Gynecologic Oncology 152 (2019) 208–217

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Review Article

The prognostic value of p16 and p53 expression for survival after vulvar cancer: A systematic review and meta-analysis

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HIGHLIGHTS

- Women with p16 positive vulvar cancers had better survival compared to p16 negative.
- p53 positive vulvar cancers had a less favorable survival compared to p53 negative.
- p16 and p53 may be clinically useful prognostic markers for vulvar cancer patients.

ARTICLE INFO

Article history:
Received 27 August 2018
Received in revised form 11 October 2018
Accepted 15 October 2018
Available online 8 November 2018

Keywords:
Vulvar cancer
p16
p53
Survival
Review
Meta-analysis

ABSTRACT

The tumor suppressor proteins p16 and p53 have been suggested to have prognostic value in some human papillomavirus (HPV)-associated cancers, however, this has been less well established for vulvar cancer. The aim of this review and meta-analysis was to examine the prognostic value of p16 and p53 expression status on survival after vulvar squamous cell carcinoma (VSCC). We conducted a thorough systematic literature search of multiple databases to identify studies examining survival after histolocally verified VSCC that were tested for p16 and/or p53. A total of 18 eligible studies were included. Using a fixed-effects model we calculated study-specific and pooled hazard ratios (HRs) of 5-year overall survival (OS). In the analyses of OS, we included 475 VSCC cases tested for p16 expression of which 38% were p16 positive. The pooled HR_{p16} was 0.40 (95% CI: 0.29–0.55). In addition, the majority of results from studies with adjusted analyses on the prognostic value of p16 indicated that p16 expression status could be an independent prognostic marker for OS in women diagnosed with VSCC, and the same pattern was seen for disease specific survival (DSS). We also included 310 VSCC cases tested for p53 expression of which 54% were p53 positive. The pooled HR_{p53} was 1.81 (95% CI: 1.22–2.68) indicating that p53 positive VSCC have a significantly lower 5-year OS compared to p53 negative. The results in relation to p53 reported from adjusted analyses OS and on DSS and disease free survival were more equivocal. This meta-analysis and review suggests that p53 and especially p16 expression status are of prognostic importance in women diagnosed with VSCC. This may be clinically important in the future design of targeted therapy and when planning the optimal follow-up strategy. Future studies should include the combined use of biomarkers such as p16, p53 and HPV status.

Abbreviations: SCC, squamous cell carcinoma; VSCC, vulvar squamous cell carcinoma; HPV, human papillomavirus; pRb, phosphorylated retinoblastoma protein; PRISMA, preferred reporting items for systematic reviews and meta-analysis; FIGO, International Federation of Gynecology and Obstetrics; OS, overall survival; DFS, disease free survival; DSS, disease specific survival; REMARK, Reporting Recommendations for Tumor Marker Prognostic Studies; HR, hazard ratio; CI, confidence interval; RR, relative risk; IHC, Immunohistochemistry; EGFR, epidermal growth factor receptor.

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https://doi.org/10.1016/j.ygyno.2018.10.015
0090-8258/

Contents

1. Introduction

Vulvar cancer accounts for approximately 5% of gynecological cancers and in 2012, 34,000 women were diagnosed with vulvar cancer worldwide [1,2]. In recent years, the incidence of vulvar cancer has increased, especially among younger women [3]. Squamous cell carcinoma (SCC) is the most common histological type of vulvar cancer, and represents 80%–90% of the cases [4]. Vulvar squamous cell carcinoma (VSCC) develop through two distinct pathways [5,6], of which one is associated with human papillomavirus (HPV), has basaloid and warty morphology and occurs in younger women. The other type is non-HPV related, and occurs more frequently in older women and is often associated with lichen sclerosis [7–9].

In a recent meta-analysis we found that HPV positive vulvar cancers have a significantly more favorable survival compared to HPV negative [10]. In HPV-associated cancers, the tumor suppressor proteins p16 and p53 have also been suggested to be possible prognostic markers [11,12]. Integration of high-risk HPV DNA into host genome causes overexpression of E6 and E7 oncoproteins. E7 binds to hypophosphorylated retinoblastoma protein (pRb), which affects the cell cycle control and consequently leads to upregulation of the tumor suppressor protein p16 [13]. The oncoprotein E6 promotes rapid degradation of p53 protein [14,15]. In addition, mutation of the p53 gene can result in dysfunctional p53 protein expression, which is often seen in HPV negative vulvar cancers [16]. Immunohistochemical (IHC) detection of p53 expression represents the presence of dysfunctional p53 protein, as normal p53 protein has a very short half-life and consequently often is undetectable by IHC. The prognostic significance of p16 is relatively well established in some HPV-related cancers e.g. head and neck cancer and penile cancer [11,12,17], however, for vulvar cancer this is less well established. Furthermore, p53 has been shown to be a possible prognostic marker in head and neck cancer [11,17], but it is uncertain whether the same applies to vulvar cancer.

In a previous meta-analysis, including three studies based on 143 vulvar cancer cases, Cao et al. [18] found that overexpression of p16 was correlated with a superior survival (combining two studies of overall survival (OS) and one study of disease specific survival (DSS)). We have conducted a thorough review of all existing literature on multiple survival outcomes (OS, DSS, and disease free survival (DFS)) after VSCC according to p16 expression status and made an updated meta-analysis, including four new studies of overall survival and more than three times more cases compared to the previous meta-analysis. In addition, we conducted, to our knowledge, the first systematic review and meta-analysis on the prognostic significance of p53 in women diagnosed with VSCC.

2. Materials and method

2.1. Search strategy

We conducted a systematic literature search of the databases PubMed, Embase and Cochrane covering the period up to April 4, 2018. We used a combination of search terms for vulvar cancer, survival, p16 and p53. Medical subject headings and Emtree headings as well as text and keywords were applied in the search. Publications were eligible for inclusion if they were published in English language, included more than five samples and if survival outcomes after histologically verified VSCC in relation to p16 or p53 protein expression were evaluated. Only peer-reviewed studies were included. Case reports, conference abstracts and publications not containing primary data were excluded. Two authors (DMBN and FLS) reviewed all titles, abstracts and full texts independently. Any inconsistencies in the identification of relevant articles were discussed until consensus was reached. The reference lists of retrieved publications were reviewed to identify other relevant studies. Study populations described in more than one paper were only included once and data from the study with the most complete information was used. However, if two papers reported different survival outcomes on the same study populations both papers were included. The study was carried out in accordance with the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines [19].

2.2. Data extraction

Two authors (DMBN and FLS) independently extracted data from the included studies with disagreements discussed until consensus. For each study we extracted information on first author, year of publication and sample collection, country, age (mean, median, range), follow-up time, histology of tumor, International Federation of Gynecology and

Obstetrics (FIGO) stage, treatment modality, tissue type, p16 and p53 testing method, definition of p16 and p53 positivity (overexpression), number of reviewers of staining, sample size, number of p16/p53 positive and survival endpoints. We extracted data on overall survival (OS), disease free survival (DFS), and disease specific survival (DSS). Studies reporting recurrence or relapse free survival were included under DFS because of similar definitions.

2.3. Quality assessment

Evaluation of the quality of the studies was based on guidelines from Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) [20,21]. By means of a predefined form with evaluation criteria (supplementary Table S1) each study was assessed and scored independently by two authors (DMBN and FLS). The studies were scored from zero to seven and a high score indicated a high quality of the study. No study was excluded based on the quality assessment score.

2.4. Statistical analysis

Study-specific hazard ratios (HRs) with 95% confidence intervals (CI) were used to evaluate the association between p16 and p53 expression status and OS. We estimated the HRs using a method previously reported by Moodie et al. [22]. Five year OS was extracted from the studies and if only data was available from survival curves, the five year survival was read manually from the survival plots. We used a fixed effects meta-analysis model to pool the data weighing the studies according to size and significance of results [22]. Statistical heterogeneity was examined with I^2 statistics, which describes the percentage of total variation that is caused by heterogeneity rather than chance [23]. Significance of heterogeneity was evaluated by Cochran's Q-test and a p-value <0.05 was

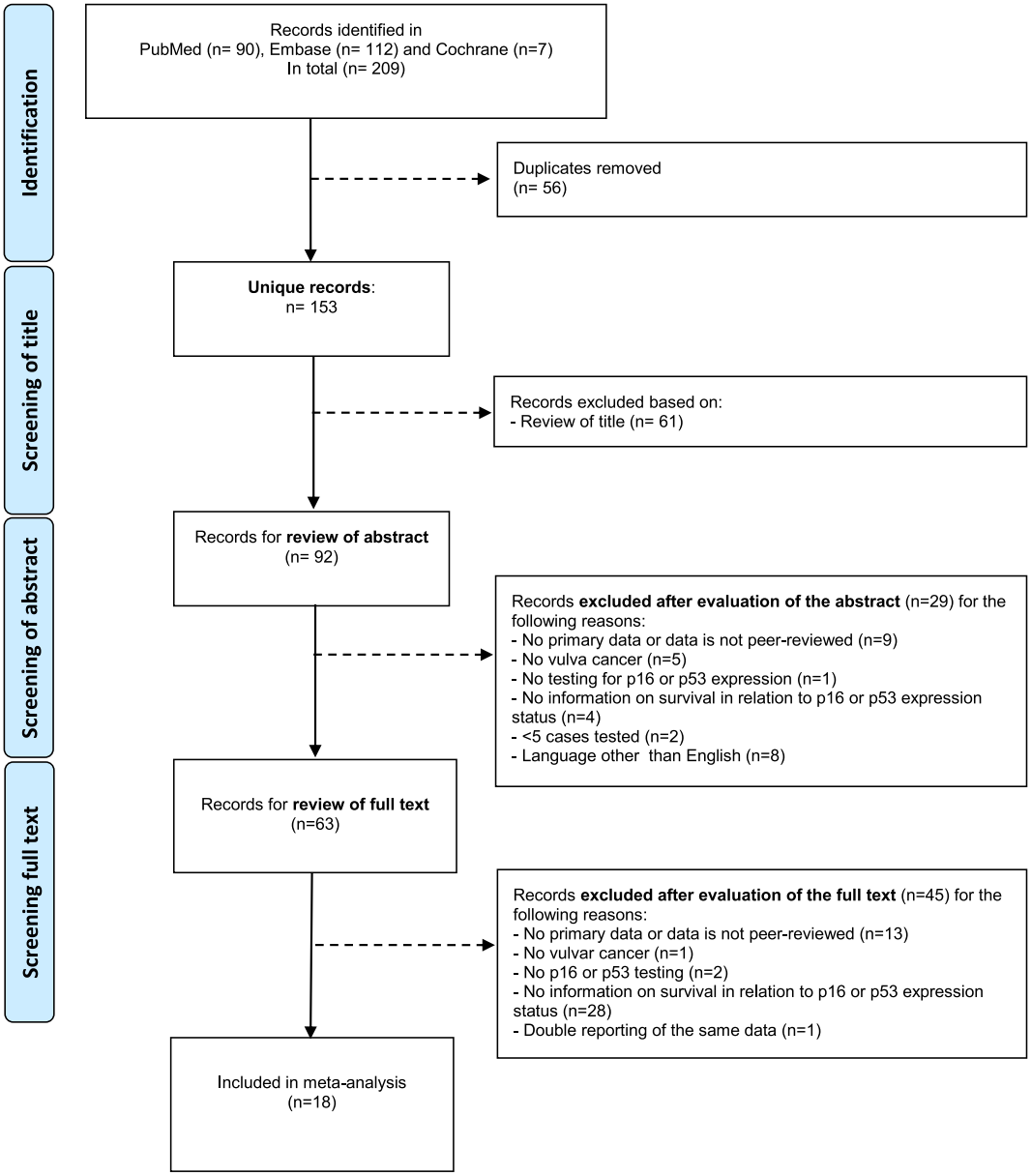


Fig. 1. Flow diagram of study identification and selection.

Table 1
Characteristics of the studies examining survival after vulvar cancer according to p16 expression status.

Author, year, (ref)	Year of sample collection	Country	Age (range), years	FIGO stage	Treatment	Tissue type	p16 testing			No. of women with VSCC	Prevalence of p16 positivity (%)	Survival outcomes reported	Quality assessment score
							p16 test	Definition of p16 positivity (overexpression)	No. of test evaluators				
Knopp et al., 2004 [29]	1977–1991	Norway	Median 70 (27–96)	I-IV	Mixed[1]	Fixed	IHC	>5% of the cells show staining	Three	223	31	DSS[2]	6
Tringler et al., 2007 [30]	NA	Austria and Australia	Mean 68.3	NA	Mixed[1]	Fixed	IHC	At least minimal focal staining within the tumor area	NA	80	43	OS, DFS	3
Fons et al., 2009 [31]	1999–2003	Netherlands	Median 76 (37–92)	I-IV	Mixed[1]	Fixed	IHC	>10% of the cells show staining	Two	80	24	DFS, DSS	5
Alonso et al., 2011 [32]	1995–2008	Spain	(25–96)	I-IV	Mixed[1]	Fixed	IHC	Diffuse nuclear and cytoplasmic expression.	NA	98	20	OS[3]	4
Lavorato-Rocha et al., 2013 [38]	1979–2006	Brazil	Mean 69 Median 71 (15–98)	I-IV	NA	Fixed	IHC	>5% of the cells show staining.	Automated slide analyzer	139	29	DFS, DSS	3
Dong et al., 2015 [33]	1990–2004	USA	Median78 (36–99)	I-IV	Mixed[1]	Fixed	IHC	>70% of the cells show staining	Two	97	34	OS, DFS[4]	4
Hay et al., 2016 [34]	1998–2007	USA	Mean 63.8	IA-IB	Mixed[1]	Fixed	IHC	>60% of the cells show staining	One	50	54	DFS[4]	3
Lee et al., 2016 [35]	1991–2011	USA	Median 75 (44–91)	I-IV	Mixed[1]	Fixed	IHC	>75% of the cells show staining	One	57	37	OS, DFS[4]	5
Sznurkowski et al[5], 2016 [27]	2002–2007	Poland	Median 68 (36–85)	I-IV	Mixed[1]	Fixed	IHC	Strong diffuse and continous nuclear/cytoplasmic expression	Two	85	41	OS	4
Sznurkowski et al[5], 2017 [28]	2002–2007	Poland	Median 68 (36–85)	I-IV	Mixed[1]	Fixed	IHC	Strong diffuse and continous nuclear/cytoplasmic expression	Two	85	41	DFS	3
McAlpine et al., 2017 [36]	1985–2005	Canada	Mean 66.4	I-IV	Mixed[1]	Fixed	IHC	Moderate to intense nuclear and cytoplasmic expression	Two	197	40	OS, DSS	4
Yap et al., 2018 [37]	2000–2009	Canada	NA	I-IV	Mixed[1]	Fixed	IHC	Strong diffuse cytoplasmic and/or nuclear staining.	Three	39	33	OS, DFS	4

VSCC, vulvar squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; NA, not available; IHC, immunohistochemistry; OS, overall survival; DFS, disease free survival; DSS, disease specific survival.

[1] Mixed treatment includes surgery and/or radiation and/or chemotherapy.

[2] Defined as disease specific mortality in the study.

[3] Defined as overall mortality in the study.

[4] Defined as vulvar cancer recurrence in the study.

[5] The studies by Sznurkowski et al. (2016) and Sznurkowski et al. (2017) have overlapping study populations but rapport different survival outcomes.

considered significant [24]. All analyses were conducted using the statistical software R [25] with the package "meta" [26].

3. Results

3.1. Search results

We identified 209 records in PubMed, Embase and Cochrane Library (Fig. 1). After removal of duplicates, 153 records were screened for potential relevance, of which 61 records were excluded after review of title, and 29 were excluded after evaluation of abstract. Of the remaining 63 records, we excluded 45 after full text review. The reasons for excluding records based on abstract and full text are specified in Fig. 1. A total of 18 studies were included in the review and meta-analysis. Two studies [27,28] had overlapping study populations, but reported different survival outcomes and were therefore both included. Among the included studies, 12 studies [27–38] examined survival according to p16 expression status of the tumor and 10 studies [29,32–34,39–44] according to p53 expression status. Four studies [29,32–34] reported information on survival according to both p16 and p53 expression status.

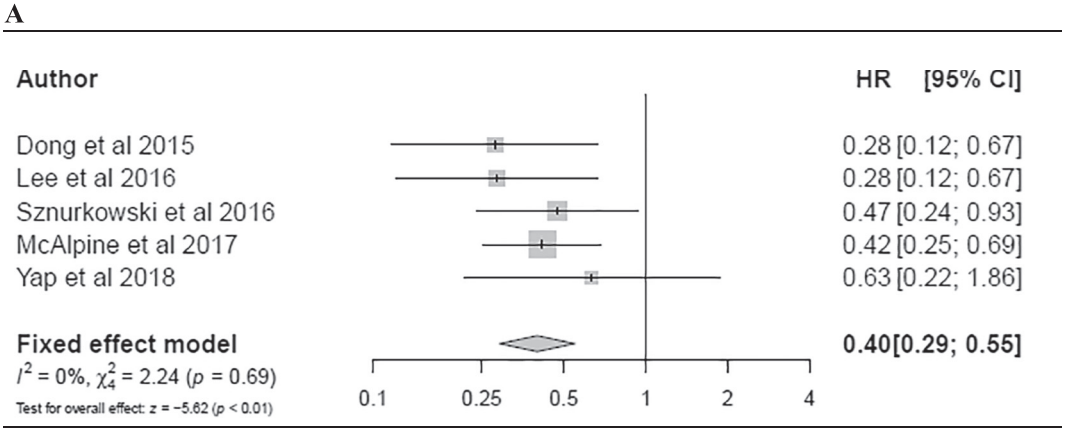
3.2. Survival according to p16 expression status

3.2.1. Study characteristics

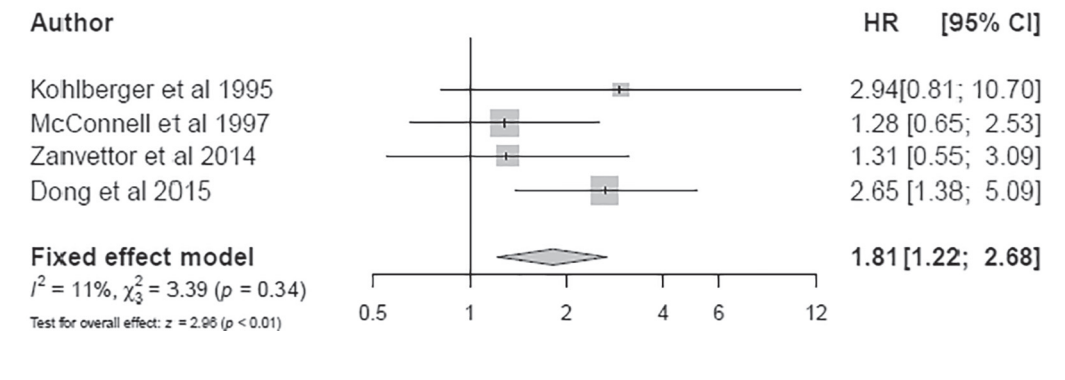
The characteristics of the studies ($n = 12$) examining survival after VSCC according to p16 expression status are presented in Table 1. The studies were published between 2004 and 2018 and the VSCC samples were collected from 1977 to 2009. Five studies originated from Europe [27–29,31,32], five from North America [33–37] and one from South America [38]. One study was a multi-country study containing data from both Austria and Australia [30]. The mean or median age of the women was mostly around the late 60s ranging from 66.4 to 78 years. The majority of studies ($n = 11$) [27–33,35–38] included VSCC of FIGO stage I–IV whereas one study [34] only included tumors of stages IA–IB. In 11 studies, the women with vulvar cancer were treated with a combination of surgery and/or radiation and/or chemotherapy. One study did not report information on treatment [38]. All studies used fixed tissue samples and IHC to determine p16 positivity. Description of the definition of p16 positivity (overexpression) applied in the studies, number of evaluators of the p16 test, survival outcomes reported and quality assessment scores are presented in Table 1. The number of women with VSCC included in the studies ranged from 39 to 223 with the majority having <100 cases. The prevalence of p16 positivity in the vulvar carcinomas ranged from 20% to 54%.

3.2.2. Overall survival

Among the seven studies [27,30,32,33,35–37] investigating OS, five studies [27,33,35–37] reported information on 5-year OS according to p16 expression status. A total of 475 women with VSCC were included in the meta-analysis of which 181 (38.1%) were p16 positive and 294 were p16 negative. The proportion of women alive after five years ranged from 62% to 81% for p16 positive and 22% to 47% for p16 negative. Fig. 2A presents the study-specific and pooled HR of the 5-year OS of p16 positive VSCC compared to p16-negative VSCC. All study-specific HRs were below 1.0, ranging from 0.28 to 0.63. Women with p16 positive VSCC had a significantly more favorable OS compared to



HR, hazard ratio; CI, confidence interval; VSCC, vulvar squamous cell carcinoma



HR, hazard ratio; CI, confidence interval; VSCC, vulvar squamous cell carcinoma

Fig. 2. Forest plot of overall survival in women with p16 positive VSCC compared to p16 negative (Fig. 2A), and p53 positive VSCC compared to p53 negative (Fig. 2B).

Table 2
Overview of the results reported in the studies examining survival for women with p16 positive VSCC compared to p16 negative.

Author, year, (ref.)	Results reported from unadjusted analyses	Results reported from adjusted analyses	Variables included in multivariate analysis								Other variables
			Age	FIGO stage	Grade	Lymph node status	Tumor size	Invasion depth	Surgical margin	Adjuvant radiotherapy	
Overall survival											
Tringler et al., 2007 [30]	*p*-value = 0.05	*p*-value = 0.4	x	x	x	x					
Alonso et al., 2011 [32][1]	HR = 1.04 (95% CI: 0.39–2.78)	*p*-value >0.05[4]	x	x		x	x	x	x	x	Ulceration and p53 and HPV status
Dong et al., 2015 [33]	HR = 0.41 (95% CI: 0.21–0.81)	HR = 0.32 (95% CI: 0.16–0.66)	x	x					x		p53 and EGFR status
Lee et al., 2016 [35]	HR = 0.5 (95% CI: 0.2–0.9)	HR = 0.6 (95% CI: 0.3–1.2)	x	x							
Sznurkowski et al., 2016 [27][2]	HR = 2.06 (95% CI: 1.11–3.81)[3]	HR = 2.11 (95% CI: 1.13–3.95)[3]	x	x	x	x				x	
McAlpine et al., 2017 [36]	HR = 0.35 (95% CI: 0.21–0.59)	HR = 0.60 (95% CI: 0.26–1.27)	x	x							
Yap et al., 2018 [37]	*p*-value = 0.33	NA									
Disease specific survival											
Knopp et al., 2004 [29]	RR = 0.5 (95% CI: 0.2–0.8)	RR = 0.5 (95% CI: 0.3–1.0)		x	x			x			Vessel infiltration and p53, p21 and p27 status
Fons et al., 2009 [31]	HR = 0.54 (95% CI: 0.16–1.84)	NA[7]									
Lavorato-Rocha et al., 2013 [38]	*p*-value >0.05[4]	NA									
McAlpine et al., 2017 [36]	HR = 0.19 (95% CI:0.08–0.41)	HR = 0.21 (95% CI: 0.04–0.78)	x	x							
Disease free survival											
Tringler et al., 2007 [30]	*p*-value = 0.03	*p*-value = 0.3	x	x	x	x					
Fons et al., 2009 [31]	HR = 0.43 (95% CI: 0.12–1.44)	NA (g)									
Lavorato-Rocha et al., 2013 [38]	*p*-value >0.05[4]	NA									
Dong et al., 2015 [33][5]	*p*-value >0.05[4]	NA									
Hay et al., 2016 [34][5]	HR = 0.31 (95% CI: 0.11–0.86)	NA									
Lee et al., 2016 [35][6]	HR = 0.2 (95% CI: 0.05–0.5)	HR = 0.2 (95% CI: 0.06–0.6)	x	x							
Sznurkowski et al., 2017 [28][2]	*p*-value = 0.413	NA									
Yap et al., 2018 [37]	*p*-value = 0.02	NA									

VSCC, vulvar squamous cell carcinoma; FIGO, International federation of Gynecology and obstetrics; HPV, human papillomavirus; EGFR, epidermal growth factor receptor; RR, relative risk; HR, hazard ratio; CI, confidence interval; NA, not available;
[1] Overall survival is reported as mortality in the study.
[2] The studies by Sznurkowski et al. (2016) and Sznurkowski et al. (2017) have overlapping study populations but rapport different survival outcomes.
[3] The relative risk is calculated as p16 negative VSCC compared to p16 positive VSCC.
[4] The study only reports that the results did not reach statistical significance.
[5] Reported as vulvar cancer recurrence in the study.
[6] Disease free survival is reported as in-field relapse in the study.
[7] The study only included variables in the multivariate analyses that contributed significantly in the univariate analysis.

women with p16 negative VSCC with a pooled HR of 0.40 (95% CI: 0.29–0.55). The inter-study heterogeneity was low (I^2 = 0% (p = 0.69)).

Multiple factors have been implicated in the prognostication of vulvar cancer including e.g. age at diagnosis, FIGO stage and lymph node involvement [45]. Table 2 presents the results in the respective studies from the unadjusted and adjusted analyses and an overview of the variables included in the adjusted analyses. Six studies [27,30,32,33,35,36] conducted analyses on OS adjusting for at least age at diagnosis and FIGO stage. In four studies [27,33,35,36], the survival rates remained higher in the adjusted analyses for women with p16 positive VSCC compared to p16 negative VSCC. However, in the studies by McAlpine et al. [36] and Lee et al. [35], who included 197 and 57 women with VSCC respectively, the relative risk estimates were no longer significant. Tringler et al. [30] only reported a *p*-value for the association between p16 expression status and survival. They found that p16 positivity was associated with a better OS in unadjusted analyses (p-value = 0.05), but was not a prognostic marker after adjusting for clinicopathological factors (age at diagnosis, FIGO stage, grade, and lymph node involvement) (p-value = 0.4). In a slightly larger study including 98 women diagnosed with VSCC, Alonso et al. [30] reported that the mortality rate was similar for women with p16 positive and negative VSCC in both the unadjusted and adjusted analyses.

3.2.3. Disease specific survival

Four studies examined DSS after VSCC according to p16 expression status [29,31,36,38]. The study by Knopp et al. [29] was the largest study including 223 VSCC cases and had the highest quality assessment score. They found that women with p16 positive VSCC were younger and less likely to have lymph node metastasis compared to the women with p16 negative VSCC. In an unadjusted analysis, the relative risk (RR) of dying of vulvar cancer for p16 positive women compared to

p16 negative was 0.5 (95% CI: 0.2–0.8). The result remained significant after adjusting for FIGO stage, grade, tumor size, vascular infiltration and p53, p21 and p27 expression status of the tumor (HR = 0.5; 95% CI: 0.3–1.0), and they concluded that p16 could be useful as a prognostic marker in VSCC. In line with this, McAlpine et al. [36] reported p16 expression status to be a prognostic marker for DSS in women with VSCC. Despite of receiving a lower score in the quality assessment, because of lack of information on specimen characteristics, follow-up time and definition of DSS, this study demonstrated detailed results on DSS in association with p16 expression status. They included 197 women of whom 79 and 118 were p16 positive and negative, respectively. In an unadjusted analysis they found that women with p16 positive tumors had a significantly more favorable DSS compared to women with p16 negative tumors (HR: 0.19, 95% CI: 0.08–0.41). When adjusting for age at diagnosis and stage, the result remained significant (HR: 0.21, 95%CI: 0.04–0.78). Furthermore, McAlpine et al. [36] also assessed whether the shift towards more conservative treatment during the recent years (after 1995) affected the general prognosis and prognostic value of p16 and found that the difference in DSS according to p16 expression status primarily occurred among women who underwent surgery after 1995.

Finally, Fons et al. [31] and Lavorato-Rocha et al. [38] both investigated DSS according to p16 expression status of women with VSCC, but none of them succeeded in demonstrating a significant association. Fons et al. [31] reported an unadjusted HR of 0.54 (95% CI: 0.16–1.84) and Lavorato- Rocha et al. [38] stated that the DSS rates did not reach statistical significance, however, no risk estimates were provided in this paper. The only statistically significant association reported in the study was between p16 expression status and FIGO stage ($p = 0.02$).

3.2.4. Disease free survival

Eight studies [28,30,31,33–35,37,38] examined a possible association between p16 expression status of women diagnosed with VSCC and DFS. The main results from the studies are presented in Table 2. Five studies [30,31,34,35,37] reported that women with p16 positive VSCC had a more favorable DFS compared to p16 negative in the unadjusted analysis, however, in one study by Fons et al. [31] the result was not statistically significant. Generally, the number of women included in the studies was small, ranging from 39 to 97 VSCC cases. The prevalence of p16 positivity ranged from 24% to 54%. Two studies [30,35] conducted adjusted analyses. Lee et al. [35] controlled for age at diagnosis and FIGO stage and found that women with p16 positive VSCC had significantly lower rates of relapse than those with p16 negative tumors (HR = 0.2; 95% CI: 0.06–0.6). In contrast, Tringler et al. [30] found no significant association between p16 expression status and DFS after adjusting for age at diagnosis, FIGO stage, grade and lymph node involvement (p-value = 0.3). A larger study by Lavorato-Rocha et al. [38] ($n = 139$ VSCC cases) reported no difference in DFS according to p16 expression status of the tumor, but found that p16 expression status was significantly associated with FIGO stage ($p = 0.02$). These results were later supported study by two other studies [28,33].

Table 3
Characteristics of the studies examining survival after vulvar cancer according to p53 expression status.

Author, year, (ref)	Year of sample collection	Country	Age (range), years	FIGO stage	Treatment	Tissue type	p53 testing			No. of women with VSCC	p53 prevalence (%)	Survival outcomes reported	Quality assessment score
							p53 test	Definition of p53 positivity (overexpression)	No. of test evaluators				
Kohlberger et al., 1995 [39]	1985–1990	Austria	Mean 69 (43–89)	I-III	Mixed[1]	Fixed	IHC	>10% of the cells nuclei showed staining	NA	25	48	OS	1
McConnell et al., 1997 [40]	1974–1994	Scotland	Median 72 (25–96)	NA	Mixed[1]	Fixed	IHC	>10% of the cells showed staining	Two	115	67	OS, DFS	4
Kagie et al., 1997 [41]	1984–1993	Netherlands	Mean 68 (29–90)	I-IV	Mixed[1]	Frozen	IHC	>10% of the cells nuclei showed staining	NA	66	53	DFS	3
Lerma et al., 1999 [42]	1981–1992	Spain	Mean 70.6 (43–89)	I-IV	Surgery only	Fixed	IHC	>10% of the cells showed staining	NA	71	56	OS	2
Knopp et al., 2004 [29]	1977–1991	Norway	Median 70 (27–96)	I-IV	Mixed[1]	Fixed	IHC	>5% of the cells nuclei showed staining	Three	223	45	DSS[5]	6
Alonso et al., 2011 [32]	1995–2008	Spain	(25–96)	I-IV	Mixed[1]	Fixed	IHC	>25% of the cells showed staining	NA	98	56	OS[3]	4
Lavorato-Rocha et al., 2013 [43]	1979–2006	Brazil	Mean 69 Median 71 (15–98)	I-IV	NA	Frozen and Fixed	IHC	>5% of the cells showed staining of intensity 2 or 3	Scanscope software	134	53	DFS, DSS	4
Zanvettor et al., 2014 [44]	1993–2011	Brazil	Mean 67.4	I-III	Mixed[1]	Fixed	IHC	>10% of the cells showed staining	NA	75	65	OS	5
Dong et al., 2015 [33]	1990–2004	USA	Median 73 (36–99)	I-IV	Mixed[1]	Fixed	IHC	>50% of cells nuclei showed staining	Two	95	29	OS, DFS[4]	4
Hay et al., 2016 [34]	1998–2007	USA	Mean 63.8	IA-IB	Mixed[1]	Fixed	IHC	A score of 2+ or 3+[2]	One	47	28	DFS[4], DSS[5]	3

VSCC, vulvar squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; NA, not available; IHC, immunohistochemistry; OS, overall survival; DFS, disease free survival; DSS, disease specific survival.

[1] Mixed treatment includes surgery and/or radiation and/or chemotherapy.
[2] In the study p53 was scored semiquantitatively as negative (no staining), 1+, 2+ or 3+. Only dark, intense nuclear staining was graded.
[3] Defined as overall mortality in the study.
[4] Defined as vulvar cancer recurrence in the study.
[5] Defined as disease specific mortality in the study.

3.3. Survival according to p53 expression status

3.3.1. Study characteristics

The characteristics of the studies ($n = 10$) examining survival after vulvar cancer according to p53 expression status are presented in Table 3. The VSCC samples were collected between 1974 and 2011 and the studies were published in the period 1995–2016. Six studies were from Europe [29,32,39–42], two from North America [33,34] and two were from South America [43,44]. The mean and median age was in ranging from 63.8 to 73 years. Fixed tissue samples was used in most studies ($n = 8$). Six studies [29,32,33,41–43] included tumors of all FIGO stages (I-IV), two studies [39,44] included FIGO stage I–III, one study [34] included tumors of FIGO stage IA–IB, and one study [40] did not report information on FIGO stage. One study did not report information on type of treatment [43], and in all the other studies the women were treated with a combination of surgery and/or radiation and/or chemotherapy in all of the studies, except for one [42] where the women only underwent surgery. All studies used IHC to determine p53 expression status of the VSCCs. In Table 3, a description of the definition of p53 positivity (overexpression) applied in the studies, number of evaluators of the p53 test, survival outcomes reported and quality assessment scores is presented. The study populations consisted of 25 to 223 women diagnosed with VSCC, with most studies including <100 cases, and the p53 positivity prevalence ranged from 28% to 67%.

3.3.2. Overall survival

Fig. 2B shows the study-specific and pooled HR for 5-year OS according to p53 expression status. Four studies were included in the meta-analysis [33,39,40,44]; including a total of 310 women with VSCC of which 166 (53.5%) were p53 positive and 144 were p53 negative. The percentage of women alive after five years ranged from 35% to 63% for p53 positive and 68% to 70% for p53 negative women. The study-specific HRs were all above 1.0, ranging from 1.28 to 2.94. Women with p53 positive VSCC had a significantly worse OS compared to p53 negative with a pooled HR of 1.81 (95% CI: 1.22–2.68). The inter-study heterogeneity was low ($I^2 = 11\%$ ($p = 0.34$)).

In Table 4, the results of the unadjusted and adjusted analyses are presented. Four studies examining OS [32,33,42,44] adjusted for variables of prognostic relevance such as age, FIGO stage, lymph node involvement, tumor size and invasion depth. Dong et al. [33] included 95 women with VSCC of which 29% were p53 positive and found p53 expression status to be an independent prognostic predictor in the analysis adjusted for age, stage, surgical margin, p16 and EGFR status (HR = 2.23; 95% CI: 1.16–4.28). They concluded that p53 expression patterns may offer additional information on survival along with age and FIGO stage. In contrast, three other studies [32,42,44] of similar size reported no association between p53 expression status and OS in the adjusted analysis.

Table 4
Overview of the results reported in the studies examining survival for women with p53 positive VSCC compared to p53 negative.

Author, year (ref.)	Results reported from univariate analyses	Results reported from multivariate analyses	Variables included in the multivariate analysis								Other variables
			Age	FIGO stage	Grade	Lymph node status	Tumor size	Invasion depth	Surgical margin	Adjuvant radiotherapy	
Overall survival											
Kohlberger et al., 1995 [39]	p-value ≤ 0.05	NA									
McConnell et al., 1997 [40]	p-value > 0.05[4]	NA									
Lerma et al., 1999 [42]	p-value > 0.05[4]	p-value > 0.05[4]		x	x	x					Vascular/perineural invasion and pRb status
Alonso et al., 2011 [32][1]	HR = 1.02 (95% CI: 0.46–2.45)	p-value > 0.05[4]	x	x		x	x	x	x	x	Ulceration and p16 and HPV status
Zanvettor et al., 2014 [44]	p-value = 0.456	p-value > 0.05[4]		x		x	x	x			MMP-2 status
Dong et al., 2015 [33]	HR = 2.05 (95% CI: 1.09–3.84)	HR = 2.23 (95% CI: 1.16–4.28)	x	x					x		p16 and EGFR status
Disease specific survival											
Lavorato-Rocha et al., 2013 [43]	RR = 1.1, p-value = 0.74	NA									
Knopp et al., 2004 [29][3]	RR = 1.7 (95% CI: 1.1–2.9)	NA[5]									
Hay et al., 2016 [34][3]	HR = 6.85 (95% CI: 1.7–27.68)	NA									
Disease free survival											
Kagie et al., 1997 [41]	p-value = 0.79	NA									
McConnell et al., 1997 [40]	p-value > 0.05[4]	NA									
Lavorato-Rocha et al., 2013 [43]	RR = 1.22, p-value = 0.49	NA									
Dong et al., 2015 [33][2]	p-value > 0.05[4]	NA									
Hay et al., 2016 [34][2]	HR = 3.23 (95% CI: 1.15–9.06)	NA									

VSCC, vulvar squamous cell carcinoma; FIGO, International federation of Gynecology and obstetrics; HPV, human papillomavirus; EGFR, epidermal growth factor receptor; RR, relative risk; HR, hazard ratio; CI, confidence interval; NA, not available; MMP-2; matrix metalloproteinase 2.

[1] Overall survival is reported as mortality in the study.
[3] Reported as disease specific mortality in the study.
[2] Reported as disease specific recurrence in the study.
[4] Study only reports that the results did not reach statistical significance.
[5] Study only included variables in the multivariate analyses that contributed significantly in the univariate analysis.

3.3.3. Disease specific survival

Three studies [29,34,43] investigated p53 expression status according to DSS. In the largest study, including 223 VSCC cases, Knopp et al. [29] examined the relative risk of dying of vulvar cancer comparing p53 positive and negative VSCC and found a statistically significant association (RR = 1.7, 95% CI: 1.1–2.9). Similarly, in a study including 47 vulvar cancer cases, Hay et al. [34] found that p53 positive women were nearly seven times more likely to die from vulvar cancer compared to women with p53 negative VSCC (HR: 6.85, 95% CI: 1.70–27.68). In contrast, Lavorato-Rocha et al. [43] reported no association between p53 expression status and DSS (RR: 1.1, $p = 0.74$) or clinicopathological variables. Unfortunately, none of the studies examining DSS adjusted for other prognostic factors.

3.3.4. Disease free survival

Five studies investigated the association between DFS and p53 expression status [33,34,40,41,43]. Hay et al. [34] examined vulvar cancer recurrence and found that p53 positive tumors were three times more likely to recur compared to p53 negative tumors (HR = 3.23, 95% CI: 1.15–9.06). They were not able to perform an adjusted analysis to control for confounding factors, however, they did not find any statistical differences between the p53 positive and negative women and clinicopathological parameters when comparing the groups. Two of the larger studies by McConnell et al. [40] and Lavorato-Rocha et al. [43], including respectively 115 and 134 women with VSCC, reported no significant association between p53 expression status and DFS and concluded that p53 expression status should not be considered a prognostic tool in the clinical management of women diagnosed with VSCC. Lavorato-Rocha et al. [43] did not find any association between p53 expression status and DFS (RR: 1.22, $p = 0.49$) or clinicopathological variables. Smaller studies [33,41] have supported their conclusion. None of the studies examining DFS conducted adjusted analyses.

4. Discussion

In the present review and meta-analysis of the association between different measures of survival and p16 expression status, we found that women with p16 positive VSCC had a significantly better OS compared to women with p16 negative VSCC. The majority of the studies on OS performed analyses with adjustment for other prognostic factors, supporting that p16 expression status could be an independent prognostic marker for OS in women diagnosed with VSCC. The same pattern was observed when the outcome of interest was disease specific survival. Even though most of the studies were based on a low number of VSCC cases, the variation in results between the studies, was relatively limited. Knopp et al. [29] and McAlpine et al. [36] appeared to have the most reliable results based on our assessment of the quality of the analysis and the number of included cases and we therefore find it likely that p16 expression status could be a prognostic marker for DSS in women with VSCC. For disease free survival, the pattern was less clear.

By pooling the studies reporting 5-year OS according to p53 expression status, we found that women with p53 positive VSCC had a significantly lower OS rate compared to women with p53 negative VSCC. Unfortunately, only very few studies were able to adjust for prognostic factors and the results on other survival outcomes were diverse and inconclusive. Interestingly, one study by Scheistrøen et al. [46] performed survival analyses according to stage. They included a total of 167 stage I-III patients and found that only in stage III patients, p53 was an independent prognostic factor. However, larger studies would still be needed to further confirm whether p53 is an independent prognostic marker.

Our results on p16 expression status and survival are in agreement with a previous meta-analysis by Cao et al. [18]. In one combined analysis they included three studies; one reporting DSS [29] and two [33,47] reporting OS of which one of the studies [47] is published in Chinese. In our meta-analysis, we exclusively included studies reporting OS in relation to p16 expression status published in English language and by combining the results of five studies including a total of 475 VSCC cases, we were able to present a robust estimate of the association between OS and p16 expression. In addition, we also conducted a systematic review and meta-analysis on survival after vulvar cancer according to p53 expression status of the tumor, which, to our knowledge, has not been done before. Another strength of our study was the comprehensive literature search with two authors independently reviewing the titles, abstracts and full-text and conducting data extraction. However, there were also some limitations that should be considered. In four of the studies [28,36,39,40] included in the meta-analysis the estimates were manually read from Kaplan-Meier curves, which could add some inaccuracy to the estimates. Furthermore, the definitions of p16 and p53 positivity varied between studies, which we were unable to take into account in the analyses.

An association between p16 and p53 expression status and survival could be of clinical relevance, by potentially making it possible to reduce treatment intensity in the HPV/p16 positive group in order to decrease long-term morbidity without compromising survival. This possibility is currently being investigated in head and neck cancer [48]. Furthermore, testing for p16 and p53 expression could be important in relation to design of targeted therapy regimes and planning the most optimal follow-up strategy of women diagnosed with vulvar cancer. In the future, it is essential also to establish the potential value of combined testing of different biomarkers, as this might add further knowledge on the prognosis of women diagnosed with vulvar cancer. It has recently been demonstrated that HPV DNA and p16 positive tonsillar and base of tongue cancers have an improved prognosis compared to using HPV and p16 expression as single markers [49]. However, studies on the combined testing of biomarkers such as p16, p53 and HPV status in vulvar cancer are warranted.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2018.10.015.

Conflict of interest statement

SKK has received lecture fees from Sanofi Pasteur MSD and Merck, scientific advisory board fee from Merck, and research grants through her institution from Merck.

FLS has received support for conference participation and speakers' fees from Becton Dickinson Diagnostics GmbH.

Author contribution section

SKK designed the study. DMBN and FLS performed the literature search and reviewed titles, abstracts and full-text articles. DMBN, FLS, CLR and SKK designed the data extraction. DMBN and FLS extracted the data. MHF performed the statistical analyses. DMBN, FLS, and SKK drafted the manuscript and CLR, and MHF critically revised subsequent drafts.

Financial support

No specific funding was obtained for this study.

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