumor stage, and tumor grade as well as failing to adjust for the potential effects of

these confounders in their analysis by using multivariate statistical tests.

The remainder of the studies received an AHRQ standard quality rating of FAIR.[14,26,28–30,33–37,40,43] The justification behind this was that the exposures were always assessed by the authors rather than obtained independently from a third-party source or secure record. This may lead authors to overstate exposures in groups with the outcome of interest and understate exposures in groups without the outcome of interest due to publication bias. A summary of individual risk of bias assessment is provided in supplementary table 8.

Discussion

LNM is the most important prognostic indicator for penile SCC, and the successful identification of biomarkers indicating a higher risk of metastasis is essential to avoid unnecessary lymphadenectomy. The identification of biomarkers that also correspond to CSS is also critical to enable better risk-stratification of patients who receive a diagnosis. This review identified 20 observational studies reporting on a possible relationship between either p53, Ki-67 of SCCAg and one of LNM or CSS.

Four out of the six papers identified showed a significant relationship between p53 overexpression and LNM with calculations of relative risk ranging widely from 1.04 to 266.4. Six out of eight total papers showed a significant association between p53 overexpression and decreased CSS with hazard ratios ranging from 3.20 to 15.28. Studies of p53 also had the highest proportion of studies favoring the use of this biomarker by vote counting. Studies evaluating Ki-67 had the largest number of participants at 630 and 629 for the evaluation of LNM and CSS, respectively. Out of the seven studies analyzing the relationship between Ki-67 expression and LNM, only four identified a statistically significant relationship, and only one of these calculated the risk ratio, simply opposing percentage comparisons. Less than half of the studies analyzing the relationship between ki-67 and CSS reported a statistically significant relationship between the two. Finally, studies investigating SCCAg had the smallest number of participants with 382 total participants in studies of LNM and 171 in the two studies of CSS. Only one of five studies identified showed a statistically significant relationship between SCCAg expression and LNM, while the relationship between SCCAg and CSS was only shown to be statistically significant on multivariate analysis in patients with LNM in one of the two studies.

Combining these findings with the GRADE approach for the evaluation of each outcome demonstrated p53 to be the best biomarker currently available with a quality rating of moderate and high for predicting LNM and CSS, respectively. Clinically, this suggests routine p53 immunohistochemistry of biopsy samples may provide a valuable prognostic indicator in penile SCC patients and be more reliable than Ki-67 and SCCAg. However, prior to widespread clinical use, the multiple limitations of included studies must be considered. First, effect estimates were heterogenous across the studies and outcomes with large differences in possible effect estimates. Additionally, the majority of studies were retrospective, meaning the most used convenience samples, and were therefore subject to selection bias. There were also discrepancies in methods, variations in thresholds used for each biomarker, and variable timings used which all introduced uncertainty. Finally, the differences in the outcome measures themselves, such as confining LNM to only inguinal lymph nodes, or defining CSS at 3-years versus 5-years, all reduce the certainty of the use of these biomarkers on a widespread scale.

Further research is required to address the current limitations in evidence. The findings of this review suggest that p53 may warrant the greatest focus as compared to other biomarkers. Future studies should also focus on using a prospective methodology with clinically well-matched cohorts and more consistent definitions of outcomes to further prove any possible relationship between the three biomarkers and prognosis. Furthermore, there is a need for a standardized technique of measuring the biomarker being investigated, so that methodological discrepancies between studies do not affect results. However, what is truly required is that the investigation of the use of markers, such as p53, in routine clinical pathways for risk stratification of penile SCC patients is, in particularly, difficult in managing the subset which are clinically lymph node negative. The ultimate evaluation impact that utilizing these biomarkers as a stratification tool has on the clinical outcomes of patients remains the final target for future studies.

This is the first review to systematically evaluate the use of p53, Ki-67, and SCCAg as biomarkers to predict LNM and CSS in penile SCC. We have critically assessed the evidence for these outcomes and thereby their potential use as clinical markers for evaluating the need for lymphadenectomy in this group. However, as with any review, there are limitations. The previously mentioned limitations of the evidence base itself is important to consider. The heterogeneity in reporting results and methods meant that a meta-analysis for this review could not be carried out: some studies reported only descriptive statistics, others risk ratios, and others odds ratios with variation in the amount of these data included in the papers to support the results. This unfortunately also means the utility of each

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biomarker could not be directly compared, and individual recommendations were made on quality of the evidence of each biomarker separately. Furthermore, the use of vote counting does not account for papers with insignificant results, which are still counted as favoring use of the biomarker. Finally, as with any review, there is a possibility of studies missed by this review, although we have attempted to minimize this through our broad and comprehensive search strategy.

A good basis of evidence exists for the relationship between p53 expression and LNM in penile SCC, suggesting that this may be a useful adjunct as a predictive marker in this cohort. This has the potential to aid decision making difficult to treat clinically node negative subgroup for early inguinal lymphadenectomy. There is, however, contradictory and unreliable evidence for the prognostic value of Ki-67 and SCCAg in penile SCC both in terms of LNM and CSS. Furthermore, prospective, high quality studies with standardized methods and outcomes of identifying each biomarker are required to assess their full prognostic potential. Additionally, the evaluation of the impact of utilizing these markers as a risk stratification tool on clinical outcomes is required prior to widespread clinical use.

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